Circulating palmitoleic acid and risk of metabolic abnormalities and new-onset diabetes[1–4]

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ABSTRACT

Background: Animal experiments suggest that circulating palmitoleic acid (*cis*-16:1n–7) from adipocyte de novo fatty acid synthesis may directly regulate insulin resistance and metabolic dysregulation.

Objective: We investigated the independent determinants of circulating palmitoleate in free-living humans and whether palmitoleate is related to lower metabolic risk and the incidence of diabetes.

Design: In a prospective cohort of 3630 US men and women in the Cardiovascular Health Study, plasma phospholipid fatty acids, anthropometric variables, blood lipids, inflammatory markers, and glucose and insulin concentrations were measured between 1992 and 2006 by using standardized methods. Independent determinants of plasma phospholipid palmitoleate and relations of palmitoleate with metabolic risk factors were investigated by using multivariable-adjusted linear regression. Relations with incident diabetes (296 incident cases) were investigated by using Cox proportional hazards.

Results: The mean (±SD) palmitoleate value was 0.49 ± 0.20% (range: 0.11–2.55%) of total fatty acids. Greater body mass index, carbohydrate intake, protein intake, and alcohol use were each independent lifestyle correlates of higher palmitoleate concentrations. In multivariable analyses that adjusted for these factors and other potential confounders, higher palmitoleate concentrations were independently associated with lower LDL cholesterol ($P < 0.001$), higher HDL cholesterol ($P < 0.001$), lower total:HDL-cholesterol ratio ($P = 0.04$), and lower fibrinogen ($P < 0.001$). However, palmitoleate was also associated with higher triglycerides ($P < 0.001$) and (in men only) with greater insulin resistance ($P < 0.001$). Palmitoleate was not significantly associated with incident diabetes.

Conclusions: Adiposity (energy imbalance), carbohydrate consumption, and alcohol use—even within typical ranges—are associated with higher circulating palmitoleate concentrations. Circulating palmitoleate is robustly associated with multiple metabolic risk factors but in mixed directions, perhaps related to divergent lifestyle determinants or endogenous sources (liver, adipose tissue) of fatty acid synthesis. *Am J Clin Nutr* 2010;92:1350–8.

INTRODUCTION

Palmitoleic acid (*cis*-16:1n−7) is produced during de novo lipogenesis, also termed *fatty acid synthesis* (FS)—the process of converting glucose into fatty acids. In recent murine experiments, genomic deletion of adipose tissue fatty acid–binding protein4 activated adipose tissue FS, which resulted in higher circulating palmitoleate concentrations that directly protected against hepatic and skeletal muscle insulin resistance and related metabolic abnormalities (1). These effects appeared related, at least in part, to suppression of hepatic FS, which suggested that palmitoleate derived from adipose FS may act in a counter-regulatory feedback loop against FS in the liver. Other experimental models further support active metabolic benefits of palmitoleate on adipocyte, macrophage, skeletal muscle, and pancreatic β cells (2–5).

On the basis of these findings, we hypothesized that circulating palmitoleate is an important fatty acid regulator, or lipokine, that is directly involved in the regulation and pathophysiology of lipid and glucose-insulin metabolism. However, we recognized that animal experiments may not be generalizable to humans and that prior investigation of the potential metabolic benefits of palmitoleate in humans has been limited, with few studies evaluating these relations in large samples or with detailed multivariable adjustment. Multivariable adjustment for potentially different drivers of FS and palmitoleate production is particularly important in humans. Generally, the liver is the principal site of FS and palmitoleate production, driven by low-fat, high-carbohydrate diets; heavy alcohol use; or excess energy consumption, which leads to conversion of excess carbohydrate energy into fat for long-term storage (1, 6–12). Thus, adjustment for carbohydrate consumption, alcohol use, and adiposity may be especially important to elucidate the potential causal metabolic effects of palmitoleate

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in humans. We investigated the relations of plasma phospholipid palmitoleate as a proportion of total fatty acids with metabolic risk factors and incidence of diabetes in the Cardiovascular Health Study (CHS)—a community-based, multicenter, prospective study of US adults sponsored by the National Heart, Lung, and Blood Institute. We hypothesized that higher concentrations of palmitoleate would be associated with an improved lipid profile and lower levels of insulin resistance and diabetes.

SUBJECTS AND METHODS

Design and population

The CHS design and recruitment were described previously (13). Briefly, 5201 ambulatory, noninstitutionalized men and women aged ≥65 y were randomly selected and enrolled in 1989–1990 from Medicare eligibility lists in 4 US communities (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Allegheny County, PA); an additional 687 black participants were similarly recruited and enrolled from these communities in 1992. Of all eligible adults contacted, 57% agreed to enroll. Baseline in-clinic evaluations included a standardized physical examination, diagnostic testing, laboratory evaluation, and questionnaires on health status, medical history, and cardiovascular and lifestyle risk factors (13). Each center's institutional review committee approved the study, and all participants gave informed written consent.

Fatty acid measurements

Plasma phospholipid fatty acids were measured in 3630 individuals in blood samples collected in 1992, which was considered to be the baseline year for all present analyses. This included 3130 CHS participants randomly selected from individuals with available blood samples and an additional 500 CHS participants from a prior nested case-control study of incident myocardial infarction. All fatty acid measurements, metabolic phenotypes, and covariates were assessed similarly in these 2 groups, and analyses accounted for this within-cohort sampling by using inverse probability of sampling weights. Plasma phospholipids were measured as an excellent marker of longer-term (4–8 wk) circulating fatty acids, with responses similar to those in erythrocyte membranes (9). Blood was drawn after a 12-h fast and was stored at −70°C before shipment, on dry ice, to the CHS Central Blood Analysis Laboratory (University of Vermont) for long-term storage at −80°C. Under these storage conditions, plasma phospholipid fatty acids are stable long-term, and, in prior studies, we observed no degradation, lipolysis, or oxidation after 10 y of storage (14). Measurements were performed by the Fred Hutchinson Cancer Research Center biomarker laboratory, providing quantitative measurement of 45 fatty acids as a percentage of total fatty acids. Total lipids were extracted from plasma by using the methods of Folch, and phospholipids separated from neutral lipids by one-dimensional thin-layer chromatography. Fatty acid methyl ester (FAME) samples were prepared by direct transesterification using the methods of Lepage and separated by using gas chromatography (Agilent 5890 gas-chromatograph/flame-ionization-detector; Agilent Technologies, Palo Alto, CA; SP-2560 fused-silica 100m capillary column; Supelco, Belefonte, PA; initial 160° Cx16min, ramp 3.0°C/min to 240°C, hold 15 min). Identification, precision, and accuracy were continuously evaluated by using model mixtures of known FAMEs and established in-house control pools, with identification confirmed by gas chromatography–mass spectrometry at the US Department of Agriculture Lipid Laboratory (Peoria, IL). CVs were <3% for most fatty acids and 2.5% for palmitoleate.

Metabolic outcomes and covariates

Risk factors were measured, during the same clinic visit at which fatty acid blood sampling was conducted, by trained personnel using standardized methods (13). Anthropometric measures, including weight, height, and waist circumference, were collected by using standard procedures and equipment; body mass index (BMI) was calculated as weight (in kg) divided by height squared (in m). Fasting blood total cholesterol, HDL cholesterol, and triglycerides were measured according to Centers for Disease Control and Prevention methods. LDL cholesterol was calculated according to the Friedewald equation, excluding individuals with hypertriglyceridemia. Fasting glucose and insulin were measured (Ektacham 700 Analyzer; Eastman Kodak Corp, Rochester, NY), and homeostasis assessment model of insulin resistance (HOMA-IR) and β cell function (HOMA-B) were calculated [(glucose in mg/dL × insulin mU/L)/405; 20 × insulin mU/L/(0.056 × glucose in mg/dL − 3.5); respectively]. Fibrinogen was measured by using standard methods and C-reactive protein (CRP) by using validated in-house high-sensitivity enzyme-linked immunosorbent assay. Seated resting blood pressure (BP) was measured by trained personnel using an appropriately sized cuff and Hawksley random-zero sphygmomanometer (model 7076; Hawksley and Sons Limited, Sussex, United Kingdom). Systolic and diastolic BPs were based on averages of 2 readings taken ≥30 s apart after a 5-min rest. Hypertension was defined as a systolic BP ≥ 140 mm Hg, a diastolic BP ≥ 90 mm Hg, or physician diagnosis of hypertension plus use of antihypertensive medication. Prevalent ischemic heart disease (IHD) was classified based on validated physician-diagnosed myocardial infarction, history of coronary revascularization, or angina. Usual consumption of different alcoholic beverages (wine, beer, liquor) was assessed, including usual frequency of consumption and usual amount consumed on each occasion. Leisure-time activity (kcal/wk) was assessed by using a modified Minnesota Leisure-Time Activities questionnaire that evaluated the frequency and duration of 15 different activities during the prior 2 wk. Usual dietary habits, including foods, macronutrients, and micronutrients, were assessed in 1989–1990 (3 y before the blood draw used as baseline in the present analysis) by using a picture-sort food-frequency questionnaire that was validated against 6 detailed 24-h dietary recall interviews spaced ≈1 mo apart (15) and against plasma phospholipid fatty acids (16).

Assessment of incident diabetes

Participants were followed by means of annual examinations with interim 6-mo telephone contacts for 10 y and subsequent 6-mo telephone contacts thereafter. At each visit, detailed medication information was obtained by using medication inventories. At baseline, study interviewers visited participants in their homes and were asked to see all prescription medications they had taken

in the previous 2 wk. At follow-up exams, participants brought to the clinic all prescription medications taken in the previous 2 wk. Information on drug name, dose, and frequency were recorded and coded according to prescription medication Medispan files. Prevalent diabetes at baseline was defined by use of insulin or hypoglycemic medication, fasting glucose ≥126 mg/dL, or (for participants fasting <8 h; 1.7%) nonfasting glucose ≥200 mg/dL. Incident diabetes was defined by new use of insulin or hypoglycemic medication (assessed annually), fasting glucose ≥126 mg/dL (assessed in 1996), or 2-h postchallenge glucose ≥200 mg/dL (assessed in 1996).

Statistical analysis

Relations between plasma phospholipid fatty acids were evaluated by using Spearman's correlations. Palmitoleate concentrations were evaluated in sex-specific quintiles as indicator variables and continuously per 1-SD difference. Independent demographic and lifestyle determinants of palmitoleate concentrations were evaluated by using multivariable-adjusted linear regression with palmitoleate as the dependent variable. Multivariable-adjusted relations of palmitoleate with metabolic risk factors were evaluated by using linear regression with palmitoleate as the independent variable, with transformation of metabolic risk factors to approximate normality as appropriate. Quintiles were evaluated as ordinal variables to assess trend, to assess effect modification using a multiplicative interaction term and the Wald test, and to assess differences in baseline characteristics by means of linear or logistic regression for continuous or dichotomous characteristics, respectively. Cox proportional hazards were used to estimate the hazard ratio 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 currently available medication data. Medication information was complete for 96.4% of person-time; follow-up for vital status was 100% complete. The proportional-hazards assumption was tested and 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 in older adults, or associations with exposures or outcomes in the current cohort. Missing covariates (most factors = 0.1–1.9%; dietary factors = 4–15%) were imputed by best-subset regression using age, sex, race, education, prevalent IHD, stroke, diabetes, smoking status, alcohol use, physical activity, and BMI; results that excluded missing values were similar. Analyses were performed by using Stata 10.1 (College Station, TX), 2-tailed $\alpha = 0.05$.

RESULTS

The mean (±SD) value of palmitoleate was 0.49 ± 0.20% (range: 0.11–2.55%). Of the other phospholipid fatty acids, palmitoleate concentrations correlated most strongly with 14:0 (r = 0.59), 16:0 (r = 0.54), and 18:1n−9 (r = 0.56), each of which are fatty acid end products of FS. In bivariate (unadjusted) analyses (**Table 1**), palmitoleate was associated with white race and less-prevalent IHD. Palmitoleate was also associated with alcohol use in a dose-dependent fashion, slightly lower fat consumption, and slightly higher carbohydrate consumption.

To elucidate and quantify the multivariable determinants of circulating palmitoleate in this large cohort of free-living adults, we evaluated how major demographic, clinical, and lifestyle factors independently related to palmitoleate (**Table 2**). In multivariable-adjusted analyses, nonwhite race strongly predicted higher palmitoleate (0.71 SD) in women, but not in men (P for interaction < 0.001). Otherwise, independent determinants were very similar by sex. For example, neither age, educational status, nor prevalent diabetes were strongly associated with palmitoleate. Both men and women with IHD had significantly lower concentrations (−0.16 SD), whereas current smokers had higher concentrations (0.18 SD in men, 0.23 SD in women). Of other lifestyle determinants, BMI was associated with modestly higher palmitoleate in both men and women,

TABLE 1
Baseline characteristics according to plasma phospholipid palmitoleic acid (*cis*-16:1n−7) concentrations in 3630 US adults

	Sex-specific quintiles of palmitoleic acid					
	1	2	3	4	5	P for trend[1]
n	732	721	729	724	724	—
Percentage of total fatty acids	0.29 ± 0.05[2]	0.38 ± 0.04	0.45 ± 0.05	0.54 ± 0.06	0.78 ± 0.21	
Age (y)	75 ± 6	75 ± 5	76 ± 5	76 ± 5	75 ± 5	—
Sex (% male)	47	42	46	45	47	—
Race (% white)	70	85	90	93	93	<0.01
Education > high school (%)	41	45	41	47	51	—
Current smoking (%)	7	9	7	8	9	—
Coronary heart disease (%)	33	25	19	23	21	<0.01
Diabetes mellitus (%)	18	18	15	17	15	—
Leisure-time activity (kcal/wk)	914 ± 1161	1085 ± 1513	1154 ± 1506	1131 ± 1524	1016 ± 1307	—
Alcohol (drinks/wk)	1.0 ± 2.9	1.3 ± 3.6	1.1 ± 3.1	2.1 ± 4.7	4.7 ± 10.7	<0.01
Total fat (% of energy)	32.2 ± 6	32.5 ± 6	32.4 ± 6	31.7 ± 6	31.3 ± 7	<0.01
Saturated fat (% of energy)	10.2 ± 2.0	10.3 ± 2.1	10.4 ± 2.2	10.3 ± 2.1	10.2 ± 2.4	—
Polyunsaturated fat (% of energy)	7.5 ± 2.1	7.5 ± 2.0	7.4 ± 2.2	7.1 ± 2.1	7.0 ± 2.2	<0.01
Carbohydrate (% of energy)	52.2 ± 7	51.8 ± 7	52.3 ± 8	52.8 ± 8	53.0 ± 8	<0.01

[1] P for trend across quintiles of palmitoleic acid concentrations evaluated as an ordinal variable in linear or logistic regression for continuous or dichotomous characteristics, respectively.

[2] Mean ± SD (all such values).

TABLE 2
Multivariable-adjusted predictors of plasma phospholipid palmitoleic acid (*cis*-16:1n−7) in 3630 US adults[1]

	SD differences (95% CI) in palmitoleic acid concentrations			
	Men (n = 1510)	P value	Women (n = 2120)	P value
Age, each 5 y	0.05 (0.00, 0.09)	0.04	−0.01 (−0.07, 0.04)	0.60
Race				
White	Reference			
Nonwhite	0.08 (−0.12, 0.29)	0.43	0.71 (0.53, 0.88)	<0.001
Education				
Less than high school	Reference			
High school graduate	−0.03 (−0.17, 0.11)	0.72	−0.03 (−0.15, 0.09)	0.58
Some college	0.00 (−0.15, 0.15)	0.99	0.01 (−0.13, 0.15)	0.87
College graduate	−0.04 (−0.18, 0.13)	0.63	−0.00 (−0.15, 0.14)	0.99
Prevalent diabetes				
No	Reference			
Yes	0.07 (−0.05, 0.19)	0.25	0.04 (−0.09, 0.17)	0.58
Prevalent IHD				
No	Reference			
Yes	−0.16 (−0.27, −0.05)	0.004	−0.16 (−0.27, −0.04)	0.004
Smoking				
Never	Reference			
Former	0.01 (−0.10, 0.11)	0.92	0.08 (−0.03, 0.19)	0.15
Current	0.18 (−0.04, 0.41)	0.11	0.23 (0.04, 0.42)	0.02
BMI, each kg/m^2	0.01 (−0.00, 0.03)	0.07	0.02 (0.01, 0.03)	<0.001
Leisure-time activity, each 500 kcal/wk	−0.01 (−0.02, 0.00)	0.21	−0.02 (−0.04, 0.00)	0.11
Alcohol				
None	Reference			
<1 drink/wk	0.05 (−0.06, 0.15)	0.33	0.02 (−0.14, 0.14)	0.97
1–2 drinks/wk	0.22 (0.07, 0.35)	0.002	−0.02 (0.17, 0.13)	0.69
3–7 drinks/wk	0.53 (0.32, 0.77)	<0.001	0.18 (−0.05, 0.35)	0.08
1–2 drinks/d	0.78 (0.48, 1.10)	<0.001	0.68 (0.31, 0.99)	<0.001
>2 drinks/d[2]	1.17 (0.84, 1.50)	<0.001	1.06 (0.71, 1.52)	<0.001
Carbohydrate, each higher 5% of energy replacing fat[3]	0.06 (0.03, 0.09)	<0.001	0.08 (0.04, 0.12)	<0.001
Protein, each higher 5% of energy replacing fat[3]	0.11 (0.02, 0.20)	0.02	0.10 (0.01, 0.19)	0.03

[1] Values are multivariable-adjusted linear regression coefficients for a 1-SD difference in palmitoleic acid concentrations as a percentage of fatty acids, adjusted for all variables in the table simultaneously. IHD, ischemic heart disease.
[2] Few individuals reported heavy alcohol use (only 1.6% reported >3 drinks/d), which limited the evaluation of higher intakes.
[3] Dietary habits were assessed 3 y earlier, in 1989–1990, which underestimated the magnitude of associations.

whereas greater physical activity was associated with modestly lower palmitoleate (P = 0.02 in men and women combined). Alcohol use and carbohydrate intake were each significant independent determinants of higher palmitoleate, consistent with these factors each driving hepatic FS. Protein intake, in exchange for dietary fat, was also associated with higher palmitoleate. In additional multivariable-adjusted nutrient-density models estimating isocaloric exchange of different types of fat for carbohydrate, palmitoleate concentrations in men were associated inversely with polyunsaturated fat consumption (−0.06 SD per % of energy; 95% CI: −0.10, −0.03) and in women were inversely associated with monounsaturated fat consumption (−0.09 SD per % of energy; 95% CI: −0.14, −0.03) and positively with saturated fat consumption (0.06 SD per % of energy; 95% CI: 0.02, 0.11).

In multivariable analyses that adjusted for the independent determinants of palmitoleate concentrations as well as other potential confounders, palmitoleate concentrations were independently associated with several major metabolic risk factors (**Table 3**). Higher palmitoleate was independently associated with both higher BMI and greater waist circumference ($P <$ 0.001 each). Palmitoleate was also independently associated with generally more favorable lipid profiles, including lower LDL cholesterol ($P <$ 0.001), higher HDL cholesterol ($P <$ 0.001), and lower total:HDL cholesterol ratio (P = 0.04). Palmitoleate was also independently associated with higher triglycerides ($P <$ 0.001), consistent with this circulating fatty acid being a marker of greater hepatic FS. Palmitoleate was also independently associated with lower fibrinogen ($P <$ 0.001) and with higher CRP (P = 0.01), although the latter association appeared limited to higher concentrations in the top quintile only. Palmitoleate was also associated with greater insulin resistance as assessed by HOMA-IR (P = 0.003).

When we assessed relations by sex, many of these findings appeared qualitatively similar in men and women (Table 3). In both men and women, palmitoleate was independently associated with both higher BMI, greater waist circumference, and higher triglycerides. Palmitoleate was also associated with lower LDL cholesterol, higher HDL cholesterol, and lower fibrinogen (P = 0.09 in men) in both men and women; a lower total:HDL cholesterol ratio was only seen in women. Palmitoleate was generally unassociated with CRP, except for higher concentrations in the top quintile in women.

Interestingly, relations of palmitoleate with glucose-insulin homeostasis were different by sex. Palmitoleate was unassociated

TABLE 3
Multivariable-adjusted relations of plasma phospholipid palmitoleic acid (*cis*-16:1n−7) with metabolic risk factors in 3630 US adults[1]

	Quintiles of palmitoleic acid					*P* for trend	*P* for interaction by sex
	1	2	3	4	5		
All (n = 3630)							
Median percentage of total fatty acids	0.29	0.38	0.45	0.54	0.73		
Adiposity							
BMI (kg/m^2)	25.9 ± 0.2	26.3 ± 0.2	27.0 ± 0.2[2]	27.4 ± 0.2[2]	27.0 ± 0.2[2]	<0.001	0.79
Waist circumference (cm)	95.1 ± 0.5	95.9 ± 0.5	97.8 ± 0.5[2]	99.1 ± 0.6[2]	98.4 ± 0.5[2]	<0.001	0.86
Blood lipids							
LDL-C (mg/dL)	132 ± 2	130 ± 1	128 ± 2	125 ± 2[3]	120 ± 2[2]	<0.001	0.04
HDL-C (mg/dL)	51.7 ± 0.5	51.6 ± 0.5	52.2 ± 0.5	54.3 ± 0.6[2]	55.0 ± 0.6[2]	<0.001	0.06
Triglycerides (mg/dL)	112 ± 3	118 ± 2	128 ± 3[2]	128 ± 3[2]	161 ± 4[2]	<0.001	0.006
Total:HDL-C ratio	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.1[4]	4.1 ± 0.1	0.04	0.003
Inflammation							
CRP (mg/L)	2.5 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	3.1 ± 0.2[3]	0.01	0.01
Fibrinogen (mg/dL)	333 ± 3	336 ± 3	327 ± 3	323 ± 3[4]	318 ± 3[3]	<0.001	0.07
Glucose-insulin homeostasis							
Fasting glucose (mg/dL)	101 ± 1	104 ± 1[3]	103 ± 1	104 ± 1[3]	104 ± 1[3]	0.007	0.04
Fasting insulin (mU/L)	10.0 ± 0.2	10.4 ± 0.3	10.8 ± 0.2[4]	10.7 ± 0.3[4]	10.9 ± 0.3[3]	0.003	0.009
HOMA-IR (units)	2.6 ± 0.1	2.8 ± 0.1	2.8 ± 0.1[4]	2.8 ± 0.1[4]	2.9 ± 0.1[3]	0.003	0.003
Men only (n = 1510)							
Median percentage of total fatty acids	0.27	0.34	0.40	0.48	0.64		
Adiposity							
BMI (kg/m^2)	25.8 ± 0.2	26.4 ± 0.2	26.7 ± 0.33[3]	27.0 ± 0.2[2]	26.5 ± 0.3[4]	0.004	—
Waist circumference (cm)	97.2 ± 0.6	98.3 ± 0.6	99.4 ± 0.8[4]	100.8 ± 0.7[2]	99.5 ± 0.7[4]	0.001	—
Blood lipids							
LDL-C (mg/dL)	123 ± 2	124 ± 2	125 ± 3	119 ± 2	116 ± 3	0.03	—
HDL-C (mg/dL)	45.8 ± 0.7	45.3 ± 0.7	46.7 ± 0.7	48.2 ± 0.7[4]	47.5 ± 0.9	0.02	—
Triglycerides (mg/dL)	106 ± 4	110 ± 3	128 ± 5[2]	125 ± 5[2]	163 ± 7[2]	<0.001	—
Total:HDL-C ratio	4.3 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.5 ± 0.1	0.52	—
Inflammation							
CRP (mg/L)	2.6 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	0.59	—
Fibrinogen (mg/dL)	327 ± 4	331 ± 4	331 ± 5	326 ± 5	316 ± 5	0.09	—
Glucose-insulin homeostasis							
Fasting glucose (mg/dL)	104 ± 1	107 ± 1	105 ± 2	107 ± 1[4]	109 ± 1[3]	0.007	—
Fasting insulin (mU/L)	9.8 ± 0.3	10.2 ± 0.4	11.1 ± 0.4[3]	11.1 ± 0.4[3]	11.5 ± 0.4[2]	<0.001	—
HOMA-IR (units)	2.5 ± 0.1	2.8 ± 0.2	2.9 ± 0.2[2]	3.0 ± 0.2[2]	3.1 ± 0.2[2]	<0.001	—
Women only (n = 2120)							
Median percentage of total fatty acids	0.32	0.42	0.49	0.59	0.80		
Adiposity							
BMI (kg/m^2)	25.8 ± 0.3	26.3 ± 0.3	27.3 ± 0.2[2]	27.7 ± 0.3[2]	27.4 ± 0.2[2]	<0.001	—
Waist circumference (cm)	93.2 ± 0.7	94.3 ± 0.7	97.1 ± 0.7[2]	97.8 ± 0.9[2]	97.9 ± 0.7[2]	<0.001	—
Blood lipids							
LDL-C (mg/dL)	138 ± 2	134 ± 2	131 ± 2[3]	130 ± 2[3]	122 ± 2[2]	<0.001	—
HDL-C (mg/dL)	55.8 ± 0.7	56.2 ± 0.7	56.1 ± 0.7	58.6 ± 0.9[3]	60.4 ± 0.8[2]	<0.001	—
Triglycerides (mg/dL)	118 ± 3	123 ± 3	127 ± 3	131 ± 4[3]	158 ± 4[2]	<0.001	—
Total:HDL-C ratio	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	3.9 ± 0.1[3]	3.9 ± 0.1[2]	<0.001	—
Inflammation							
CRP (mg/L)	2.4 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	2.5 ± 0.2	3.7 ± 0.2[2]	<0.001	—
Fibrinogen (mg/dL)	340 ± 4	340 ± 5	324 ± 4[3]	319 ± 3[2]	321 ± 4[2]	<0.001	—
Glucose-insulin homeostasis							
Fasting glucose (mg/dL)	100 ± 1	102 ± 1	101 ± 1	103 ± 1	101 ± 1	0.54	—
Fasting insulin (mU/L)	10.3 ± 0.3	10.5 ± 0.3	10.5 ± 0.3	10.4 ± 0.3	10.5 ± 0.4	0.74	—
HOMA-IR (units)	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	0.76	—

[1] All values are adjusted means ± SEs from multivariable linear regression with dependent variables transformed to approximate normality for analyses and retransformed as necessary, adjusted for age (y), sex, race (white or nonwhite), education (<high school, high school, some college, or college graduate), enrollment site (4 sites), smoking (never, former, or current), diabetes (yes or no), ischemic heart disease (yes or no), physical activity (kcal/wk), alcohol use (6 categories), and consumption of carbohydrate (% of energy), protein (% of energy), and total energy (kcal/d). Results for blood lipid, inflammatory, and glucose-insulin measures were also adjusted for BMI (kg/m^2) and waist circumference (cm). LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; CRP, C-reactive protein; HOMA-IR, homeostasis model of assessment of insulin resistance.

[2–4] Significantly different from quintile 1: [2]$P < 0.001$, [3]$P < 0.01$, [4]$P < 0.05$.

with measures of glucose-insulin homeostasis in women, whereas in men, palmitoleate was associated with significantly higher fasting glucose ($P = 0.007$), fasting insulin ($P < 0.001$), and HOMA-IR ($P < 0.001$; P for interaction by sex < 0.05 for each). Each of these overall and sex-specific findings were similar when restricted to nondrinkers ($n = 1968$; data not shown), which indicated that these associations were not driven by alcohol use.

All findings were similar when we removed diabetes and IHD from the multivariable model (data not shown), which could be either confounders or mediators (consequences) of metabolic effects of palmitoleate. All findings were also similar after additional adjustment for dietary consumption of saturated fat, monounsaturated fat, and polyunsaturated fat (data not shown). We also further adjusted for potential confounding from phospholipid concentrations of palmitic acid (16:0), a fatty acid derived from both diet and FS that is correlated with palmitoleate ($r = 0.54$). Associations with metabolic risk factors were generally partly attenuated but still statistically significant; for example, adjusted mean BMI in quintile 1 compared with quintile 5 of palmitoleate was now 26.1 compared with 26.7 (P for trend = 0.002); waist circumference, 95.8 compared with 97.5 cm (P for trend = 0.001); LDL cholesterol, 130 compared with 122 mg/dL (P for trend = 0.002); HDL cholesterol, 52.0 compared with 54.7 mg/dL (P for trend < 0.001); triglycerides, 117 compared with 153 mg/dL (P for trend < 0.001); fibrinogen, 332 compared with 320 mg/dL (P for trend = 0.003); and HOMA-IR in men, 2.5 compared with 3.1 (P for trend = 0.002). Interestingly, the positive association between palmitoleate and CRP wholly disappeared with further adjustment for palmitic acid: in quintile 1 compared with 5, 2.8 compared with 2.7 in men and women combined (P for trend = 0.16) and 2.8 compared with 2.9 in women (P for trend = 0.60).

Of 2905 participants free of prevalent diabetes at baseline, 296 new cases of diabetes occurred during 27,100 person-years of follow-up (incidence rate: 10.9/1000 person-years). In multivariable-adjusted analyses (covariates as in Table 2), palmitoleate was not significantly associated with incidence of diabetes in either men or women. Per each 1-SD higher palmitoleate concentration, the multivariable-adjusted hazard ratio of diabetes was 1.13 (95% CI: 0.89, 1.43) in men; 1.14 (95% CI: 0.97, 1.34) in women; and 1.12 (95% CI: 0.98, 1.29) in men and women combined. With further adjustment for fasting glucose, which could be either a confounder or mediator of this relation, the corresponding hazard ratios were as follows: 1.17 (95% CI: 0.88, 1.55), 1.15 (95% CI: 0.97, 1.35), and 1.12 (95% CI: 0.96, 1.30). In contrast, with further adjustment for palmitic acid, the corresponding hazard ratios for palmitoleate were as follows: 0.92 (95% CI: 0.69, 1.22), 0.99 (95% CI: 0.76, 1.28), and 0.96 (95% CI: 0.78, 1.18).

On the basis of both biological considerations and independent correlates in the present cohort (Table 2), carbohydrate intake, alcohol use, and adiposity/weight gain could each confound the relation between palmitoleate and incident diabetes. We therefore performed exploratory analyses in the subset of participants with carbohydrate consumption below the median (<51.8% energy), alcohol use <1 drink/wk, and no weight gain during the prior 3 y ($n = 379$). In these participants, higher palmitoleate was associated with lower incident diabetes: compared with quintile 1, the multivariable-adjusted relative risks (95% CIs) in quintiles 2–5 were 0.79 (0.32, 1.92), 0.33 (0.10, 1.05), 0.15 (0.02, 0.92), and 0.28 (0.06, 1.40), respectively (P for trend = 0.007). These latter findings should be interpreted with caution because of their exploratory nature and the relatively small number of included participants.

DISCUSSION

In this large cohort of US adults, independent lifestyle correlates of higher circulating palmitoleate included alcohol use and higher carbohydrate or protein intakes in place of fat. Notably, these relations were seen at typical ranges of macronutrient consumption and at low levels of alcohol use, providing evidence that stimulation of hepatic FS and palmitoleate production in humans does not require extreme exposures, ie, very-low-fat diets or high alcohol use. In experimental models, palmitoleic acid has been implicated as a direct regulator of metabolism, with actions including suppression of adipocyte cytokine expression (1), promotion of pancreatic β cell proliferation and secretory function (4), enhancement of skeletal muscle glucose uptake (1, 2), and stimulation of adipocyte peroxisome proliferator–activated receptor-γ transcriptional activity (3). Each of these findings suggests direct metabolic benefits of this fatty acid. Consistent with this, we found plasma phospholipid palmitoleate to be independently associated with more favorable HDL cholesterol, total:HDL cholesterol, and fibrinogen, which supports the potential metabolic benefits seen in animal experiments. Conversely, palmitoleate was associated with greater adiposity, higher triglycerides, and (in men only) greater insulin resistance.

These opposing findings may relate to different endogenous pathways and drivers of FS. In our murine model in which palmitoleic acid was protective, higher concentrations were related to constitutively up-regulated adipose FS, which suppressed hepatic FS and improved peripheral insulin resistance (1). However, when FS has been experimentally up-regulated in humans, hepatic FS, rather than adipose FS, is the major source of de novo lipogenesis (6–11). Because these studies generally evaluated extremes of macronutrient composition, the extent to which hepatic or adipose FS is activated with habitual diets has not been well understood. Our finding of independent relations of carbohydrate and protein consumption, as well as alcohol use, with palmitoleate concentrations suggests that FS is activated at usual ranges of intake. In addition, lower physical activity and adiposity were independent lifestyle correlates of higher palmitoleate concentrations, which suggests additional independent effects of energy imbalance on activation of FS. Thus, with typical modern diets, metabolic effects of palmitoleate could be confounded by habitual carbohydrate or energy overconsumption and related weight gain, and a combination of some direct protective effects of palmitoleate and residual confounding by these lifestyle determinants of FS could explain why palmitoleate was associated with many but not all markers of lower metabolic risk. Consistent with such counterbalancing pathways, although palmitoleate was associated with both favorable and unfavorable metabolic risk factors, palmitoleate concentrations were not associated with incident diabetes overall. Adjustment for palmitic acid, which could partly reduce confounding from FS, fully abolished relations and even nonsignificant trends of palmitoleate with higher CRP and incident diabetes.

The source of palmitoleate—hepatic or adipose tissue—could also partly alter its systemic effects. In experimental models, adipose-derived palmitoleate inhibited hepatic FS (1), and disruption of

hepatic FS reduced body adiposity and insulin resistance, potentially mediated by suppression of hepatic peroxisome proliferator–activated receptor-α for which FS-derived fatty acids may be preferred regulators (17–19). In humans, evidence suggests that the contribution of adipose-derived palmitoleate to tissue concentrations is low (6–11), so that even adipose tissue concentrations may largely reflect storage of hepatically produced palmitoleate. Consistent with this, among obese females who substantially reduced energy consumption and maintained 1-y weight loss, the most consistent fatty acid change in all measured lipid compartments—including adipose tissue—was reduced palmitoleate (20), which suggests that, even with weight loss, any increases in adipose-produced palmitoleate may be smaller than decreases in hepatic-produced palmitoleate. Among Swedish men, adipose tissue palmitoleate was associated with lower insulin sensitivity after adjustment for BMI, smoking, physical activity, and alcohol use (21). Two small studies that evaluated adipose tissue palmitoleate in nonobese individuals, which could partly reflect adipose FS, found inverse associations with risk of coronary restenosis after angioplasty (n = 100) (22) and ventricular arrhythmias during index hospitalization for acute myocardial infarction (n = 106) (23).

In 294 healthy subjects, nonesterified palmitoleate, which may best represent adipose FS, was associated with a nonsignificant trend toward lower HOMA-IR (24). Similarly, in another small study (n = 100), nonesterified palmitoleate correlated positively with insulin sensitivity (r = 0.31) after adjustment for age, sex, and body fat (25). However, analyses were not adjusted for lifestyle behaviors, alcohol use, or dietary factors, and temporal changes in nonesterified palmitoleate did not correlate with changes in insulin sensitivity (25). On the basis of these prior experimental and small human studies and the present findings, we hypothesize that liver- and adipose-produced palmitoleate might have partly differing effects, with specific activation of adipose FS and adipose-derived palmitoleate suppressing hepatic FS and producing the greatest metabolic benefit.

Other studies that assessed palmitoleate in phospholipids, cholesterol esters, or platelet membranes were often small, cross-sectional, and typically considered palmitoleate as only one of a panel of numerous fatty acids, frequently with little multivariable adjustment. These studies generally observed positive, although not always consistent, associations or trends toward associations with obesity (26–28), cardiometabolic risk factors (29–36), or cardiovascular disease (37–41). The extent to which such findings might be confounded by carbohydrate or calorie consumption driving hepatic FS and weight gain was not assessed in these studies. Taken together with our findings, these results highlight the challenges in linking steady state palmitoleate concentrations to metabolic effects in humans because of the likely dominance of hepatic FS and its complex determinants. These challenges support the need for new methods to assess metabolic effects in humans of 16:1n−7 from nonhepatic sources, including both adipose-derived and exogenous (eg, dietary) sources.

Even low levels of alcohol intake independently predicted higher palmitoleate concentrations, consistent with stimulation of hepatic FS by alcohol. If palmitoleate has protective metabolic effects, then the production of palmitoleate due to moderate alcohol intake, unencumbered by excess carbohydrate or calories that typically drive hepatic FS, could be a putative mediator of some of the metabolic benefits of alcohol, which include higher HDL cholesterol, lower fasting glucose, improved insulin sensitivity, and inverse associations with incident diabetes (42–45).

Direct dietary sources of palmitoleate are uncommon; macadamia nuts are one source (17% of fatty acids). A small trial (n = 34) using partially hydrogenated macadamia oil found no benefits on serum lipids compared with partially hydrogenated high–oleic acid or palm oil (46). In 4 other small trials, short-term macadamia nut consumption improved serum lipid concentrations, generally lowering total cholesterol and triglycerides and, depending on the comparison diet, sometimes also lowering LDL cholesterol and raising or lowering HDL cholesterol (47–50). Interpreting specific effects of dietary palmitoleate in these trials is problematic, because intakes of total, saturated, polyunsaturated, and other monounsaturated fats were also often concomitantly (and variably) altered. We did not identify any prior trials evaluating effects of macadamia nut consumption on glucose-insulin homeostasis. Further investigation of metabolic effects of palmitoleate, and whether such effects vary depending on hepatic, adipose, or dietary origin, is needed.

Our analysis has several strengths. Information on fatty acid levels, metabolic risk factors, other sociodemographic and clinical factors, and diabetes incidence were prospectively collected in a well-established multicenter cohort study with little loss to follow-up. Biomarker measurements provided objective measures of exposure. Nearly 4000 participants provided statistical power to detect relevant associations. Participants were randomly selected and enrolled from Medicare eligibility lists in several US communities, providing a population-based sample of older adults and increasing generalizability. Multiple demographic, lifestyle, and dietary covariates were evaluated in multivariable models, which minimized residual confounding.

Potential limitations should be considered. Associations between fatty acid concentrations and metabolic risk factors were cross-sectional, which limited the assessment of temporality. Because plasma phospholipid palmitoleate concentrations are likely determined by several processes, including liver and adipose tissue function, nutrition, and interaction of phospholipids with various cell membranes, caution is warranted before definitive conclusions are drawn about causal effects. Although we adjusted for several major metabolic risk factors simultaneously, residual confounding by unknown or unmeasured factors may occur. Conversely, magnitudes of many of the multivariable-adjusted findings makes it improbable that residual confounding could fully account for these relations. As is common in large free-living populations, fasting and postchallenge glucose were not measured annually; thus, diabetes incidence might be underestimated, which prospectively would be unrelated to baseline fatty acid measurements and not introduce bias but could reduce the statistical power to detect associations.

Overall, relations of palmitoleate with metabolic risk factors were robust, but in mixed directions, including more favorable LDL cholesterol, HDL cholesterol, total:HDL cholesterol, and fibrinogen but also greater adiposity, triglycerides, and (in men) insulin resistance, without a overall associations with incident diabetes. These findings suggest that circulating palmitoleate may have direct regulatory benefits on some metabolic pathways, consistent with animal experiments, yet may also be a marker of other underlying lifestyle traits such as carbohydrate intake and energy imbalance (adiposity) that could confound these direct

effects. Our results further suggest that extremes of carbohydrate and alcohol intake are not required to stimulate palmitoleate production from hepatic FS, given independent relations between these lifestyle factors and phospholipid palmitoleate at usual ranges of intake. Thus, future studies will need to carefully evaluate both potential direct effects of palmitoleate and how carbohydrate intake, alcohol use, adiposity, and possibly sex may modify or confound its relations with metabolic risk. Our findings indicate that these relations are likely complex, with effects perhaps dependent on both the lifestyle drivers and endogenous sources (eg, liver or adipose) of palmitoleate production. Further focus on these pathways and effects in different tissues and lipid compartments and in intervention studies may produce paradigm shifts in our understanding of how circulating palmitoleate and tissue FS affect pathways of metabolic risk.

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