Circulating Biomarkers of Dairy Fat and Risk of Incident Diabetes Mellitus Among Men and Women in the United States in Two Large Prospective Cohorts

Mohammad Y. Yakoob, MD, MS, PhD; Peilin Shi, PhD; Walter C. Willett, MD, DrPH; Kathryn M. Rexrode, MD, MPH; Hannia Campos, PhD; E. John Orav, PhD; Frank B. Hu, MD, MPH, PhD; Dariush Mozaffarian, MD, DrPH

Background—In prospective studies, the relationship of self-reported consumption of dairy foods with risk of diabetes mellitus is inconsistent. Few studies have assessed dairy fat, using circulating biomarkers, and incident diabetes mellitus. We tested the hypothesis that circulating fatty acid biomarkers of dairy fat, 15:0, 17:0, and t-16:1n-7, are associated with lower incident diabetes mellitus.

Methods and Results—Among 3333 adults aged 30 to 75 years and free of prevalent diabetes mellitus at baseline, total plasma and erythrocyte fatty acids were measured in blood collected in 1989 to 1990 (Nurses' Health Study) and 1993 to 1994 (Health Professionals Follow-Up Study). Incident diabetes mellitus through 2010 was confirmed by a validated supplementary questionnaire based on symptoms, diagnostic tests, and medications. Risk was assessed by using Cox proportional hazards, with cohort findings combined by meta-analysis. During mean±standard deviation follow-up of 15.2±5.6 years, 277 new cases of diabetes mellitus were diagnosed. In pooled multivariate analyses adjusting for demographics, metabolic risk factors, lifestyle, diet, and other circulating fatty acids, individuals with higher plasma 15:0 had a 44% lower risk of diabetes mellitus (quartiles 4 versus 1, hazard ratio, 0.56; 95% confidence interval, 0.37–0.86; *P*-trend=0.01); higher plasma 17:0, 43% lower risk (hazard ratio, 0.57; 95% confidence interval, 0.39–0.83; *P*-trend=0.01); and higher t-16:1n-7, 52% lower risk (hazard ratio, 0.48; 95% confidence interval, 0.33–0.70; *P*-trend <0.001). Findings were similar for erythrocyte 15:0, 17:0, and t-16:1n-7, although with broader confidence intervals that only achieved statistical significance for 17:0.

Conclusions—In 2 prospective cohorts, higher plasma dairy fatty acid concentrations were associated with lower incident diabetes mellitus. Results were similar for erythrocyte 17:0. Our findings highlight the need to better understand the potential health effects of dairy fat, and the dietary and metabolic determinants of these fatty acids.

(*Circulation*. 2016;133:1645-1654. DOI: 10.1161/CIRCULATIONAHA.115.018410.)

Key Words: biomarkers ■ dairy ■ diabetes mellitus ■ fatty acid

For decades, dietary recommendations for dairy foods have been based on predicted health effects of isolated individual nutrients, such as for bone health (eg, expected benefits of calcium and vitamin D), and cardiovascular disease (CVD; eg, expected harms of total fat and saturated fat). Based on these considerations, major dietary guidelines recommend low-fat dairy products and avoidance of whole-fat dairy.[1] However, neither low-fat nor whole-fat dairy foods have major effects on traditional cardiovascular risk factors[2] nor are appreciably linked to risk of coronary heart disease events.[3] Interestingly, self-reported consumption of dairy foods has mixed associations with risk of type 2 diabetes mellitus, without consistently different results for low-fat versus whole-fat products.[4–6] For instance, several studies suggest that yogurt may be beneficial for diabetes mellitus,[4–6] whereas some large studies,[4,5] but not others,[6] further suggest that cheese, which is highest in dairy fat, may also be protective.

Clinical Perspective on p 1654

Most previous investigations have relied on self-reported dietary information from questionnaires, which can be limited by errors in memory or subjective reporting, especially for dairy fat. In addition, dairy fat is consumed not only from whole foods such as milk, cheese, yogurt, and major dishes

Received July 31, 2015; accepted March 9, 2016.

From Department of Epidemiology, Harvard School of Public Health, Boston, MA (M.Y.Y., D.M.); Friedman School of Nutrition Science & Policy, Tufts University, Boston, MA (P.S., D.M.); Department of Nutrition, Harvard School of Public Health, Boston, MA (W.C.W., H.C., F.B.H.); Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (K.M.R.); and Division of General Internal Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (E.J.O.).

Guest Editor for this article was Mercedes R. Carnethon, PhD.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.115.018410/-/DC1.

Correspondence to Dariush Mozaffarian, MD, DrPH, Tufts University, Friedman School of Nutrition Science & Policy, Dean's Office, 150 Harrison Ave, Boston, MA 02111. E-mail dariush.mozaffarian@tufts.edu

such as pizzas, but also throughout the food supply in smaller amounts in mixed dishes, bakery products, and prepared foods. This may increase challenges of accurately capturing the intake of dairy fat from all sources. In comparison, circulating blood biomarkers of fatty acids may provide a more complete measure of dairy fat consumption. These include specific fatty acids obtained primarily from dairy fat including the odd-chain saturated fats pentadecanoic acid (15:0) and heptadecanoic acid (17:0)[7,8] and the natural ruminant *trans* fat *trans*-palmitoleate (t-16:1n-7).[9] These fatty acids are not endogenously synthesized and are obtained only from diet, particularly dairy, making them reasonable biomarkers of dairy fat consumption.[10,11] In addition to their role as biomarkers, it has been hypothesized that these fatty acids could have direct metabolic effects, eg, t-16:1n-7 could mimic experimentally observed effects of adipose-produced c-16:n-7 to increase muscle glucose uptake and suppress hepatic de novo lipogenesis.[12] Yet, few prospective studies have investigated these biomarkers and incident diabetes mellitus, with mixed results (see Discussion).

Thus, the effects of dairy fat, and specific dairy fatty acids, on risk of diabetes mellitus remain unclear. Understanding these relationships is crucial for informing dietary guidelines, and designing additional interventional and experimental studies, as well, to explore biological pathways and mechanisms. We tested the hypothesis that total plasma and erythrocyte biomarkers of dairy fat, 15:0, 17:0, and t-16:1n-7, are associated with a lower incidence of diabetes mellitus in 2 large prospective cohorts.

Methods

Study Design and Population

This investigation was derived from 2 large US prospective studies, the Nurses' Health Study and Health Professionals Follow-Up Study (HPFS). Details of the cohorts and blood sample collection have been reported.[13]

We used previously measured fatty acid concentrations in stored blood used for nested case-control studies of incident CVD. Among Nurses' Health Study and HPFS subjects who provided blood samples and were free of prevalent CVD or cancer at the time of blood sampling, we measured total plasma and erythrocyte fatty acid concentrations in 3499 men and women. Approximately 71% of participants were fasting at the time of blood collection. For present analysis focused on incident diabetes mellitus, participants with diabetes mellitus at the time of blood collection were excluded (n=166), leaving a total of 3333 participants for analysis. This included 1364 participants who developed CVD during follow-up and 1969 participants who did not develop CVD during follow-up. All analyses were first conducted in these 2 groups separately, and then combined after confirming that all associations were similar in magnitude and direction. This investigation was approved by the human subjects committees of all participating institutions, and all participants gave implied consent by providing blood samples and returning completed questionnaires.

Fatty Acids

In both cohorts, fatty acid concentrations were measured in stored total plasma and erythrocyte samples in the same laboratory using gas-liquid chromatography.[13,14] Concentrations of individual circulating fatty acids were expressed as a percentage of total fatty acids in plasma or erythrocyte membranes. Technicians and laboratory personnel were unaware of participant clinical information including disease status. Forty fatty acids in plasma and erythrocytes were quantified (Table I in the online-only Data Supplement). For this investigation, primary biomarkers of interest were pentadecanoic acid (15:0), heptadecanoic acid (17:0), and *trans*-palmitoleic acid (t-16:1n-7). Myristic acid, a much less specific biomarker for dairy fat that is found in dairy, beef, and some plant oils[15] and also endogenously synthesized by the liver (eg, in response to excess carbohydrate intake), was secondarily analyzed. All the participants had levels of these 4 fatty acids above detectable limits.

Reproducibility of these fatty acids (intraclass correlation coefficients) in plasma and red blood cells over time has been reported.[13,16] Average interassay CVs for quality controls in plasma were 16.0% for 15:0, 7.2% for 17:0, 10.4% for t-16:1n-7, and 30.8% for 14:0. Corresponding CVs in erythrocyte measurements were 22.4%, 11.3%, 14.2%, and 39.0%.

Incident Diabetes Mellitus

In both cohorts, participants were asked to report physician-diagnosed diabetes mellitus and the calendar year of diagnosis. To validate self-reports, a supplementary questionnaire was sent obtaining further information on symptoms, diagnosis, and drug treatment. Suspected cases were labeled as confirmed if, according to the supplementary questionnaire, they met ≥1 of 3 National Diabetes Data Group criteria: (1) classic symptoms plus fasting glucose ≥140 mg/dL or random glucose ≥200 mg/dL; (2) at least 2 separate elevated plasma glucose levels (fasting ≥140, random ≥200, or 2-hour challenge ≥200 mg/dL); or (3) medical prescription of oral hypoglycemic agents or insulin. These diagnostic criteria were modified after June 1996 to incorporate lower diagnostic cut offs for fasting glucose of 126 mg/dL. Validity of supplementary questionnaire for diagnosing diabetes mellitus was confirmed in subsets of participants in each cohort by comparison with direct review of medical records: 98% were confirmed in Nurses' Health Study, and 97% were confirmed in HPFS.[17,18]

Covariates and Other Risk Factors

Data on medical history, risk factors, and lifestyle were obtained in both cohorts via validated self-administered questionnaires (online-only Data Supplement). Usual alcohol use and dietary habits were assessed through validated semiquantitative food frequency questionnaires.

Statistical Analysis

Fatty acid levels were evaluated in quartiles as indicator variables, continuously comparing the medians of the highest versus lowest quartile (87.5th versus 12.5th percentiles). Linear trend was assessed by assigning the median value of each quartile with participants and evaluating this as a continuous variable. Cox proportional hazards evaluated associations of each fatty acid with incident diabetes mellitus through 2010, with time at risk from blood collection until first event, death, or censoring at the return of the last questionnaire. Analyses were conducted separately in each cohort and then combined using fixed-effects inverse-variance–weighted meta-analysis;[19] with pooled *P*-for-trend calculated by using generalized least-squares trend meta-analysis.[20] Proportional hazards assumptions were tested by an interaction term with time and not rejected for any fatty acid, with the exception of 14:0 (*P*-interaction=0.01 in Nurses' Health Study, 0.01 in HPFS). Visualization of Schoenfeld residuals for each fatty acid, including 14:0, did not suggest a nonzero slope, suggesting that any potential time interaction was small.

Multivariable models were used to minimize potential confounding, with covariates included based on biological relevance, clinical interest, strength of associations with exposure or outcome, or percentage of change in the risk estimate of interest (>5%). For example, because dairy fat intake could be associated with other dietary habits that may also influence diabetes mellitus, we adjusted for fruits, vegetables, fish, meats, whole grains, sugar-sweetened beverages, polyunsaturated fat, calcium, and glycemic load. We also adjusted for biomarker levels of *trans*-18:1 and *trans*-18:2, because dairy fat can contain ruminant *trans* fats; and for palmitic acid (16:0) and stearic acid (18:0) synthesized by hepatic de novo lipogenesis, because

Table 1. Baseline Characteristics of 3333 US Men and Women With Fatty Acid Measurements and Free of Prevalent Diabetes Mellitus in the Nurses' Health Study (1990) and Health Professionals Follow Up Study (1994)

	Women (n=1864)	Men (n=1469)
Age, y	60.4±6.3	64.6±8.6
Age range, y	44–70	48–83
Race/ethnicity, %		
White	99.0	93.6
Black	0.4	0.1
Asian/other	0.6	6.3
Weight status, %		
Normal (BMI <25 kg/m^2)	55.2	42.4
Overweight (BMI 25 to <30 kg/m^2)	31.6	46.8
Obese (BMI ≥30 kg/m^2)	13.2	10.8
BMI, kg/m^2	25.3±4.5	25.8±3.3
Smoking status, %		
Current smoker	21.8	8.4
Past smoker	38.7	49.1
Never smoker	39.5	42.5
Physical activity, MET-hours/wk	16.0±18.9	36.4±39.0
Medical history, %		
Hypertension	22.9	24.8
Hypercholesterolemia	35.5	26.7
Parental MI before 60 y	22.6	12.5
Family history of diabetes mellitus	27.6	22.7
Plasma fatty acids, % of total fatty acids*		
14:0	0.55 (0.25, 1.01)	0.51 (0.25, 0.98)
15:0	0.16 (0.11, 0.22)	0.14 (0.10, 0.20)
17:0	0.32 (0.26, 0.39)	0.31 (0.24, 0.38)
t-16:1n-7	0.19 (0.13, 0.28)	0.15 (0.10, 0.23)
Erythrocyte fatty acids, % of total fatty acids*		
14:0	0.27 (0.12, 0.69)	0.25 (0.12, 0.56)
15:0	0.12 (0.07, 0.19)	0.11 (0.07, 0.17)
17:0	0.39 (0.31, 0.61)	0.36 (0.29, 0.50)
t-16:1n-7	0.16 (0.11, 0.23)	0.13 (0.09, 0.18)
Dietary factors, servings/d		
Total dairy	2.1±1.5	2.1±1.6
Whole-fat dairy†	0.95±1.17	0.97±1.28
Low-fat dairy‡	1.2±1.0	1.1±1.1
Processed meats	0.21±0.29	0.32±0.42
Unprocessed meats	0.90±0.49	0.94±0.55
Fruits and vegetables	5.5±3.0	5.9±3.2
Fish	0.31±0.29	0.27±0.25
Alcohol	0.44±0.79	0.98±1.28

Values are mean±SD for continuous variables and percent for categorical variables. Missing values range: from 0.0% for age to 8.6% for parental history of MI (women), and from 0.0% for age to 4.6% for smoking (men). BMI indicates body mass index; MET, metabolic equivalent; MI, myocardial infarction; and SD, standard deviation.

*Fatty acid concentrations are reported as medians (12.5th, 87.5th percentiles, representing midpoint of bottom and top quartiles).

†Whole milk, ice cream, butter, cream, sour cream, cream cheese, and other cheese.

‡Low-fat or skim milk, yogurt, and cottage cheese.

these fatty acids can alter relative circulating proportions of trace fatty acids and are linked to insulin resistance and diabetes mellitus in experimental and prospective studies.[13,14,21] Dairy consumption may causally influence body mass index (BMI) – eg, yogurt and cheese[22,23] (the latter, if replacing refined carbohydrates) are linked to lower long-term weight gain and dairy intake reduces weight and fat mass in short-term trials[24] – suggesting that BMI could partly mediate any associations between dairy intake and diabetes mellitus. Individuals with different BMIs may also select different dairy foods, which could confound associations. We therefore separately considered BMI as a potential mediator and confounder in an additional multivariable model.

We explored effect modification by age and BMI by using the Wald test for a multiplicative interaction term, with Bonferroni-corrected significance (4 fatty acids × 2 effect modifiers: α=0.05/8=0.006). To minimize misclassification attributable to exposure changes over time, we performed sensitivity analyses restricted to the first 8 years of follow-up. To minimize reverse causation attributable to unrecognized subclinical disease or presence of clinical risk factors at the time of fatty acid measurement, we also excluded cases occurring during the first 2 years. We evaluated self-reported yogurt, cheese, and dairy fat consumption as additional covariates to assess if the associations were independent of these dietary factors outside their specific fatty acid contents. Multiple (10-fold) imputation was performed for missing continuous, and indicator variables for missing categorical covariates. Nonlinear relationships were assessed by semiparametric restricted cubic splines, excluding participants with fatty acid levels <0.5th or >99.5th percentiles to minimize the influence of outliers. Analyses were conducted using SAS 9.3 (Cary, NC), 2-tailed α=0.05.

Results

At baseline, mean±standard deviation age was 64.6±8.6 years among men (range, 48–83 years) and 60.4±6.3 years among women (range, 44–70 years; Table 1, Table II in the online-only Data Supplement). About half of participants were overweight or obese; 1 in 4 had hypertension or a family history of diabetes mellitus; 1 in 3 had hypercholesterolemia; and 1 in 12 men and 1 in 5 women were current smokers. Average total dairy consumption was 2.1 servings/d, with similar contributions from whole-fat (≈45%) and low-fat (≈55%) products.

Within each circulating lipid compartment (plasma, erythrocyte), the different fatty acid biomarkers were modestly intercorrelated: in plasma, adjusted intercorrelations (r) ranged from 0.10 to 0.59 (Table III in the online-only Data Supplement); and in erythrocytes, from 0.47 to 0.69 (Table IV in the online-only Data Supplement). As expected for these different lipid compartments, correlations between plasma and erythrocyte fatty acid biomarkers were moderate: 0.32

for 15:0, 0.44 for 17:0, 0.42 for t-16:1n-7, and 0.24 for 14:0. Partial correlations with self-reported dairy fat consumption were modest for plasma 15:0 (r=0.29), 17:0 (r=0.21), and t-16:1n-7 (r=0.22); and weaker for 14:0 (r=0.11; Table III and Figure I in the online-only Data Supplement); and similar but somewhat lower for erythrocyte fatty acids (Table IV in the online-only Data Supplement). These correlations were similar among participants who were fasting at the time of blood collection (data not shown).

Plasma Fatty Acid Biomarkers and Risk of Diabetes Mellitus

During mean±standard deviation follow-up of 15.2±5.6 years, 277 new cases of diabetes mellitus were diagnosed. After adjustment for demographics, metabolic risk factors, lifestyle, dietary habits, and other circulating fatty acids, in comparison with the lowest quartile, individuals in the highest quartile of plasma 15:0 had 44% lower risk of diabetes mellitus (hazard ratio [HR],0.56; 95% confidence interval [CI], 0.37–0.86; P-trend=0.01); of plasma 17:0, 43% lower risk (HR, 0.57; 95% CI, 0.39–0.83; P-trend<0.01); and of t-16:1n-7, 52% lower risk (HR, 0.48; 95% CI, 0.33–0.70; P-trend <0.001; Table 2). Findings were generally similar in magnitude and direction in the 2 separate cohorts, without statistically significant effect modification by sex. 14:0 was not significantly associated with diabetes mellitus (P-trend=0.36).

Evaluated continuously, 15:0 was associated with 38% lower risk (pooled HR, 0.62; 95% CI, 0.46–0.85); 17:0, with 32% lower risk (HR, 0.68; 95% CI, 0.50–0.91); and t-16:1n-7, with 46% lower risk (HR, 0.54; 95% CI, 0.40–0.73; Table 3). Similar to categorical analyses, plasma 14:0 was not associated with diabetes risk (HR, 0.82; 95% CI, 0.60–1.11).

Because BMI is a major predictor of insulin resistance and diabetes mellitus, we conducted further multivariable-adjusted analyses to evaluate the potential independent associations between BMI and dairy fatty acid biomarker levels at baseline. In both cohorts combined, higher 15:0 was not associated with BMI (Q1 versus Q4: 25.3 versus 25.3 kg/m^2, P-trend=0.97; Table II in the online-only Data Supplement). Higher 17:0 (25.8 versus 25.0, P-trend<0.001) and t-16:1n-7 (25.9 versus 25.2, P-trend<0.001) were associated with lower BMI, although absolute differences were modest. 14:0 was associated with higher BMI (24.9 versus 25.9, P-trend<0.001). After further adjustment for baseline BMI as a potential mediator or confounder, associations with incident diabetes mellitus were slightly attenuated for 17:0 and not appreciably altered for other fatty acids (Table 3). Results were also similar after further multivariable adjustment for conjugated linoleic acid, another ruminant-derived fatty acid (data not shown).

We further examined the potential influence of each individual covariate in the model. Adjustment for fatty acid biomarkers of de novo lipogenesis, 16:0 and 18:0,[25,26] had the greatest effect on plasma 15:0, which was no longer significantly associated with diabetes mellitus when 16:0 and 18:0 were removed as covariates (continuous HR, 1.10; 95% CI, 0.85–1.42). Conversely, removal of these fatty acid covariates had smaller influence on associations with diabetes mellitus for plasma 17:0 (HR, 0.67; 95% CI, 0.51–0.89) or t-16:1n-7 (HR, 0.68; 95% CI, 0.51–0.92).

With simultaneous mutual adjustment for all 3 dairy fat biomarkers, associations were nonsignificant for plasma 15:0 (continuous HR, 0.83; 95% CI, 0.53–1.29) and 17:0 (HR, 0.93; 95% CI, 0.64–1.36), and unchanged for t-16:1n-7 (HR, 0.59; 95% CI, 0.43–0.80).

Semiparametric Analyses

Restricted cubic splines demonstrated an inverse linear relationship for plasma 15:0, 17:0, and t-16:1n-7 and incident diabetes mellitus (Figure). Plasma 14:0 was not associated with risk (P-linearity=0.48).

Erythrocyte Fatty Acid Biomarkers

When we evaluated erythrocyte fatty acids, a lower incidence of diabetes mellitus was seen with higher levels of 17:0 (HR, 0.54; 95% CI, 0.34–0.87; P-trend <0.001), and t-16:1n-7 (HR, 0.78; 95% CI, 0.51–1.18; P-trend=0.05; Table V in the online-only Data Supplement). Erythrocyte 15:0 was not significantly associated with incident diabetes (HR, 0.83; 95% CI, 0.55–1.25; P-trend=0.63), and erythrocyte 14:0 was associated with a nonsignificant trend toward higher risk (HR, 1.56; 95% CI, 0.98–2.49; P-trend=0.13). The latter association was driven by findings in HPFS.

When these erythrocyte fatty acids were evaluated continuously, similar findings were seen for 15:0 and 17:0; t-16:1n-7 was no longer associated with lower risk (HR, 0.80; 95% CI, 0.57–1.13); and 14:0 was associated with 36% higher risk (HR, 1.36; 95% CI, 1.11–1.67; Table VI in the online-only Data Supplement). As with the categorical analyses, the latter association was driven by findings in HPFS only (data not shown). As seen with plasma 17:0, further adjustment for BMI as a mediator or confounder partly attenuated the observed inverse association for erythrocyte 17:0. Among other fatty acid covariates in the multivariable model, removal of adjustment for fatty acid biomarkers of de novo lipogenesis had little effect on these risk estimates (data not shown).

Sensitivity Analyses

When we evaluated potential effect modification, the associations of plasma and erythrocyte fatty acids with incident diabetes mellitus did not significantly vary according to differences in age or BMI (P>Bonferroni-corrected α=0.006 each). Associations were also similar after further adjustment for self-reported consumption of yogurt, cheese, or dairy fat (Table VII in the online-only Data Supplement). For example, in comparison with the association of plasma t-16:1n-7 with diabetes mellitus in the main model (continuous HR, 0.54; 95% CI, 0.40–0.73), findings were not appreciably altered by additional adjustment for reported consumption of yogurt (HR, 0.55; 95% CI, 0.41–0.73), cheese (HR, 0.53; 95% CI, 0.40–0.71), or dairy fat (HR, 0.52; 95% CI, 0.38–0.70). Results were also generally similar after excluding cases occurring within the first 2 years and restricting to the first 8 years of follow-up (Table VIII in the online-only Data Supplement).

Discussion

In 2 separate cohorts of US men and women, 3 plasma biomarkers of dairy fat, 15:0, 17:0, and *trans*-16:1n-7, were associated with lower risk of incident diabetes mellitus. Findings were generally similar for erythrocyte fatty acids, although with

Table 2. Risk of Incident Diabetes Mellitus According to Plasma Fatty Acid Biomarkers of Dairy Fat Consumption Among 3333 Men and Women in the NHS (n=184 Cases), HPFS (n=93 Cases), and Both Cohorts Combined

	Cohort-Specific Fatty Acid Quartiles				*P* for Trend*
	1	2	3	4	
15:0, NHS					
% of total FA, median	0.11	0.14	0.17	0.22	
No. cases	33	49	55	47	
Person-months	95002	89388	98482	95695	
Multivariable HR (95% CI)†	Reference	1.26 (0.80–2.00)	0.89 (0.56–1.43)	0.60 (0.36–1.01)	0.01
15:0, HPFS					
% of total FA, median	0.10	0.13	0.15	0.20	
No. cases	18	14	34	27	
Person-months	55559	51928	58919	61348	
Multivariable HR (95% CI)	Reference	0.63 (0.31–1.30)	1.12 (0.59–2.12)	0.49 (0.23–1.04)	0.09
15:0, pooled	Reference	1.03 (0.70–1.52)	0.96 (0.66–1.41)	0.56 (0.37–0.86)	0.01
17:0, NHS					
% of total FA, median	0.26	0.30	0.33	0.39	
No. cases	59	47	44	34	
Person-months	94103	92604	94510	97350	
Multivariable HR (95% CI)	Reference	0.79 (0.53–1.19)	0.75 (0.49–1.14)	0.50 (0.31–0.81)	0.01
17:0, HPFS					
% of total FA, median	0.24	0.29	0.32	0.38	
No. cases	23	24	17	29	
Person-months	56574	55212	57264	58704	
Multivariable HR (95% CI)	Reference	0.80 (0.43–1.47)	0.48 (0.25–0.94)	0.69 (0.38–1.26)	0.18
17:0, pooled	Reference	0.79 (0.57–1.11)	0.66 (0.46–0.94)	0.57 (0.39–0.83)	<0.01
t-16:1n-7, NHS					
% of total FA, median	0.13	0.17	0.21	0.28	
No. cases	54	38	44	48	
Person-months	90854	83770	95906	108037	
Multivariable HR (95% CI)	Reference	0.79 (0.51–1.22)	0.69 (0.46–1.05)	0.48 (0.30–0.76)	0.002
t-16:1n-7, HPFS					
% of total FA, median	0.10	0.13	0.16	0.22	
No. cases	27	17	23	26	
Person-months	57935	52101	60989	56729	
Multivariable HR (95% CI)	Reference	0.66 (0.35–1.24)	0.64 (0.36–1.16)	0.49 (0.26–0.90)	0.03
t-16:1n-7, pooled	Reference	0.75 (0.52–1.07)	0.67 (0.48–0.94)	0.48 (0.33–0.70)	<0.001
14:0, NHS					
% of total FA, median	0.24	0.44	0.64	0.98	
No. cases	29	46	38	71	
Person-months	94398	93023	93812	97334	
Multivariable HR (95% CI)	Reference	1.55 (0.97–2.49)	0.95 (0.57–1.57)	0.93 (0.55–1.57)	0.35
14:0, HPFS					
% of total FA, median	0.25	0.40	0.57	0.90	

(*Continued*)

Table 2. Continued

	Cohort-Specific Fatty Acid Quartiles				
	1	2	3	4	*P* for Trend*
No. cases	10	22	27	34	
Person-months	53 011	56 008	56 340	62 395	
Multivariable HR (95% CI)	Reference	1.98 (0.90–4.33)	1.85 (0.83–4.15)	1.50 (0.56–3.98)	0.92
14:0, pooled	Reference	1.65 (1.10–2.48)	1.15 (0.75–1.76)	1.03 (0.65–1.64)	0.36

CI indicates confidence interval; FA, fatty acid; HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; MET, metabolic equivalent; MI, myocardial infarction; and NHS, Nurses' Health Study.

*Computed within each cohort by assigning median level in each quartile to participants and evaluating this variable continuously. Pooled *P*-for-trend was calculated by using generalized least-squares trend (GLST) meta-analysis.[20]

†Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/wk), alcohol (servings/d), family history of diabetes mellitus (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/d), processed meats (servings/d), unprocessed meats (servings/d), fruits (servings/d), vegetables (servings/d), whole grains (g/d), coffee (servings/d), sugar-sweetened beverages (servings/d), glycemic load (continuous), dietary calcium (mg/d), polyunsaturated fat (g/d), total energy (kcal/d), and plasma *trans*-18:1, *trans*-18:2, 16:0, and 18:0 (each as % of total fatty acids).

smaller and not always significant magnitudes of association. These results provide new evidence on associations of dairy-derived circulating fatty acids and risk of diabetes mellitus.

Our findings suggest that either these dairy fatty acids themselves or other correlated factors in dairy fat could reduce the risk of diabetes mellitus. For instance, short- and medium-chain saturated fats, vitamin D (a fat-soluble vitamin), omega-3 fats, or gangliosides in dairy fat could each play a role.[27] The magnitudes of our associations, linear dose response, independence from established diabetes risk factors and other dietary habits, and consistency of findings across sensitivity analyses argue against residual confounding as the sole explanation for our findings. These results highlight the need for intensive experimental and mechanistic evaluation on health effects of dairy fat, and dietary and potential metabolic determinants of these circulating fatty acids, as well, including their sensitivity to indicators of de novo lipogenesis.

In observational analyses, self-reported consumption of high-fat dairy foods such as cheese have beneficial or neutral associations with diabetes mellitus,[4–6] whereas dairy fat consumption and dairy fat biomarkers correlate with improved hepatic and systemic insulin sensitivity and lower hepatic steatosis.[28,29] When we included all 3 plasma fatty acids in 1 model, only t-16:1n-7 retained independent significant association with diabetes mellitus. We have hypothesized[12] that benefits could be attributable to isomeric similarities between

Table 3. Risk of Incident Diabetes Mellitus According to Plasma Fatty Acid Biomarkers of Dairy Fat Consumption, Evaluated Continuously, Among 3333 Men and Women in the NHS (n=184 Cases), HPFS (n=93 Cases), and Both Cohorts Combined

	Multivariable HR (95% CI) Standardized to Difference Between Midpoints of Highest Versus Lowest Quartiles (87.5th–12.5th Percentiles)			
	NHS	HPFS	Pooled	*P* Value
15:0	Range=0.10*	Range=0.10		
Multivariable HR (95% CI)†	0.63 (0.42–0.92)	0.61 (0.36–1.03)	0.62 (0.46–0.85)	<0.01
+ BMI‡	0.60 (0.40–0.89)	0.73 (0.43–1.25)	0.64 (0.47–0.89)	0.01
17:0	Range=0.13	Range=0.14		
Multivariable HR (95% CI)	0.71 (0.49–1.04)	0.63 (0.39–1.02)	0.68 (0.50–0.91)	0.01
+ BMI	0.76 (0.52–1.10)	0.69 (0.42–1.11)	0.73 (0.55–0.99)	0.04
t-16:1n-7	Range=0.15	Range=0.12		
Multivariable HR (95% CI)	0.52 (0.36–0.76)	0.58 (0.36–0.93)	0.54 (0.40–0.73)	<0.001
+ BMI	0.51 (0.36–0.74)	0.61 (0.37–0.99)	0.54 (0.41–0.73)	<0.001
14:0	Range=0.74	Range=0.65		
Multivariable HR (95% CI)	0.92 (0.64–1.32)	0.61 (0.35–1.09)	0.82 (0.60–1.11)	0.19
+ BMI	0.90 (0.63–1.31)	0.75 (0.42–1.32)	0.85 (0.63–1.16)	0.32

BMI indicates body mass index; CI, confidence interval; HPFS, Health Professionals Follow-Up Study; HR, hazard ratio; and NHS, Nurses' Health Study.

*The difference in percentage of total fatty acids between midpoint of the highest versus the lowest quartile.

†Multivariable adjustments as in Table 2.

‡Further adjusted for BMI (kg/m^2) as a potential mediator or confounder of the association.

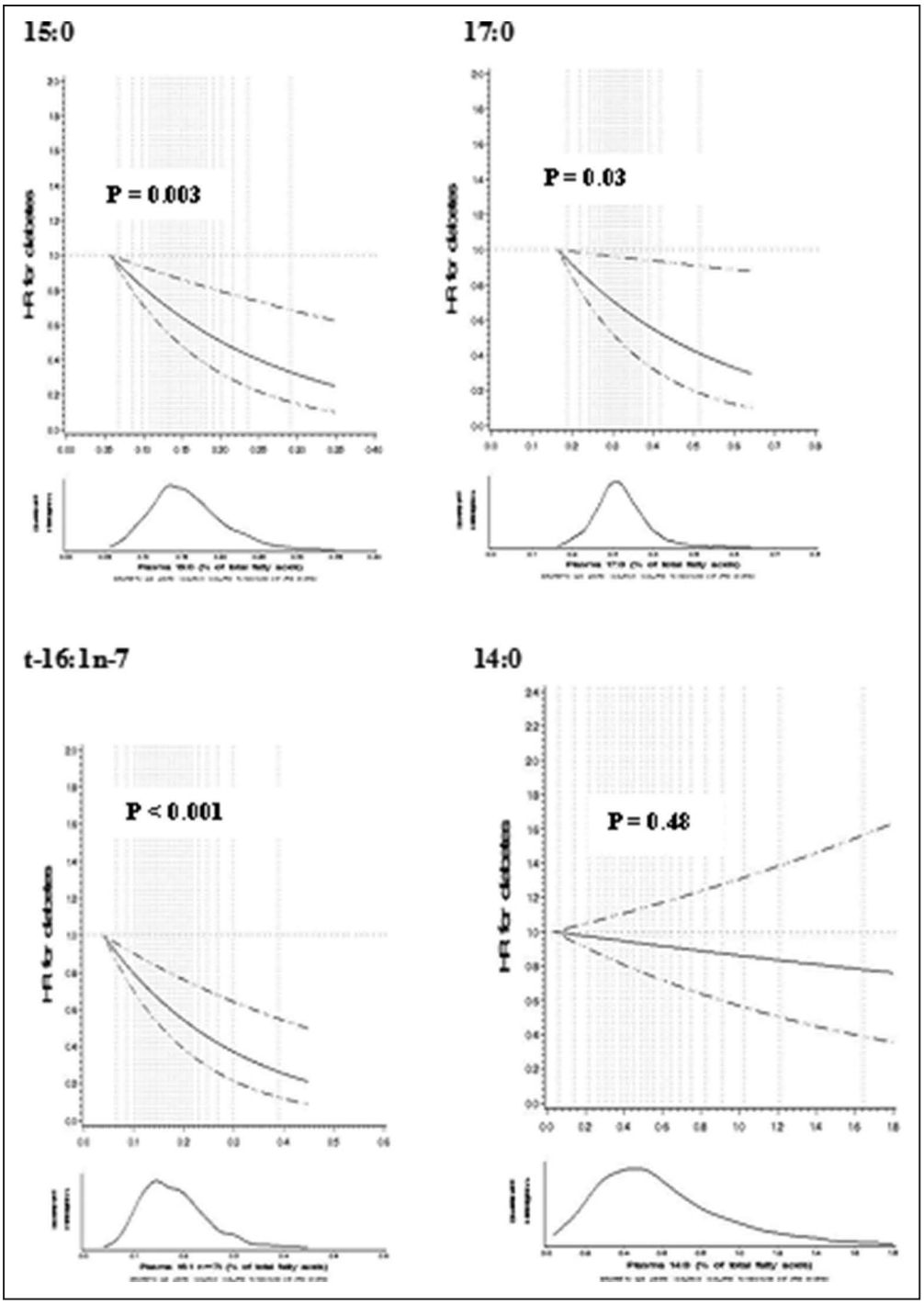


Figure. Semiparametric multivariable-adjusted associations of plasma 15:0, 17:0, t-16:1n-7, and 14:0 with incident diabetes mellitus among 3333 US men and women in 2 separate cohorts, evaluated using restricted cubic splines with covariate adjustments as in Table 2. Solid and dashed lines represent hazard ratios (HRs) and 95% confidence intervals, respectively; dotted vertical lines represent 21 knots. *P* values for linear association are shown. *P* values for nonlinearity were nonsignificant in all analyses; the automatic selection process did not identify any significant spline variables.

t-16:1n-7, a natural ruminant trans fat, and *cis*-16:1n-7 that, when derived from adipose tissue through de novo lipogenesis, may act in feedback loops to reduce hepatic fat synthesis and increase muscle insulin sensitivity.[30,31] We wonder whether t-16:1n-7, consumed in the diet, might have similar effects.

Fatty acids 15:0, 17:0, and t-16:1n-7 are predominantly obtained from diet and not synthesized, making reverse causation because of abnormal metabolism at baseline unlikely. One small study (n=12) found that providing a mixed oil high in *trans*-18:1 (vaccenic acid) also increased serum t-16:1n-7, whereas human peripheral blood mononuclear cells cultured with t-18:1 incorporated small amounts of t-16:1n-7 into cellular lipids.[32] These findings suggest small amounts of t-16:1n-7 could derive from partial β-oxidation (endogenous

Table 4. Summary of Prospective Studies Evaluating Fatty Acid Biomarkers of Dairy Fat and Incidence of Diabetes Mellitus

Study	Design	Lipid Compartment	No. of Cases	Multivariable Adjusted Hazard Ratio (95% CI)			
				15:0	17:0	t-16:1n-7	14:0
CHS[12]*	Prospective cohort	Plasma phospholipid	304	NS	NS	0.38 (0.24–0.62)*	NS†
EPIC-Interact[21]	Nested case subcohort	Plasma phospholipid	12 403	0.79 (0.73–0.85)‡	0.67 (0.63–0.71)‡	-	1.15 (1.09–1.22)‡
MCCS[36]	Case cohort	Plasma phospholipid	364	0.26 (0.17–0.40)§	-	-	-
MESA[35]	Prospective cohort	Plasma phospholipid	205	NS	-	0.52 (0.32–0.85)*	NS
VIP[37]	Nested case-control study	Erythrocyte membrane	237	0.65 (0.50–0.85)¶	0.47 (0.35–0.63)¶	-	1.25 (1.01–1.53)¶
NHS/HPFS	Prospective cohort	Total plasma	277	0.56 (0.37–0.86)*	0.57 (0.39–0.83)*	0.48 (0.65–0.70)*	1.03 (0.65–1.64)*

CHS indicates Cardiovascular Health Study; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; HPFS, Health Professionals Follow-up Study; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses Health Study; NS, not significant; SD, standard deviation; and VIP, Vasterbotten Intervention Program. Previous smaller subset reports from EPIC are not shown.

*Comparing the highest versus the lowest quintile of levels.

†Separately reported by Ma et al., *Am J Clin Nutr* 2015;101:153–163.

‡Per 1 SD increment in levels.

§Odds ratio comparing the highest versus the lowest quintile of levels.

¶Odds ratio per 1 SD increment in levels.

chain shortening) of t-18:1; labeled fatty acid tracer studies are needed to confirm this result. In the present study, among all dietary factors, circulating t-16:1n-7 correlated most with dairy fat rather than sources of industrial trans fat (vaccenic acid), suggesting that direct dairy consumption remains a major source. Genome-wide association studies have not identified significant genetic determinants of circulating t-16:1n-7,[33] further suggesting the absence of strong endogenous influences. Body weight and insulin resistance produce no known effects on levels of these circulating fatty acids, and findings were generally similar following adjustment for BMI. Reverse causation could play a role in behaviors, for example, if higher-risk participants with subclinical prediabetes elected to avoid whole-fat dairy products. Yet, the prospective nature of our analysis minimizes this possibility; and 15:0, 17:0, and t-16:1n-7 also remained inversely associated with diabetes mellitus after excluding cases occurring in the first 2 years.

In these cohorts, plasma fatty acid biomarkers correlated more strongly with dairy fat intake than did erythrocyte biomarkers, and were also more strongly inversely associated with diabetes mellitus. Correlations of all these fatty acids with self-reported dairy consumption were modest. This may be attributable to random or systematic errors in self-reported diet, variability of these fatty acids in different dairy foods,[34] laboratory error in fatty acid measures, within-person variation in diet or circulating fatty acids, or other unknown influences on bioavailability, metabolism, or incorporation into specific lipid compartments of these fatty acids. Notably, dairy fat is consumed not just as whole foods (milk, cheese, yogurt, butter) but mixed into numerous foods, dishes, and recipes as major and minor ingredients. In our analysis of National Health and Nutrition Examination Survey (NHANES) data based on detailed, product-specific dietary recalls (2005–2012), 51% of cheese and 30% of total dairy is consumed in mixed dishes, especially grain products but also mixed with meats, sweets, vegetables, and eggs (data not shown). Food frequency questionnaires that estimate dairy fat intakes from whole foods and major mixed sources (eg, pasta dishes, burritos, pizza) may not accurately capture quantities in these mixed dishes nor the multitude of smaller amounts in many other products. Thus, the observed modest correlations of 15:0, 17:0, and t-16:1n-7 with self-reported dairy fat may appropriately reflect the challenges in fully estimating dairy fat from questionnaires.

Fatty acid 14:0 had weak correlations with self-reported intakes of dairy fat, meat, and carbohydrate-rich foods (the latter being drivers of endogenous hepatic fatty acid synthesis). Plasma 14:0 was unassociated with diabetes risk; erythrocyte 14:0 was associated with increased risk in 1 cohort. Higher laboratory imprecision in 14:0 measures could limit the ability to detect associations. Trend toward higher risk could also relate to derivation of 14:0 from endogenous de novo lipogenesis, a correlate of insulin resistance and diabetes mellitus.[21] This could also be a chance finding and should be interpreted with caution. Recent analysis in the European Prospective Investigation into Cancer and Nutrition (EPIC) study identified a positive association between plasma phospholipid 14:0 and incident diabetes mellitus.[21] Our findings support the need for further studies on 14:0, especially on endogenous metabolic determinants of its circulating levels.

In pooled analyses of observational studies, self-reported intakes of total dairy, low-fat dairy, whole-fat dairy, milk, and cheese have each shown mixed (beneficial or neutral) associations with diabetes mellitus; yogurt consumption is more consistently associated with lower risk.[4–6] Interestingly, none of these studies identified harms of dairy, including whole-fat dairy, for diabetes mellitus. A handful of prior prospective studies have evaluated circulating biomarkers of dairy fat consumption in relation to incident diabetes mellitus (Table 4).

Only 2 studies[12,35] reported on t-16:1n-7; both found substantially lower risk, consistent with our findings. Three of 5 previous studies evaluating 15:0 and 2 of 3 studies evaluating 17:0 observed significant inverse associations with diabetes mellitus; none observed higher risk. In the present analysis, t-16:1n-7 remained associated with diabetes mellitus after adjustment for the other dairy fat biomarkers, suggesting it may be particularly relevant for further investigation of its determinants and biological effects. Our findings build on and extend previous studies by evaluating these fatty acid biomarkers, including t-16:1n-7, in total plasma and erythrocytes in 2 large, well-established cohorts of US men and women and including adjustment for a range of potential confounding factors.

Our investigation has several strengths. We assessed circulating fatty acids, which may more fully capture all dietary sources of dairy fat. We evaluated 2 separate lipid compartments, in which generally similar associations support validity of the findings, especially in light of their potential differential temporal and metabolic responses to dietary fats intake. The prospective cohort design minimized reverse causation, selection bias, and recall bias. Inclusion of 2 separate cohorts including both men and women provided for replication, and pooling, as well, of cohort findings.

Potential limitations should be considered. Misclassification because of laboratory error and within-person changes over time would attenuate results, making it more difficult to detect true associations. Despite this, we were able to detect associations with diabetes mellitus, suggesting true relationships could be even stronger. Residual confounding attributable to unmeasured or mismeasured covariates cannot be excluded. However, magnitudes of associations and linear dose responses suggest that residual confounding is unlikely to fully explain the results. These cohorts comprised educated US health professionals. Yet, although absolute levels of fatty acids and rates of diabetes mellitus may vary by education, geography, or race/ethnicity, we do not suspect that biological effects of these fatty acids should differ on a relative scale across different populations.

In summary, we found that plasma 15:0, 17:0, and *trans*-16:1n-7 were associated with a lower incidence of diabetes mellitus in 2 separate prospective cohort studies.

Acknowledgments

Drs Yakoob, Hu, and Mozaffarian participated in project conception and development of research methods; Drs Hu and Mozaffarian obtained funding and provided oversight; Drs Yakoob, Shi, and Orav analyzed data and performed analysis; Drs Yakoob and Mozaffarian drafted the manuscript; and Drs Willett, Rexrode, Campos, Orav, Hu, and Mozaffarian provided critical feedback on revisions and other intellectual input.

Sources of Funding

This study was supported by the National Institute of Environmental Health Sciences, NIH (R01-ES014433 and ES013692), and NIH research grants HL-60712, HL-034594, HL-088521, HL-35464, DK-58845, CA-186107, CA-49449, CA-87969, CA-55075, and CA-167552, as well. Dr Yakoob was supported by a Harvard University Scholarship, Founders Affiliate American Heart Association Pre-Doctoral Training Fellowship 2013–14, and Harvard Lown Cardiovascular Research Foundation Scholarship.

Disclosures

Dr Mozaffarian reports ad hoc honoraria from Amarin, Astra Zeneca, Haas Avocado Board, Bunge, and Life Sciences Research Organization. Harvard University holds a patent, listing Dr Mozaffarian among coinventors, for use of *trans*-palmitoleic acid to prevent and treat insulin resistance, type 2 diabetes mellitus, and related conditions. The other authors report no conflicts.

References

1. Dietary Guidelines Advisory Committee. *Scientific Report of the 2015 Dietary Guidelines Advisory Committee*. Rockville, MD: Office of Disease Prevention and Health Promotion; 2015.
2. Benatar JR, Sidhu K, Stewart RA. Effects of high and low fat dairy food on cardio-metabolic risk factors: a meta-analysis of randomized studies. *PLoS One*. 2013;8:e76480. doi: 10.1371/journal.pone.0076480.
3. Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, Hu FB, Engberink MF, Willett WC, Geleijnse JM. Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. *Am J Clin Nutr*. 2011;93:158–171. doi: 10.3945/ajcn.2010.29866.
4. Aune D, Norat T, Romundstad P, Vatten LJ. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. *Am J Clin Nutr*. 2013;98:1066–1083. doi: 10.3945/ajcn.113.059030.
5. Sluijs I, Forouhi NG, Beulens JW, van der Schouw YT, Agnoli C, Arriola L, Balkau B, Barricarte A, Boeing H, Bueno-de-Mesquita HB, Clavel-Chapelon F, Crowe FL, de Lauzon-Guillain B, Drogan D, Franks PW, Gavrila D, Gonzalez C, Halkjaer J, Kaaks R, Moskal A, Nilsson P, Overvad K, Palli D, Panico S, Quirós JR, Ricceri F, Rinaldi S, Rolandsson O, Sacerdote C, Sánchez MJ, Slimani N, Spijkerman AM, Teucher B, Tjonneland A, Tormo MJ, Tumino R, van der A DL, Sharp SJ, Langenberg C, Feskens EJ, Riboli E, Wareham NJ; InterAct Consortium. The amount and type of dairy product intake and incident type 2 diabetes: results from the EPIC-InterAct Study. *Am J Clin Nutr*. 2012;96:382–390. doi: 10.3945/ajcn.111.021907.
6. Chen M, Sun Q, Giovannucci E, Mozaffarian D, Manson JE, Willett WC, Hu FB. Dairy consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *BMC Med*. 2014;12:215. doi: 10.1186/s12916-014-0215-1.
7. Smedman AE, Gustafsson IB, Berglund LG, Vessby BO. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. *Am J Clin Nutr*. 1999;69:22–29.
8. 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;131:828–833.
9. 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;91:883–893. doi: 10.3945/ajcn.2009.28877.
10. Brevik A, Veierød 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;59:1417–1422. doi: 10.1038/sj.ejcn.1602256.
11. Hirahatake KM, Slavin JL, Maki KC, Adams SH. Associations between dairy foods, diabetes, and metabolic health: potential mechanisms and future directions. *Metabolism*. 2014;63:618–627. doi: 10.1016/j.metabol.2014.02.009.
12. 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;153:790–799. doi: 10.7326/0003-4819-153-12-201012210-00005.
13. Yakoob MY, Shi P, Hu FB, Campos H, Rexrode KM, Orav EJ, Willett WC, Mozaffarian D. Circulating biomarkers of dairy fat and risk of incident stroke in U.S. men and women in 2 large prospective cohorts. *Am J Clin Nutr*. 2014;100:1437–1447. doi: 10.3945/ajcn.114.083097.
14. Sun Q, Ma J, Campos H, Hu FB. Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease. *Am J Clin Nutr*. 2007;86:929–937.
15. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *Am J Clin Nutr*. 1999;70:1001–1008.
16. Kotsopoulos J, Tworoger SS, Campos H, Chung FL, Clevenger CV, Franke AA, Mantzoros CS, Ricchiuti V, Willett WC, Hankinson SE, Eliassen AH.

Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. *Cancer Epidemiol Biomarkers Prev*. 2010;19:938–946. doi: 10.1158/1055-9965.EPI-09-1318.
17. Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, Rosner B, Hennekens CH, Speizer FE. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. *Lancet*. 1991;338:774–778.
18. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med*. 2001;345:790–797. doi: 10.1056/NEJMoa010492.
19. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7:177–188.
20. Orsini N, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. *Stata J*. 2006;6:40–57.
21. Forouhi NG, Koulman A, Sharp SJ, Imamura F, Kröger J, Schulze MB, Crowe FL, Huerta JM, Guevara M, Beulens JW, van Woudenbergh GJ, Wang L, Summerhill K, Griffin JL, Feskens EJ, Amiano P, Boeing H, Clavel-Chapelon F, Dartois L, Fagherazzi G, Franks PW, Gonzalez C, Jakobsen MU, Kaaks R, Key TJ, Khaw KT, Kühn T, Mattiello A, Nilsson PM, Overvad K, Pala V, Palli D, Quirós JR, Rolandsson O, Roswall N, Sacerdote C, Sánchez MJ, Slimani N, Spijkerman AM, Tjonneland A, Tormo MJ, Tumino R, van der A DL, van der Schouw YT, Langenberg C, Riboli E, Wareham NJ. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. *Lancet Diabetes Endocrinol*. 2014;2:810–818. doi: 10.1016/S2213-8587(14)70146-9.
22. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med*. 2011;364:2392–2404. doi: 10.1056/NEJMoa1014296.
23. Smith JD, Hou T, Ludwig DS, Rimm EB, Willett W, Hu FB, Mozaffarian D. Changes in intake of protein foods, carbohydrate amount and quality, and long-term weight change: results from 3 prospective cohorts. *Am J Clin Nutr*. 2015;101:1216–1224. doi: 10.3945/ajcn.114.100867.
24. Chen M, Pan A, Malik VS, Hu FB. Effects of dairy intake on body weight and fat: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2012;96:735–747. doi: 10.3945/ajcn.112.037119.
25. Wu JH, Lemaitre RN, Imamura F, King IB, Song X, Spiegelman D, Siscovick DS, Mozaffarian D. Fatty acids in the de novo lipogenesis pathway and risk of coronary heart disease: the Cardiovascular Health Study. *Am J Clin Nutr*. 2011;94:431–438. doi: 10.3945/ajcn.111.012054.
26. Wu JH, Lemaitre RN, Manichaikul A, Guan W, Tanaka T, Foy M, Kabagambe EK, Djousse L, Siscovick D, Fretts AM, Johnson C, King IB, Psaty BM, McKnight B, Rich SS, Chen YD, Nettleton JA, Tang W, Bandinelli S, Jacobs DR Jr, Browning BL, Laurie CC, Gu X, Tsai MY, Steffen LM, Ferrucci L, Fornage M, Mozaffarian D. Genome-wide association study identifies novel loci associated with concentrations of four plasma phospholipid fatty acids in the de novo lipogenesis pathway: results from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. *Circ Cardiovasc Genet*. 2013;6:171–183. doi: 10.1161/CIRCGENETICS.112.964619.
27. Mozaffarian D. Natural *trans* fat, dairy fat, partially hydrogenated oils, and cardiometabolic health: the Ludwigshafen Risk and Cardiovascular Health Study (published online ahead of print November 17, 2015). *Eur Heart J*. doi: 10.1093/eurheartj/ehv595. http://eurheartj.oxfordjournals.org/content/early/2015/11/17/eurheartj.ehv595.long
28. Kratz M, Marcovina S, Nelson JE, Yeh MM, Kowdley KV, Callahan HS, Song X, Di C, Utzschneider KM. Dairy fat intake is associated with glucose tolerance, hepatic and systemic insulin sensitivity, and liver fat but not β-cell function in humans. *Am J Clin Nutr*. 2014;99:1385–1396. doi: 10.3945/ajcn.113.075457.
29. Nestel PJ, Straznicky N, Mellett NA, Wong G, De Souza DP, Tull DL, Barlow CK, Grima MT, Meikle PJ. Specific plasma lipid classes and phospholipid fatty acids indicative of dairy food consumption associate with insulin sensitivity. *Am J Clin Nutr*. 2014;99:46–53. doi: 10.3945/ajcn.113.071712.
30. 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;399:473–481. doi: 10.1042/BJ20060244.
31. 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;134:933–944. doi: 10.1016/j.cell.2008.07.048.
32. Jaudszus A, Kramer R, Pfeuffer M, Roth A, Jahreis G, Kuhnt K. trans Palmitoleic acid arises endogenously from dietary vaccenic acid. *Am J Clin Nutr*. 2014;99:431–435. doi: 10.3945/ajcn.113.076117.
33. Mozaffarian D, Kabagambe EK, Johnson CO, Lemaitre RN, Manichaikul A, Sun Q, Foy M, Wang L, Wiener H, Irvin MR, Rich SS, Wu H, Jensen MK, Chasman DI, Chu AY, Fornage M, Steffen L, King IB, McKnight B, Psaty BM, Djoussé L, Chen IY, Wu JH, Siscovick DS, Ridker PM, Tsai MY, Rimm EB, Hu FB, Arnett DK. Genetic loci associated with circulating phospholipid trans fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *Am J Clin Nutr*. 2015;101:398–406. doi: 10.3945/ajcn.114.094557.
34. USDA NAL. USDA Nutrient Data Laboratory. Beltsville, MD: US Department of Agriculture National Agricultural Library. http://fnic.nal.usda.gov/food-composition/usda-nutrient-data-laboratory. Accessed November 22, 2015.
35. Mozaffarian D, de Oliveira Otto MC, Lemaitre RN, Fretts AM, Hotamisligil G, Tsai MY, Siscovick DS, Nettleton JA. trans-Palmitoleic acid, other dairy fat biomarkers, and incident diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr*. 2013;97:854–861. doi: 10.3945/ajcn.112.045468.
36. Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA, Giles GG. Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. *Am J Clin Nutr*. 2007;86:189–197.
37. 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. *Nutr Metab Cardiovasc Dis*. 2008;18:503–510. doi: 10.1016/j.numecd.2007.04.005.

CLINICAL PERSPECTIVE

Diabetes mellitus is a major preventable cause of morbidity and mortality. Growing evidence suggests that dairy foods, and even dairy fat, could reduce risk of diabetes mellitus. Most previous studies used self-reported dietary questionnaires that could be prone to subjective reporting and might not fully capture dairy fat intake, particularly from mixed dishes. Circulating fatty acid biomarkers, such as odd-chain saturated fats (15:0, 17:0) and certain natural *trans* fats (t-16:1n-7), may provide more objective measures of dairy fat intake. We prospectively evaluated the relationship between plasma concentrations of 15:0, 17:0, and t-16:1n-7 and new-onset diabetes mellitus among 3333 men and women aged 30 to 75 years in 2 separate US cohorts. Incident diabetes mellitus was confirmed by validated methods based on symptoms, diagnostic tests, and medical therapy. After adjusting for demographics, metabolic risk factors, lifestyle, diet, and other circulating fatty acids, we found that all 3 of these circulating fatty acids were associated with substantially lower risk of diabetes mellitus, with ≈45% to 50% lower risk comparing the top versus bottom quartiles of their levels. Potential limitations include the misclassification of fatty acid measurements because of laboratory error and within-person variation over time, which would attenuate results toward the null; and residual confounding because of unmeasured or mismeasured covariates, although this would seem unlikely to fully explain the magnitudes of observed associations. Our findings suggest that dairy foods, and specifically dairy fat, could help prevent diabetes mellitus, highlighting the need for intensive experimental and mechanistic evaluation on the health effects of dairy fat, and as the determinants of these circulating fatty acids, as well.

DOI: 10.1161/CIRCULATIONAHA.115.018410

Clinical Perspective

Diabetes is a major preventable cause of morbidity and mortality. Growing evidence suggests dairy foods, and even dairy fat, could reduce risk of diabetes. Most prior studies utilized self-reported dietary questionnaires, which could be prone to subjective reporting and might not fully capture dairy fat intake, particularly from mixed dishes. Circulating fatty acid biomarkers, such as odd-chain saturated fats (15:0, 17:0) and certain natural *trans* fats (t-16:1n-7), may provide more objective measures of dairy fat intake. We prospectively evaluated the relationship between plasma concentrations of 15:0, 17:0, and t-16:1n-7 and new-onset diabetes among 3,333 men and women aged 30-75 years in two separate US cohorts. Incident diabetes was confirmed by validated methods based on symptoms, diagnostic tests, and medical therapy. After adjusting for demographics, metabolic risk factors, lifestyle, diet, and other circulating fatty acids, we found that all three of these circulating fatty acids were associated with substantially lower risk of diabetes, with about 45-50% lower risk comparing the top vs. bottom quartiles of their levels. Potential limitations include misclassification of fatty acid measurements due to laboratory error and within-person variation over time, which would attenuate results toward the null; and residual confounding due to unmeasured or mismeasured covariates, although this would seem unlikely to fully explain the magnitudes of observed associations. Our findings suggest that dairy foods, and specifically dairy fat, could help prevent diabetes, highlighting the need for intensive experimental and mechanistic evaluation on health effects of dairy fat as well as determinants of these circulating fatty acids.

DOI: 10.1161/CIRCULATIONAHA.115.018410

Table 1. Baseline characteristics of 3,333 US men and women with fatty acid measurements and free of prevalent diabetes in the Nurses' Health Study (1990) and Health Professionals Follow Up Study (1994).*

	Women (n =1,864)	Men (n =1,469)
Age, years	60.4±6.3	64.6±8.6
Age range, years	44 to 70	48 to 83
Race/Ethnicity (%)		
Caucasian	99.0	93.6
African-Americans	0.4	0.1
Asian/Other	0.6	6.3
Weight status (%)		
Normal (BMI <25 kg/m2)	55.2	42.4
Overweight (BMI 25 to <30 kg/m2)	31.6	46.8
Obese (BMI ≥ 30 kg/m2)	13.2	10.8
BMI, kg/m2	25.3±4.5	25.8±3.3
Smoking status (%)		
Current smoker	21.8	8.4
Past smoker	38.7	49.1
Never smoker	39.5	42.5
Physical activity, MET-hours/week	16.0±18.9	36.4±39.0
Medical History (%)		
Hypertension	22.9	24.8
Hypercholesterolemia	35.5	26.7
Parental MI before 60 y	22.6	12.5
Family history of diabetes	27.6	22.7
Plasma fatty acids, % of total fatty acids†		
14:0	0.55 (0.25, 1.01)	0.51 (0.25, 0.98)
15:0	0.16 (0.11, 0.22)	0.14 (0.10, 0.20)
17:0	0.32 (0.26, 0.39)	0.31 (0.24, 0.38)
t-16:1n-7	0.19 (0.13, 0.28)	0.15 (0.10, 0.23)
Erythrocyte fatty acids, % of total fatty acids†		
14:0	0.27 (0.12, 0.69)	0.25 (0.12, 0.56)
15:0	0.12 (0.07, 0.19)	0.11 (0.07, 0.17)
17:0	0.39 (0.31, 0.61)	0.36 (0.29, 0.50)
t-16:1n-7	0.16 (0.11, 0.23)	0.13 (0.09, 0.18)
Dietary factors, servings/day		
Total dairy	2.1±1.5	2.1±1.6
Whole-fat dairy‡	0.95±1.17	0.97±1.28
Low-fat dairy§	1.2±1.0	1.1±1.1
Processed meats	0.21±0.29	0.32±0.42
Unprocessed meats	0.90±0.49	0.94±0.55
Fruits and vegetables	5.5±3.0	5.9±3.2
Fish	0.31±0.29	0.27±0.25
Alcohol	0.44±0.79	0.98±1.28

*Values are mean±SD for continuous variables and percent for categorical variables. Missing values range: 0.0% for age to 8.6% for parental history of MI (women), and 0.0% for age to 4.6% for smoking (men).
†Fatty acid concentrations are reported as medians (12.5^{th} , 87.5^{th} percentiles, representing midpoint of bottom and top quartiles).
‡Whole milk, ice cream, butter, cream, sour cream, cream cheese, and other cheese.
§Low-fat or skim milk, yogurt, and cottage cheese.

DOI: 10.1161/CIRCULATIONAHA.115.018410

Table 2. Risk of incident diabetes according to plasma fatty acid biomarkers of dairy fat consumption among 3,333 men and women in the NHS (N=184 cases), HPFS (N=93 cases), and both cohorts combined.

	Cohort-specific fatty acid quartiles				
	1	**2**	**3**	**4**	**P for trend***
15:0, NHS					
% of total FA, median	0.11	0.14	0.17	0.22	
No. cases	33	49	55	47	
Person-months	95,002	89,388	98,482	95,695	
Multivariable HR (95%CI)†	Reference	1.26 (0.80-2.00)	0.89 (0.56-1.43)	0.60 (0.36-1.01)	0.01
15:0, HPFS					
% of total FA, median	0.10	0.13	0.15	0.20	
No. cases	18	14	34	27	
Person-months	55,559	51,928	58,919	61,348	
Multivariable HR (95%CI)	Reference	0.63 (0.31-1.30)	1.12 (0.59-2.12)	0.49 (0.23-1.04)	0.09
15:0, pooled	**Reference**	**1.03 (0.70-1.52)**	**0.96 (0.66-1.41)**	**0.56 (0.37-0.86)**	**0.01**
17:0, NHS					
% of total FA, median	0.26	0.30	0.33	0.39	
No. cases	59	47	44	34	
Person-months	94,103	92,604	94,510	97,350	
Multivariable HR (95%CI)	Reference	0.79 (0.53-1.19)	0.75 (0.49-1.14)	0.50 (0.31-0.81)	0.01
17:0, HPFS					
% of total FA, median	0.24	0.29	0.32	0.38	
No. cases	23	24	17	29	
Person-months	56,574	55,212	57,264	58,704	
Multivariable HR (95%CI)	Reference	0.80 (0.43-1.47)	0.48 (0.25-0.94)	0.69 (0.38-1.26)	0.18
17:0, pooled	**Reference**	**0.79 (0.57-1.11)**	**0.66 (0.46-0.94)**	**0.57 (0.39-0.83)**	**<0.01**
t-16:1n-7, NHS					
% of total FA, median	0.13	0.17	0.21	0.28	
No. cases	54	38	44	48	
Person-months	90,854	83,770	95,906	108,037	
Multivariable HR (95%CI)	Reference	0.79 (0.51-1.22)	0.69 (0.46-1.05)	0.48 (0.30-0.76)	0.002
t-16:1n-7, HPFS					
% of total FA, median	0.10	0.13	0.16	0.22	
No. cases	27	17	23	26	

Person-months	57,935	52,101	60,989	56,729	
Multivariable HR (95%CI)	Reference	0.66 (0.35-1.24)	0.64 (0.36-1.16)	0.49 (0.26-0.90)	0.03
t-16:1n-7, pooled	**Reference**	**0.75 (0.52-1.07)**	**0.67 (0.48-0.94)**	**0.48 (0.33-0.70)**	**<0.001**
14:0, NHS					
% of total FA, median	0.24	0.44	0.64	0.98	
No. cases	29	46	38	71	
Person-months	94,398	93,023	93,812	97,334	
Multivariable HR (95%CI)	Reference	1.55 (0.97-2.49)	0.95 (0.57-1.57)	0.93 (0.55-1.57)	0.35
14:0, HPFS					
% of total FA, median	0.25	0.40	0.57	0.90	
No. cases	10	22	27	34	
Person-months	53,011	56,008	56,340	62,395	
Multivariable HR (95%CI)	Reference	1.98 (0.90-4.33)	1.85 (0.83-4.15)	1.50 (0.56-3.98)	0.92
14:0, pooled	**Reference**	**1.65 (1.10-2.48)**	**1.15 (0.75-1.76)**	**1.03 (0.65-1.64)**	**0.36**

*Computed within each cohort by assigning median level in each quartile to participants and evaluating this variable continuously. Pooled P-for-trend was calculated using generalized least squares trend (GLST) meta-analysis.[20]

†Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).

DOI: 10.1161/CIRCULATIONAHA.115.018410

Table 3. Risk of incident diabetes according to plasma fatty acid biomarkers of dairy fat consumption, evaluated continuously, among 3,333 men and women in the NHS (N=184 cases), HPFS (N=93 cases), and both cohorts combined.

	Multivariable HR (95%CI) standardized to difference between midpoints of highest vs. lowest quartiles (87.5th minus 12.5th percentiles)			
	NHS	**HPFS**	**Pooled**	**P-value**
15:0	range=0.10*	range=0.10		
Multivariable HR (95%CI)†	0.63 (0.42-0.92)	0.61 (0.36-1.03)	**0.62 (0.46-0.85)**	**<0.01**
+ BMI‡	0.60 (0.40-0.89)	0.73 (0.43-1.25)	**0.64 (0.47-0.89)**	**0.01**
17:0	range=0.13	range=0.14		
Multivariable HR (95%CI)	0.71 (0.49-1.04)	0.63 (0.39-1.02)	**0.68 (0.50-0.91)**	**0.01**
+ BMI	0.76 (0.52-1.10)	0.69 (0.42-1.11)	**0.73 (0.55-0.99)**	**0.04**
t-16:1n-7	range=0.15	range=0.12		
Multivariable HR (95%CI)	0.52 (0.36-0.76)	0.58 (0.36-0.93)	**0.54 (0.40-0.73)**	**<0.001**
+ BMI	0.51 (0.36-0.74)	0.61 (0.37-0.99)	**0.54 (0.41-0.73)**	**<0.001**
14:0	range=0.74	range=0.65		
Multivariable HR (95%CI)	0.92 (0.64-1.32)	0.61 (0.35-1.09)	**0.82 (0.60-1.11)**	**0.19**
+ BMI	0.90 (0.63-1.31)	0.75 (0.42-1.32)	**0.85 (0.63-1.16)**	**0.32**

*The difference in % of total fatty acids between midpoint of highest vs. lowest quartile.

†Multivariable adjustments as in Table 2.

‡Further adjusted for BMI (kg/m^2) as a potential mediator or confounder of the association.

DOI: 10.1161/CIRCULATIONAHA.115.018410

Table 4. Summary of prior prospective studies evaluating fatty acid biomarkers of dairy fat and incidence of diabetes.

Study	Design	Lipid compartment	No. of cases	Multivariable-Adjusted Hazard Ratio (95%CI)			
				15:0	17:0	t-16:1n-7	14:0
CHS[12]*	Prospective cohort	Plasma phospholipid	304	NS	NS	0.38 (0.24-0.62)*	NS†
EPIC-Interact[21]	Nested case-subcohort	Plasma phospholipid	12,403	0.79 (0.73-0.85)‡	0.67 (0.63-0.71)‡	-	1.15 (1.09-1.22)‡
MCCS[36]	Case-cohort	Plasma phospholipid	364	0.26 (0.17-0.40)§	-	-	-
MESA[35]	Prospective cohort	Plasma phospholipid	205	NS	-	0.52 (0.32-0.85)*	NS
VIP[37]	Nested case-control study	Erythrocyte membrane	237	0.65 (0.50-0.85)¶	0.47 (0.35-0.63)¶	-	1.25 (1.01-1.53)¶

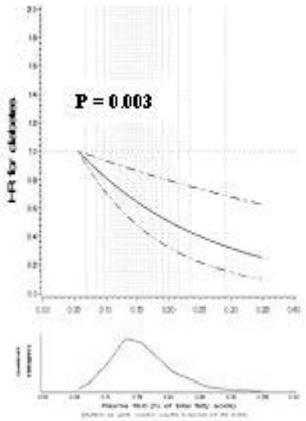
*Comparing the highest vs. lowest quintile of levels.
†Separately reported by Ma et al., Am J Clin Nutr 2015;101:153–63.
‡Per 1 SD increment in levels.
§Odds ratio comparing the highest vs. lowest quintile of levels.
¶Odds ratio per 1 SD increment in levels.
NS=not significant, CHS=Cardiovascular Health Study, EPIC=European Prospective Investigation into Cancer and Nutrition, MCCS=Melbourne Collaborative Cohort Study, MESA=Multi-Ethnic Study of Atherosclerosis, VIP=Vasterbotten Intervention Programme. Previous smaller subset reports from EPIC are not shown.

DOI: 10.1161/CIRCULATIONAHA.115.018410

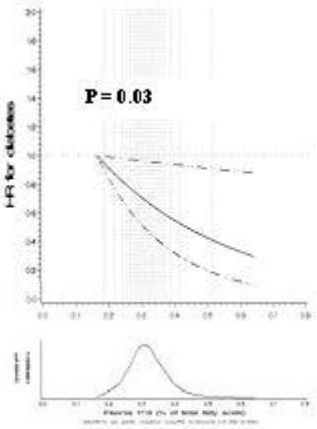
Figure Legend:

Figure 1. Semi-parametric multivariable-adjusted associations of plasma 15:0, 17:0, t-16:1n-7, and 14:0 with incident diabetes among 3,333 US men and women in two separate cohorts, evaluated using restricted cubic splines with covariate adjustments as in Table 2. Solid and dashed lines represent hazard ratios (HRs) and 95% confidence intervals, respectively; dotted vertical lines represent 21 knots. P-values for linear association are shown. P-values for non-linearity were nonsignificant in all analyses; the automatic selection process did not identify any significant spline variables.

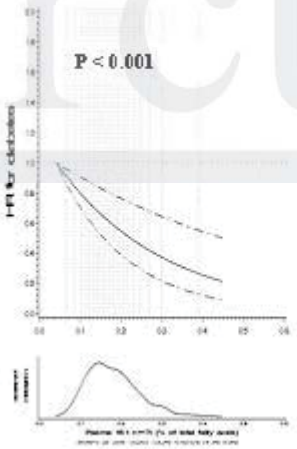
15:0



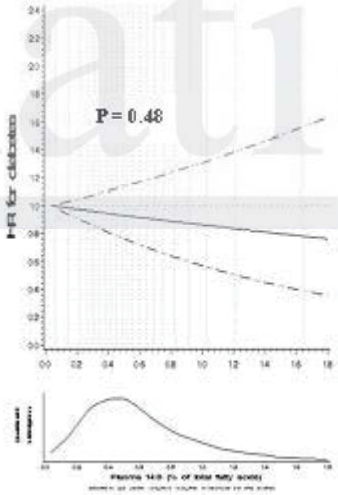
17:0



t-16:1n-7



14:0



Supplemental Material

Circulating biomarkers of dairy fat and risk of incident diabetes mellitus among US men and women in two large prospective cohorts

Table S1. Fatty acids measured by gas-liquid chromatography and methodology to compute their relative concentrations.*

	Code	Carbon Name	Systematic Name	Trivial Name
Saturated	Sa	8:0	Octanoic Acid	
	Sb	10:0	Decanoic Acid	
	S1	12:0	Dodecanoic Acid	Lauric Acid, Laurosteanic Acid
	Sc	13:0	Tridecanoic Acid	
	S2	14:0	Tetradecanoic Acid	Myristic Acid
	S3	15:0	Pentadecanoic Acid	
	S4	16:0	Hexadecanoic Acid	Palmitic Acid, Aethalic Acid
	S5	17:0	Heptadecanoic Acid	Margaric Acid, Daturinic Acid
	S6	18:0	Octadecanoic Acid	Stearic Acid
	S7	19:0	Nonadecanoic Acid	
	S8	20:0	Eicosanoic Acid	Arachidic Acid, Icosanoic Acid, Arachic Acid
	S9	21:0	Heneicosanoic Acid	
	S10	22:0	Docosanoic Acid	Behenic Acid
	S11	23:0	Tricosanoic Acid	
	S12	24:0	Tetracosanoic Acid	Lignoceric Acid
Monounsaturated	M1	14:1n-5c	9c-Tetradecenoic Acid	Mysristoleic Acid
	M2	15:1n-5c	10c-Pentadecenoic Acid	from 14:1n-5
	M3	16:1n-7c	9c-Hexadecenoic Acid	Palmitoleic Acid, Zoomaric Acid, Physetoleic Acid
	M4	17:1n-7c	10c-Heptadecenoic Acid	from 16:1n-7
	M5	18:1n-12c	6c-Octadecenoic Acid	Petroselinic Acid
	M6	18:1n-9c	9c-Octadecenoic Acid	Oleic Acid, Rapinic Acid
	M7	18:1n-7c	11c-Octadecenoic Acid	
	M8	19:1n-9c	10c-Nonadecenoic Acid	from 18:1n-9
	M9	20:1n-12c	8c-Eicosenoic Acid	from 18:1(n-12)

	Code	Carbon Name	Systematic Name	Trivial Name
	M10	20:1n-9c	11c-Eicosenoic Acid	Gondoic Acid
	M11	20:1n-7	13ct-Eicosenoic	from 16:1n-7
	M12	24:1n-9c	15c-Tetrasenoic Acid	Nervonic Acid, Selacholeic Acid
Polyunsaturated- Omega-3	P3	18:3n-3c	9c,12c,15c-Octadecatrienoic Acid	Alpha-linolenic Acid
	P7	20:3n-3c	11c,14c,17c-Eicosatrienoic Acid	from 18:3(n-3)
	P10	20:5n-3c	5c,8c,11c,14c17c-Eicosapentaenoic Acid	EPA, Timnodonic Acid
	P11	22:3n-3c	13c,16c19c-Docosantrienoic Acid	
	P13	22:5n-3c	7c,10c,13c,16c,19c-Docosapentaenoic Acid	DPA
	P14	22:6n-3c	4c,7c,10c,13c,16c,19c-Docosahexaenoic Acid	DHA, Cervanic Aicd
Polyunsaturated- Omega-6	P1	18:2n-6cc	9c,12c-Octadecadienoic Acid	Linoleic Acid, Leinlic Acid, Telfairic Acid, Linolic Acid
	P2	18:3n-6c	6c,9c,12c-Octadecatrienoic Acid	Gamma-linolenic Acid, Gamolenic Acid
	P5	20:2n-6c	11c,14c-Eicosadienoic Acid	
	P6	20:3n-6c	8c,11c,14c-Eicosatrienoic Acid	Dihomogammalinolenic Acid
	P8	20:4n-6c	5c,8c,111c,14c-Eicosatetraenoic Acid	Arachidonic Acid
	P9	22:2n-6c	13c,16c-Docosadienoic Acid	from 20:2(n-6)
	P12	22:4n-6c	7c,10c,13c,16c-Docosatetraynoic Acid	Aolrenic Acid
Trans	T1	14:1n-5t	9t-Tetadecenoic Acid	Myristelaidic Acid
	T2	16:1n-7t	9t-Hexadecenoic Acid	Palmitelaidic Acid
	T3	18:1n-12t	6t-Octadecenoic Acid	Petroselaidic Acid, Tarelaidinic Acid
	T4	18:1n-9t	9t-Octadecenoic Acid	Elaidic Acid
	T5	18:1n-7t	11t-Octadecenoic Acid	Vaccenic Acid
	T6	18:2n-6t	9t,12t-Octadecadienoic Acid	Linolelaidic Acid
	T7	18:2n-6ct	9c,12t-Octadecadienoic Acid	
	T8	18:2n-6tc	9t,12c-Octadecadienoic Acid	
	T9	18:3n-3t	9t,12t,15t-Octadecatrienoic Acid	from 18:3(n-3)c
	T10	20:1n-9t	11t-Eicosenoic Acid	
	T11	20:2n-6t	11t,14t-Eicosadienoic Acid	from 20:2(n-6)c
Others	P4	18:2n-7c	9c,11t-Octadecadienoic Acid	CLA (C14), Rumenic Acid
	U3			
	C2		believed to be trans	

Code	Carbon Name	Systematic Name	Trivial Name
U12		believed to be trans	
U13		believed to be trans	
U14		believed to be trans	

*The fatty acid identification chart lists the fatty acids identified. Peaks that were below the detection limit were scored as a zero (0). From this list, we identified 57 peaks (pre-2009) whose chromatogram area was summed to constitute the total peak area (denominator) and then each individual peak was expressed in units of normalized area percent (i.e. percent of the total peak area).The total number of fatty acids evaluated post-2009 was 40 because fatty acids with almost always below detectable limits were removed, and several trans-isomers were summed together into a single variable.

METHODS

Details of ascertainment of covariates and other risk factors

Data on medical history, major risk factors and lifestyle habits were obtained in both cohorts via validated self-administered questionnaires, including on smoking and physical activity.[1-4] Usual alcohol use and dietary habits over the last year were assessed through validated semi-quantitative food frequency questionnaire.[5, 6] Self-reported height and weight were used to calculate body mass index (BMI), after confirming high age-adjusted correlation coefficients with technician-measured values height and weight (NHS and HPFS: r=0.97).[7] Family history of myocardial infarction (MI) or diabetes and (in women) menopausal status and postmenopausal hormone use were assessed through validated self-report. Hypercholesterolemia and hypertension were evaluated by self-report, with high demonstrated validity as compared with medical records in random subsamples.[8, 9]

Table S2. Multivariable adjusted baseline characteristics of 3,333 US men and women with fatty acid measurements and free of prevalent diabetes in the Nurses' Health Study and Health Professionals Follow-Up Study cohorts (pooled) according to quartiles of total plasma fatty acid biomarkers of dairy fat.

	Quartiles of 15:0				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.11	0.14	0.16	0.21	-
Age, years	62.1	62.2	62.2	62.2	0.92
Sex, % male	14.3	19.9	29.4	37.0	<0.01
Race, % Caucasian	98.4	98.3	98.0	99.0	0.22
Body mass index, kg/m^2	25.3	25.6	25.9	25.3	0.98
Overweight or obese, %	46.9	48.8	54.9	47.6	0.71
Current smoking, %	10.9	8.5	9.6	9.3	0.50
Physical activity, MET-hours/week	24.8	25.8	24.8	24.7	0.77
Hypertension, %	23.6	22.0	22.3	19.0	0.05
Hypercholesterolemia, %	34.7	31.7	28.6	25.8	<0.001
Parental MI before 60 y, %	17.9	13.6	16.9	13.0	0.05
Family history of diabetes, %	22.5	25.4	24.0	26.2	0.21
Total dairy, servings/day	1.9	2.0	2.1	2.3	<0.001
Whole fat dairy, servings/day	0.77	0.83	0.99	1.21	<0.001
Low fat dairy, servings/day	1.2	1.2	1.1	1.1	0.07
Processed meats, servings/day	0.29	0.26	0.24	0.25	0.01
Unprocessed meats, servings/day	0.96	0.92	0.91	0.88	<0.001
Fruits, servings/day	1.7	1.8	1.7	1.7	0.73
Vegetables, servings/day	4.1	4.0	4.0	3.7	<0.001
Fish, servings/day	0.30	0.30	0.30	0.25	<0.01
Alcohol, servings/day	1.00	0.77	0.59	0.37	<0.001

	Quartiles of 17:0				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.25	0.30	0.33	0.39	-
Age, years	61.9	62.0	62.4	62.5	0.06
Sex, % male	18.2	27.8	24.4	29.3	0.05
Race, % Caucasian	98.4	98.5	98.6	98.3	0.91
Body mass index, kg/m^2	25.8	25.8	25.5	25.0	<0.001
Overweight or obese, %	52.0	53.0	49.4	44.5	<0.01
Current smoking, %	11.7	8.4	9.0	9.3	0.20
Physical activity, MET-hours/week	25.4	25.5	25.2	23.9	0.28
Hypertension, %	24.1	20.0	23.2	19.5	0.15
Hypercholesterolemia, %	35.2	30.6	28.4	26.3	<0.001
Parental MI before 60 y, %	17.4	15.2	15.7	13.2	0.04
Family history of diabetes, %	24.2	25.3	24.4	24.1	0.87
Total dairy, servings/day	1.9	2.1	2.2	2.2	<0.001
Whole fat dairy, servings/day	0.77	0.94	1.01	1.10	<0.001
Low fat dairy, servings/day	1.2	1.2	1.1	1.1	0.14
Processed meats, servings/day	0.25	0.24	0.29	0.25	0.44
Unprocessed meats, servings/day	0.92	0.93	0.91	0.91	0.19
Fruits, servings/day	1.7	1.8	1.7	1.8	0.03
Vegetables, servings/day	4.0	4.0	3.9	3.9	0.17
Fish, servings/day	0.29	0.31	0.28	0.28	0.20
Alcohol, servings/day	0.98	0.67	0.58	0.47	<0.001

	Quartiles of t-16:1n-7				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.12	0.15	0.19	0.25	-
Age, years	61.8	61.7	62.0	63.0	<0.01
Sex, % male	17.0	18.5	23.0	37.8	<0.001
Race, % Caucasian	98.6	98.5	98.6	98.2	0.61
Body mass index, kg/m^2	25.9	25.5	25.5	25.2	<0.001
Overweight or obese, %	54.4	49.2	49.2	46.2	<0.01
Current smoking, %	9.4	12.0	8.6	8.5	0.17
Physical activity, MET-hours/week	25.8	24.9	24.7	24.6	0.54
Hypertension, %	22.0	21.6	20.8	22.2	0.87
Hypercholesterolemia, %	34.8	30.0	27.8	28.1	0.01
Parental MI before 60 y, %	15.5	15.6	14.6	15.6	0.98
Family history of diabetes, %	26.4	22.6	25.3	23.5	0.34
Total dairy, servings/day	1.9	2.0	2.2	2.3	<0.001
Whole fat dairy, servings/day	0.71	0.86	1.01	1.21	<0.001
Low fat dairy, servings/day	1.2	1.2	1.2	1.1	<0.001
Processed meats, servings/day	0.26	0.25	0.28	0.25	0.48
Unprocessed meats, servings/day	0.95	0.93	0.92	0.88	<0.001
Fruits, servings/day	1.8	1.8	1.8	1.7	0.13
Vegetables, servings/day	4.1	3.9	3.9	3.9	0.49
Fish, servings/day	0.31	0.30	0.28	0.27	<0.01
Alcohol, servings/day	0.87	0.74	0.64	0.48	<0.001

	Quartiles of 14:0				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.24	0.42	0.61	0.94	-
Age, years	61.6	61.9	62.9	62.2	0.11
Sex, % male	12.1	21.5	27.2	37.6	0.02
Race, % Caucasian	98.5	97.9	98.6	98.8	0.30
Body mass index, kg/m^2	24.9	25.4	25.9	25.9	<0.001
Overweight or obese, %	42.1	45.8	53.1	56.3	<0.001
Current smoking, %	6.4	9.7	10.1	11.9	<0.01
Physical activity, MET-hours/week	24.2	25.6	24.6	25.5	0.56
Hypertension, %	20.3	20.9	22.6	22.6	0.38
Hypercholesterolemia, %	29.3	28.6	30.7	31.4	0.46
Parental MI before 60 y, %	16.2	16.9	15.2	13.4	0.16
Family history of diabetes, %	22.9	23.0	24.2	27.7	0.05
Total dairy, servings/day	2.1	2.0	2.1	2.2	<0.01
Whole fat dairy, servings/day	0.88	0.89	0.95	1.08	<0.01
Low fat dairy, servings/day	1.2	1.1	1.2	1.1	0.55
Processed meats, servings/day	0.27	0.26	0.26	0.25	0.19
Unprocessed meats, servings/day	0.94	0.93	0.92	0.89	<0.01
Fruits, servings/day	1.7	1.7	1.8	1.7	0.94
Vegetables, servings/day	4.1	4.0	3.9	3.8	0.01
Fish, servings/day	0.30	0.30	0.29	0.27	0.01
Alcohol, servings/day	0.76	0.76	0.69	0.51	<0.001

Table S3. Partial Spearman correlations between plasma fatty acid biomarkers of dairy fat and dietary factors among 3,333 participants in the Nurses' Health Study and Health Professionals Follow-Up Study.*

	Plasma fatty acids			
	15:0	**17:0**	**t-16:1n-7**	**14:0**
Dietary Factors†				
Dairy fat	0.29	0.21	0.22	0.11
Whole-fat dairy	0.26	0.19	0.24	0.06
Low-fat dairy	0.13	0.08	0.06	0.04
Sugar-sweetened beverages	0.07	0.03	0.07	0.04
Refined grains	0.07	0.04	0.01	0.03
Sweets/desserts	0.03	0.02	0.08	-0.00
Alcohol	-0.17	-0.26	-0.10	0.02
French fries	0.00	0.01	0.06	-0.01
Potato (baked, boiled, mashed)	-0.01	0.00	0.00	-0.00
Potato or corn chips	-0.04	-0.05	-0.01	-0.02
Processed meat	0.01	0.07	0.07	-0.01
Unprocessed meat	0.01	0.08	0.08	-0.05
Plasma Fatty Acids‡				
15:0	1.0	0.57	0.50	0.59
17:0		1.0	0.50	0.10
t-16:1n-7			1.0	0.20
14:0				1.0

*Values are adjusted Spearman correlations based on pooling of individual-level data from both cohorts.

†Dietary habits were assessed using the average of self-reported consumption in 1986 and 1990 in the NHS, and 1990 and 1994 in the HPFS. Correlations with diet (N=2,717 due to missing dietary questionnaire data in some participants) were adjusted for age (years), sex, body mass index (kg/m^2), smoking (never, current, former, missing), fasting status at blood draw, consumption of total energy (kcal/day), and each of the other dietary factor in the table simultaneously. Dairy fat was excluded as a covariate when evaluating whole-fat or low-fat dairy foods.

‡Fatty acid intercorrelations (N=3,289) were adjusted for age and sex.

Table S4. Partial Spearman correlations between red blood cell fatty acids and dietary factors among 3,289 women and men in the Nurses' Health Study and Health Professionals Follow-Up Study.*

	Erythrocyte fatty acids			
	15:0	**17:0**	**t-16:1n-7**	**14:0**
Dietary factors†				
Dairy fat	0.16	0.11	0.20	0.10
Whole-fat dairy	0.16	0.13	0.21	0.08
Low-fat dairy	0.06	0.04	0.07	0.02
Sugar-sweetened beverages	0.04	0.02	0.04	0.04
Refined grains	0.03	-0.01	0.00	0.02
Sweets/desserts	0.01	0.01	0.06	0.01
Alcohol	-0.10	-0.17	-0.09	0.02
French fries	0.00	0.03	0.02	-0.01
Potatoes (baked, boiled, mashed)	0.01	0.01	0.04	-0.01
Potato or corn chips	-0.03	-0.04	-0.02	-0.02
Processed meat	0.03	0.07	0.06	0.02
Unprocessed meat	0.01	0.06	0.05	0.00
Erythrocyte fatty acids‡				
15:0	1.0	0.63	0.63	0.68
17:0		1.0	0.69	0.47
t-16:1n-7			1.0	0.48
14:0				1.0

*Values are adjusted Spearman correlations based on pooling of individual-level data from both cohorts. Compared with plasma fatty acids (N=3,333), 44 fewer subjects (N=3,289) had successful measures of erythrocyte fatty acids.

†Dietary habits were assessed using the average of self-reported consumption in 1986 and 1990 in the NHS, and 1990 and 1994 in the HPFS. Correlations with diet (N=2,717 due to missing dietary questionnaire data in some participants) were adjusted for age (years), sex, body mass index (kg/m^2), smoking (never, current, former, missing), fasting status at blood draw, consumption of total energy (kcal/day), and each of the other dietary factor in the table simultaneously. Dairy fat was excluded as a covariate when evaluating whole-fat or low-fat dairy foods.

‡Fatty acid intercorrelations (N=3,289) were adjusted for age and sex.

Table S5. Risk of incident diabetes according to red blood cell fatty acid biomarkers of dairy fat consumption among 3,289 men and women in the NHS (N=179 cases), HPFS (N=97 cases), and both cohorts combined.

	Cohort-specific fatty acid quartiles				
Fatty acid	**1**	**2**	**3**	**4**	**P for trend***
15:0, NHS					
% of total FA, median	0.08	0.11	0.14	0.18	
No. of cases	43	38	53	45	
Person-months	85,143	85,284	89,701	99,253	
Multivariable hazard ratio (95%CI) †	Reference	0.73 (0.46-1.16)	0.93 (0.60-1.43)	0.65 (0.39-1.09)	0.20
15:0, HPFS					
% of total FA, median	0.07	0.09	0.12	0.16	
No. of cases	20	21	26	30	
Person-months	58,160	57,184	60,933	57,792	
Multivariable hazard ratio (95%CI)	Reference	0.87 (0.46-1.65)	1.09 (0.58-2.05)	1.27 (0.65-2.48)	0.35
15:0, pooled	**Reference**	**0.78 (0.53-1.13)**	**0.98 (0.68-1.40)**	**0.83 (0.55-1.25)**	**0.63**
17:0, NHS					
% of total FA, median	0.31	0.37	0.42	0.59	
No. of cases	65	46	23	45	
Person-months	84,278	90,276	85,591	99,236	
Multivariable hazard ratio (95%CI)	Reference	0.66 (0.44-0.98)	0.30 (0.18-0.49)	0.37 (0.20-0.66)	<0.001
17:0, HPFS					
% of total FA, median	0.29	0.34	0.38	0.48	
No. of cases	25	23	19	30	
Person-months	58,563	54,066	61,510	59,930	
Multivariable hazard ratio (95%CI)	Reference	0.88 (0.49-1.61)	0.54 (0.28-1.04)	0.99 (0.47-2.09)	0.90
17:0, pooled	**Reference**	**0.72 (0.52-1.01)**	**0.37 (0.25-0.56)**	**0.54 (0.34-0.87)**	**<0.001**
t-16:1n-7, NHS					
% of total FA, median	0.11	0.14	0.17	0.22	
No. of cases	47	52	35	45	
Person-months	83,002	86,211	89,095	101,073	
Multivariable hazard ratio (95%CI)	Reference	1.03 (0.69-1.54)	0.65 (0.41-1.02)	0.60 (0.36-1.02)	0.02

t-16:1n-7, HPFS					
% of total FA, median	0.09	0.12	0.14	0.18	
No. of cases	20	26	19	32	
Person-months	58,671	57,636	57,138	60,624	
Multivariable hazard ratio (95%CI)	Reference	1.31 (0.72-2.39)	0.82 (0.42-1.59)	1.21 (0.61-2.39)	0.80
t-16:1n-7, pooled	**Reference**	**1.11 (0.80-1.55)**	**0.70 (0.48-1.02)**	**0.78 (0.51-1.18)**	**0.05**
14:0, NHS					
% of total FA, median	0.11	0.20	0.31	0.64	
No. of cases	32	50	37	60	
Person-months	80,336	85,653	92,087	101,305	
Multivariable hazard ratio (95%CI)	Reference	1.18 (0.74-1.87)	0.76 (0.45-1.29)	1.08 (0.61-1.88)	0.82
14:0, HPFS					
% of total FA, median	0.12	0.21	0.31	0.53	
No. of cases	14	20	30	33	
Person-months	57,996	58,816	60,816	56,441	
Multivariable hazard ratio (95%CI)	Reference	1.49 (0.70-3.14)	2.29 (1.10-4.78)	3.43 (1.51-7.77)	0.001
14:0, pooled	**Reference**	**1.26 (0.85-1.87)**	**1.11 (0.72-1.70)**	**1.56 (0.98-2.49)**	**0.13**

*Computed within each cohort by assigning the median level in each quartile to participants and evaluating this as a continuous variable. The pooled P-for-trend was calculated using generalized least squares trend (GLST) meta-analysis.[10]

†Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).

Table S6. Risk of incident diabetes according to red blood cell fatty acid biomarkers, evaluated continuously, among 3,289 men and women in the Nurses' Health Study (N=179 cases), Health Professionals Follow-Up Study (N=97 cases), and both cohorts combined.

Fatty acids	Results standardized to the difference between the midpoints of the highest vs. lowest quartiles (87.5th minus 12.5th percentiles)			
	NHS	**HPFS**	**Pooled**	**P-value**
15:0	range = 0.10*	range = 0.10		
Multivariable HR (95%CI) †	0.75 (0.50-1.12)	1.33 (0.98-1.80)	**1.08 (0.85-1.38)**	**0.53**
+ BMI ‡	0.73 (0.48-1.12)	1.37 (1.01-1.86)	**1.11 (0.86-1.42)**	**0.43**
17:0	range = 0.28	range = 0.19		
Multivariable HR (95%CI)	0.42 (0.26-0.68)	0.66 (0.29-1.53)	**0.47 (0.31-0.71)**	**<0.001**
+ BMI	0.57 (0.31-1.04)	0.88 (0.38-2.06)	**0.66 (0.40-1.08)**	**0.10**
t-16:1n-7	range = 0.11	range = 0.09		
Multivariable HR (95%CI)	0.76 (0.49-1.17)	0.87 (0.50-1.53)	**0.80 (0.57-1.13)**	**0.20**
+ BMI	0.86 (0.55-1.35)	0.95 (0.53-1.70)	**0.89 (0.63-1.27)**	**0.53**
14:0	Range = 0.53	Range = 0.41		
Multivariable HR (95%CI)	1.23 (0.98-1.54)	2.11 (1.31-3.39)	**1.36 (1.11-1.67)**	**0.003**
+ BMI	1.16 (0.91-1.46)	2.11 (1.30-3.42)	**1.30 (1.05-1.61)**	**0.02**

*The difference in % of total fatty acids between the midpoint of the highest vs. lowest quartile.

† Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).

‡ Further adjusted for body mass index (BMI, kg/m^2) as a potential mediator of confounder of the association.

Table S7. Risk of incident diabetes according to plasma fatty acid biomarkers of dairy fat after further adjustment for self-reported consumption of yogurt, cheese, or dairy fat as covariates in the NHS (N=184 cases), HPFS (N=93 cases), and both cohorts combined.

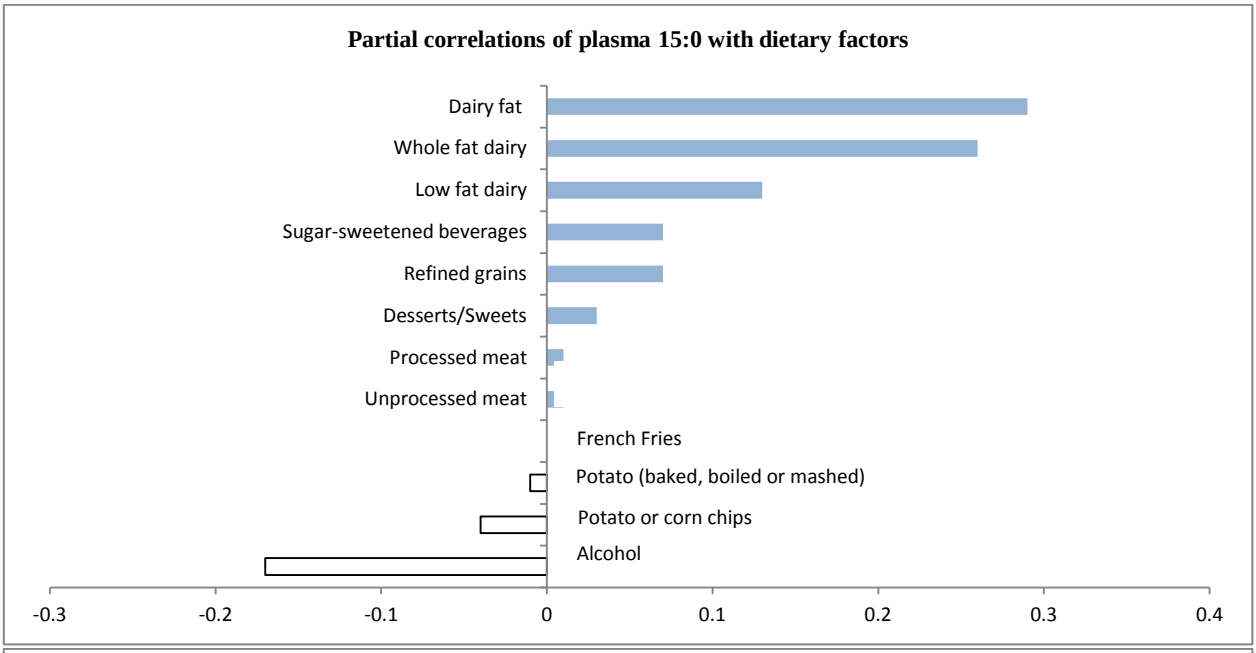
	Multivariable HR (95%CI) per increase in difference of the midpoint of the highest versus lowest quartiles (87.5^{th} minus 12.5^{th} percentiles)			
	Main results	Adding yogurt	Adding cheese	Adding dairy fat
15:0				
NHS	0.63 (0.42-0.92)	0.63 (0.43-0.94)	0.63 (0.42-0.93)	0.63 (0.42-0.94)
HPFS	0.61 (0.36-1.03)	0.62 (0.36-1.06)	0.60 (0.35-1.03)	0.56 (0.32-0.97)
Pooled	**0.62 (0.46-0.85)**	**0.63 (0.46-0.86)**	**0.62 (0.45-0.85)**	**0.61 (0.44-0.84)**
17:0				
NHS	0.71 (0.49-1.04)	0.72 (0.49-1.05)	0.72 (0.49-1.04)	0.72 (0.50-1.05)
HPFS	0.63 (0.39-1.02)	0.64 (0.39-1.03)	0.63 (0.39-1.02)	0.60 (0.37-0.99)
Pooled	**0.68 (0.50-0.91)**	**0.69 (0.51-0.93)**	**0.68 (0.51-0.92)**	**0.67 (0.50-0.91)**
t-16:1n-7				
NHS	0.52 (0.36-0.76)	0.53 (0.36-0.76)	0.52 (0.36-0.76)	0.53 (0.36-0.77)
HPFS	0.58 (0.36-0.93)	0.57 (0.35-0.92)	0.55 (0.34-0.90)	0.49 (0.30-0.82)
Pooled	**0.54 (0.40-0.73)**	**0.55 (0.41-0.73)**	**0.53 (0.40-0.71)**	**0.52 (0.38-0.70)**
14:0				
NHS	0.92 (0.64-1.32)	0.94 (0.65-1.35)	0.92 (0.64-1.33)	0.93 (0.64-1.34)
HPFS	0.61 (0.35-1.09)	0.63 (0.35-1.11)	0.61 (0.34-1.08)	0.56 (0.31-1.01)
Pooled	**0.82 (0.60-1.11)**	**0.84 (0.62-1.14)**	**0.82 (0.60-1.11)**	**0.81 (0.59-1.10)**

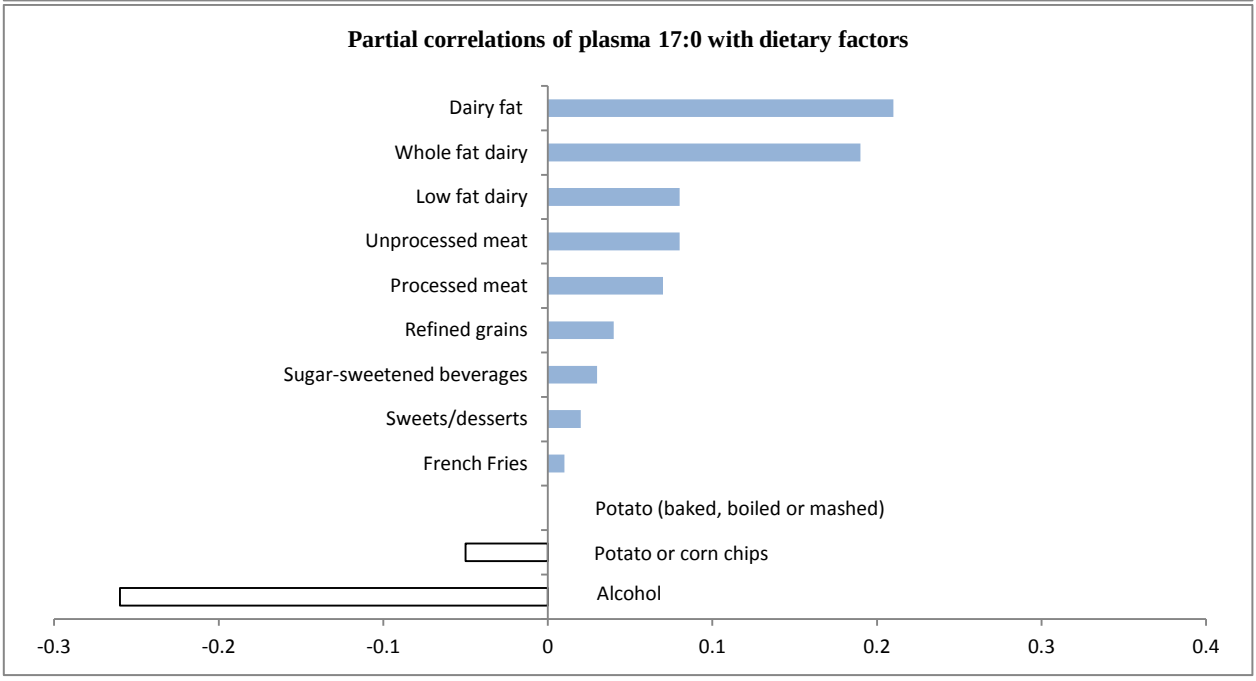
Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).

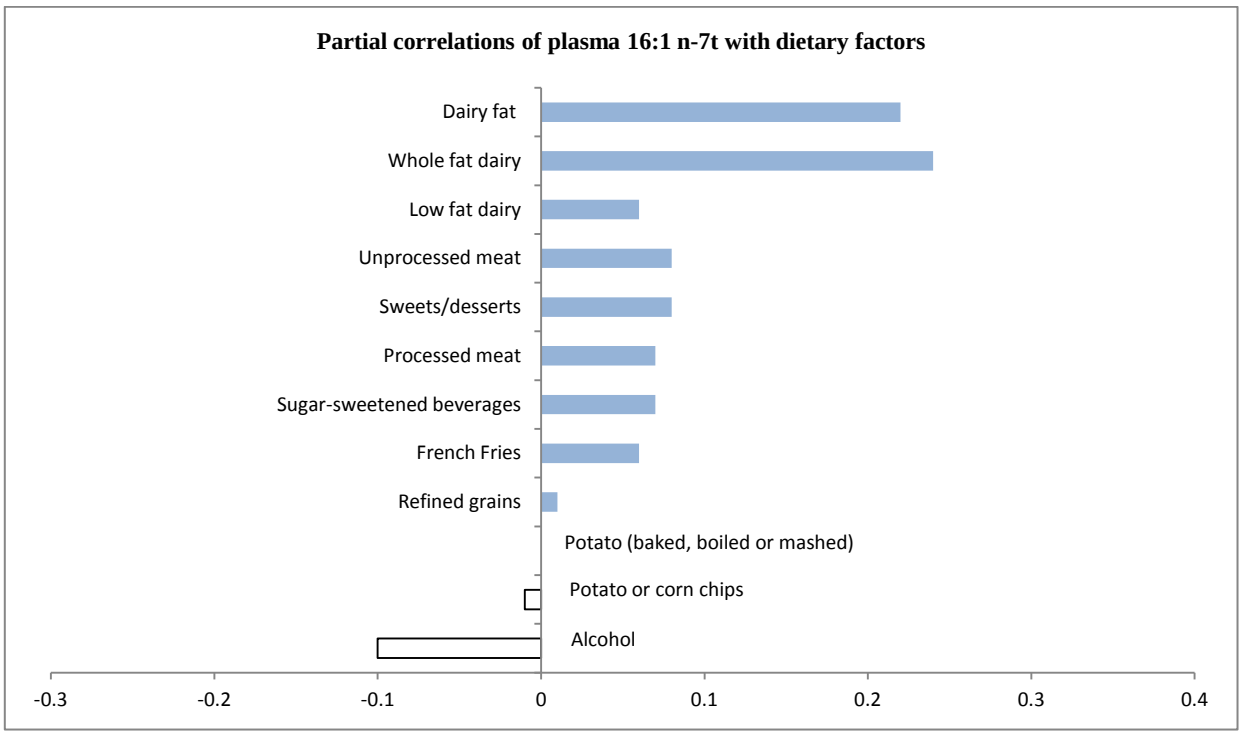
Table S8. Risk of incident diabetes according to plasma fatty acid biomarkers of dairy fat in sensitivity analyses excluding cases in the first 2 years of follow-up and restricting to the first 8 years of follow-up in the NHS (N=172 and 65 cases, respectively), HPFS (N=84 and 50 cases, respectively), and both cohorts combined.

	Results standardized to the difference between the midpoints of the highest vs. lowest quartiles (87.5th minus 12.5th percentiles)		
Fatty Acids	**Full follow-up**	**Excluding cases in the first 2 years**	**Restricting to the first 8 years**
15:0			
NHS	0.63 (0.42-0.92)	0.65 (0.43-0.97)	0.56 (0.29-1.06)
HPFS	0.61 (0.36-1.03)	0.76 (0.45-1.29)	0.71 (0.36-1.40)
Pooled	**0.62 (0.46-0.85)**	**0.69 (0.50-0.95)**	**0.63 (0.39-1.00)**
17:0			
NHS	0.71 (0.49-1.04)	0.75 (0.51-1.10)	0.66 (0.35-1.23)
HPFS	0.63 (0.39-1.02)	0.72 (0.45-1.17)	0.75 (0.40-1.39)
Pooled	**0.68 (0.50-0.91)**	**0.74 (0.55-1.00)**	**0.70 (0.45-1.10)**
t-16:1n-7			
NHS	0.52 (0.36-0.76)	0.58 (0.40-0.84)	0.28 (0.15-0.54)
HPFS	0.58 (0.36-0.93)	0.66 (0.39-1.09)	0.59 (0.31-1.12)
Pooled	**0.54 (0.40-0.73)**	**0.61 (0.45-0.82)**	**0.41 (0.26-0.64)**
14:0			
NHS	0.92 (0.64-1.32)	0.86 (0.59-1.25)	1.08 (0.58-2.00)
HPFS	0.61 (0.35-1.09)	0.67 (0.37-1.20)	0.71 (0.34-1.48)
Pooled	**0.82 (0.60-1.11)**	**0.80 (0.58-1.10)**	**0.91 (0.57-1.46)**

Values are hazard ratio (95%CI) adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).


Partial correlations of plasma 15:0 with dietary factors
Dairy fat
Whole fat dairy
Low fat dairy
Sugar-sweetened beverages
Refined grains
Desserts/Sweets
Processed meat
Unprocessed meat
French Fries
Potato (baked, boiled or mashed)
Potato or corn chips
Alcohol
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4


Partial correlations of plasma 17:0 with dietary factors
Dairy fat
Whole fat dairy
Low fat dairy
Unprocessed meat
Processed meat
Refined grains
Sugar-sweetened beverages
Sweets/desserts
French Fries
Potato (baked, boiled or mashed)
Potato or corn chips
Alcohol
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4



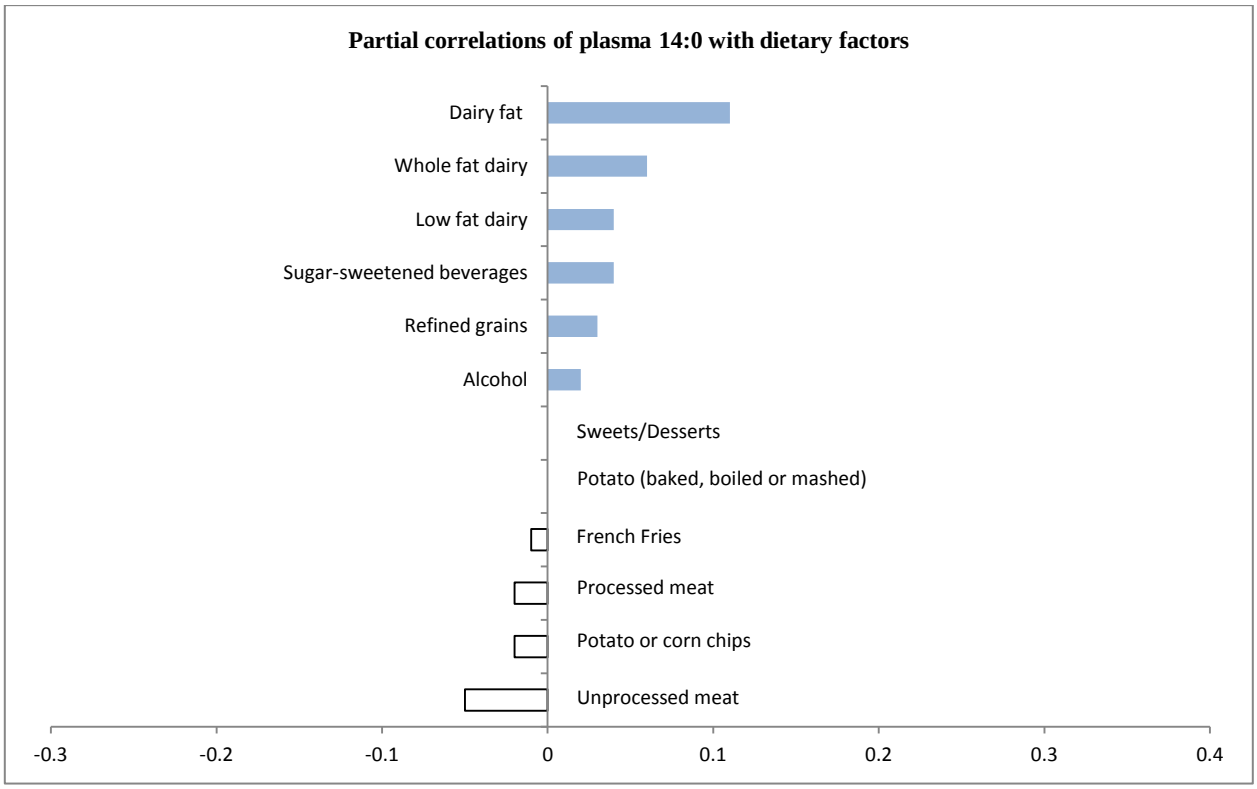


Figure S1. Partial Spearman correlations between plasma fatty acid biomarkers of dairy fat and dietary habits in the Nurses' Health Study and Health Professionals Follow-Up Study. Dietary habits assessed using the average of self-reported intake in 1986 and 1990 in NHS, and 1990 and 1994 in HPFS (total N=2,761). Correlations based on pooled individual-level data, adjusted for age (years), sex, body mass index (kg/m^2), smoking (never, current, former, missing), fasting status at blood draw, consumption of total energy (kcal/day), and each of the other dietary factor in the figure simultaneously. Dairy fat was excluded when evaluating whole-fat and low-fat dairy.

REFERENCES

1. Zhang L, Curhan GC, Hu FB, Rimm EB and Forman JP. Association between passive and active smoking and incident type 2 diabetes in women. *Diabetes Care*. 2011;34:892-7.
2. Wolf AM, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Corsano KA, Rosner B, Kriska A and Willett WC. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol*. 1994;23:991-9.
3. Chasan-Taber S, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E, Ascherio A and Willett WC. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology*. 1996;7:81-6.
4. Zhang X, Smith-Warner SA, Chan AT, Wu K, Spiegelman D, Fuchs CS, Willett WC and Giovannucci EL. Aspirin use, body mass index, physical activity, plasma C-peptide, and colon cancer risk in US health professionals. *Am J Epidemiol*. 2011;174:459-67.
5. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB and Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc*. 1993;93:790-6.
6. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B and Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol*. 1989;18:858-67.
7. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB and Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology*. 1990;1:466-73.
8. Ascherio A, Rimm EB, Giovannucci EL, Colditz GA, Rosner B, Willett WC, Sacks F and Stampfer MJ. A prospective study of nutritional factors and hypertension among US men. *Circulation*. 1992;86:1475-84.
9. Colditz GA, Martin P, Stampfer MJ, Willett WC, Sampson L, Rosner B, Hennekens CH and Speizer FE. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol*. 1986;123:894-900.
10. Orsini N, Bellocco R and Greenland S. Generalized least squares for trend estimation of summarized dose-response data. *Stata J*. 2006;6:40-57.

Supplemental Material

Circulating biomarkers of dairy fat and risk of incident diabetes mellitus among US men and women in two large prospective cohorts

Table S1. Fatty acids measured by gas-liquid chromatography and methodology to compute their relative concentrations.*

	Code	Carbon Name	Systematic Name	Trivial Name
Saturated	Sa	8:0	Octanoic Acid	
	Sb	10:0	Decanoic Acid	
	S1	12:0	Dodecanoic Acid	Lauric Acid, Laurosteanic Acid
	Sc	13:0	Tridecanoic Acid	
	S2	14:0	Tetradecanoic Acid	Myristic Acid
	S3	15:0	Pentadecanoic Acid	
	S4	16:0	Hexadecanoic Acid	Palmitic Acid, Aethalic Acid
	S5	17:0	Heptadecanoic Acid	Margaric Acid, Daturinic Acid
	S6	18:0	Octadecanoic Acid	Stearic Acid
	S7	19:0	Nonadecanoic Acid	
	S8	20:0	Eicosanoic Acid	Arachidic Acid, Icosanoic Acid, Arachic Acid
	S9	21:0	Heneicosanoic Acid	
	S10	22:0	Docosanoic Acid	Behenic Acid
	S11	23:0	Tricosanoic Acid	
	S12	24:0	Tetracosanoic Acid	Lignoceric Acid
Monounsaturated	M1	14:1n-5c	9c-Tetradecenoic Acid	Mysristoleic Acid
	M2	15:1n-5c	10c-Pentadecenoic Acid	from 14:1n-5
	M3	16:1n-7c	9c-Hexadecenoic Acid	Palmitoleic Acid, Zoomaric Acid, Physetoleic Acid
	M4	17:1n-7c	10c-Heptadecenoic Acid	from 16:1n-7
	M5	18:1n-12c	6c-Octadecenoic Acid	Petroselinic Acid
	M6	18:1n-9c	9c-Octadecenoic Acid	Oleic Acid, Rapinic Acid
	M7	18:1n-7c	11c-Octadecenoic Acid	
	M8	19:1n-9c	10c-Nonadecenoic Acid	from 18:1n-9
	M9	20:1n-12c	8c-Eicosenoic Acid	from 18:1(n-12)

	Code	Carbon Name	Systematic Name	Trivial Name
	M10	20:1n-9c	11c-Eicosenoic Acid	Gondoic Acid
	M11	20:1n-7	13ct-Eicosenoic	from 16:1n-7
	M12	24:1n-9c	15c-Tetrasenoic Acid	Nervonic Acid, Selacholeic Acid
Polyunsaturated- Omega-3	P3	18:3n-3c	9c,12c,15c-Octadecatrienoic Acid	Alpha-linolenic Acid
	P7	20:3n-3c	11c,14c,17c-Eicosatrienoic Acid	from 18:3(n-3)
	P10	20:5n-3c	5c,8c,11c,14c17c-Eicosapentaenoic Acid	EPA, Timnodonic Acid
	P11	22:3n-3c	13c,16c19c-Docosantrienoic Acid	
	P13	22:5n-3c	7c,10c,13c,16c,19c-Docosapentaenoic Acid	DPA
	P14	22:6n-3c	4c,7c,10c,13c,16c,19c-Docosahexaenoic Acid	DHA, Cervanic Aicd
Polyunsaturated- Omega-6	P1	18:2n-6cc	9c,12c-Octadecadienoic Acid	Linoleic Acid, Leinlic Acid, Telfairic Acid, Linolic Acid
	P2	18:3n-6c	6c,9c,12c-Octadecatrienoic Acid	Gamma-linolenic Acid, Gamolenic Acid
	P5	20:2n-6c	11c,14c-Eicosadienoic Acid	
	P6	20:3n-6c	8c,11c,14c-Eicosatrienoic Acid	Dihomogammalinolenic Acid
	P8	20:4n-6c	5c,8c,111c,14c-Eicosatetraenoic Acid	Arachidonic Acid
	P9	22:2n-6c	13c,16c-Docosadienoic Acid	from 20:2(n-6)
	P12	22:4n-6c	7c,10c,13c,16c-Docosatetraynoic Acid	Aolrenic Acid
Trans	T1	14:1n-5t	9t-Tetadecenoic Acid	Myristelaidic Acid
	T2	16:1n-7t	9t-Hexadecenoic Acid	Palmitelaidic Acid
	T3	18:1n-12t	6t-Octadecenoic Acid	Petroselaidic Acid, Tarelaidinic Acid
	T4	18:1n-9t	9t-Octadecenoic Acid	Elaidic Acid
	T5	18:1n-7t	11t-Octadecenoic Acid	Vaccenic Acid
	T6	18:2n-6t	9t,12t-Octadecadienoic Acid	Linolelaidic Acid
	T7	18:2n-6ct	9c,12t-Octadecadienoic Acid	
	T8	18:2n-6tc	9t,12c-Octadecadienoic Acid	
	T9	18:3n-3t	9t,12t,15t-Octadecatrienoic Acid	from 18:3(n-3)c
	T10	20:1n-9t	11t-Eicosenoic Acid	
	T11	20:2n-6t	11t,14t-Eicosadienoic Acid	from 20:2(n-6)c
Others	P4	18:2n-7c	9c,11t-Octadecadienoic Acid	CLA (C14), Rumenic Acid
	U3			
	C2		believed to be trans	

Code	Carbon Name	Systematic Name	Trivial Name
U12		believed to be trans	
U13		believed to be trans	
U14		believed to be trans	

*The fatty acid identification chart lists the fatty acids identified. Peaks that were below the detection limit were scored as a zero (0). From this list, we identified 57 peaks (pre-2009) whose chromatogram area was summed to constitute the total peak area (denominator) and then each individual peak was expressed in units of normalized area percent (i.e. percent of the total peak area).The total number of fatty acids evaluated post-2009 was 40 because fatty acids with almost always below detectable limits were removed, and several trans-isomers were summed together into a single variable.

METHODS

Details of ascertainment of covariates and other risk factors

Data on medical history, major risk factors and lifestyle habits were obtained in both cohorts via validated self-administered questionnaires, including on smoking and physical activity.[1-4] Usual alcohol use and dietary habits over the last year were assessed through validated semi-quantitative food frequency questionnaire.[5, 6] Self-reported height and weight were used to calculate body mass index (BMI), after confirming high age-adjusted correlation coefficients with technician-measured values height and weight (NHS and HPFS: r=0.97).[7] Family history of myocardial infarction (MI) or diabetes and (in women) menopausal status and postmenopausal hormone use were assessed through validated self-report. Hypercholesterolemia and hypertension were evaluated by self-report, with high demonstrated validity as compared with medical records in random subsamples.[8, 9]

Table S2. Multivariable adjusted baseline characteristics of 3,333 US men and women with fatty acid measurements and free of prevalent diabetes in the Nurses' Health Study and Health Professionals Follow-Up Study cohorts (pooled) according to quartiles of total plasma fatty acid biomarkers of dairy fat.

	Quartiles of 15:0				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.11	0.14	0.16	0.21	-
Age, years	62.1	62.2	62.2	62.2	0.92
Sex, % male	14.3	19.9	29.4	37.0	<0.01
Race, % Caucasian	98.4	98.3	98.0	99.0	0.22
Body mass index, kg/m^2	25.3	25.6	25.9	25.3	0.98
Overweight or obese, %	46.9	48.8	54.9	47.6	0.71
Current smoking, %	10.9	8.5	9.6	9.3	0.50
Physical activity, MET-hours/week	24.8	25.8	24.8	24.7	0.77
Hypertension, %	23.6	22.0	22.3	19.0	0.05
Hypercholesterolemia, %	34.7	31.7	28.6	25.8	<0.001
Parental MI before 60 y, %	17.9	13.6	16.9	13.0	0.05
Family history of diabetes, %	22.5	25.4	24.0	26.2	0.21
Total dairy, servings/day	1.9	2.0	2.1	2.3	<0.001
Whole fat dairy, servings/day	0.77	0.83	0.99	1.21	<0.001
Low fat dairy, servings/day	1.2	1.2	1.1	1.1	0.07
Processed meats, servings/day	0.29	0.26	0.24	0.25	0.01
Unprocessed meats, servings/day	0.96	0.92	0.91	0.88	<0.001
Fruits, servings/day	1.7	1.8	1.7	1.7	0.73
Vegetables, servings/day	4.1	4.0	4.0	3.7	<0.001
Fish, servings/day	0.30	0.30	0.30	0.25	<0.01
Alcohol, servings/day	1.00	0.77	0.59	0.37	<0.001

	Quartiles of 17:0				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.25	0.30	0.33	0.39	-
Age, years	61.9	62.0	62.4	62.5	0.06
Sex, % male	18.2	27.8	24.4	29.3	0.05
Race, % Caucasian	98.4	98.5	98.6	98.3	0.91
Body mass index, kg/m^2	25.8	25.8	25.5	25.0	<0.001
Overweight or obese, %	52.0	53.0	49.4	44.5	<0.01
Current smoking, %	11.7	8.4	9.0	9.3	0.20
Physical activity, MET-hours/week	25.4	25.5	25.2	23.9	0.28
Hypertension, %	24.1	20.0	23.2	19.5	0.15
Hypercholesterolemia, %	35.2	30.6	28.4	26.3	<0.001
Parental MI before 60 y, %	17.4	15.2	15.7	13.2	0.04
Family history of diabetes, %	24.2	25.3	24.4	24.1	0.87
Total dairy, servings/day	1.9	2.1	2.2	2.2	<0.001
Whole fat dairy, servings/day	0.77	0.94	1.01	1.10	<0.001
Low fat dairy, servings/day	1.2	1.2	1.1	1.1	0.14
Processed meats, servings/day	0.25	0.24	0.29	0.25	0.44
Unprocessed meats, servings/day	0.92	0.93	0.91	0.91	0.19
Fruits, servings/day	1.7	1.8	1.7	1.8	0.03
Vegetables, servings/day	4.0	4.0	3.9	3.9	0.17
Fish, servings/day	0.29	0.31	0.28	0.28	0.20
Alcohol, servings/day	0.98	0.67	0.58	0.47	<0.001

	Quartiles of t-16:1n-7				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.12	0.15	0.19	0.25	-
Age, years	61.8	61.7	62.0	63.0	<0.01
Sex, % male	17.0	18.5	23.0	37.8	<0.001
Race, % Caucasian	98.6	98.5	98.6	98.2	0.61
Body mass index, kg/m^2	25.9	25.5	25.5	25.2	<0.001
Overweight or obese, %	54.4	49.2	49.2	46.2	<0.01
Current smoking, %	9.4	12.0	8.6	8.5	0.17
Physical activity, MET-hours/week	25.8	24.9	24.7	24.6	0.54
Hypertension, %	22.0	21.6	20.8	22.2	0.87
Hypercholesterolemia, %	34.8	30.0	27.8	28.1	0.01
Parental MI before 60 y, %	15.5	15.6	14.6	15.6	0.98
Family history of diabetes, %	26.4	22.6	25.3	23.5	0.34
Total dairy, servings/day	1.9	2.0	2.2	2.3	<0.001
Whole fat dairy, servings/day	0.71	0.86	1.01	1.21	<0.001
Low fat dairy, servings/day	1.2	1.2	1.2	1.1	<0.001
Processed meats, servings/day	0.26	0.25	0.28	0.25	0.48
Unprocessed meats, servings/day	0.95	0.93	0.92	0.88	<0.001
Fruits, servings/day	1.8	1.8	1.8	1.7	0.13
Vegetables, servings/day	4.1	3.9	3.9	3.9	0.49
Fish, servings/day	0.31	0.30	0.28	0.27	<0.01
Alcohol, servings/day	0.87	0.74	0.64	0.48	<0.001

	Quartiles of 14:0				
	Q1	Q2	Q3	Q4	P-trend
Median, % of total fatty acids	0.24	0.42	0.61	0.94	-
Age, years	61.6	61.9	62.9	62.2	0.11
Sex, % male	12.1	21.5	27.2	37.6	0.02
Race, % Caucasian	98.5	97.9	98.6	98.8	0.30
Body mass index, kg/m^2	24.9	25.4	25.9	25.9	<0.001
Overweight or obese, %	42.1	45.8	53.1	56.3	<0.001
Current smoking, %	6.4	9.7	10.1	11.9	<0.01
Physical activity, MET-hours/week	24.2	25.6	24.6	25.5	0.56
Hypertension, %	20.3	20.9	22.6	22.6	0.38
Hypercholesterolemia, %	29.3	28.6	30.7	31.4	0.46
Parental MI before 60 y, %	16.2	16.9	15.2	13.4	0.16
Family history of diabetes, %	22.9	23.0	24.2	27.7	0.05
Total dairy, servings/day	2.1	2.0	2.1	2.2	<0.01
Whole fat dairy, servings/day	0.88	0.89	0.95	1.08	<0.01
Low fat dairy, servings/day	1.2	1.1	1.2	1.1	0.55
Processed meats, servings/day	0.27	0.26	0.26	0.25	0.19
Unprocessed meats, servings/day	0.94	0.93	0.92	0.89	<0.01
Fruits, servings/day	1.7	1.7	1.8	1.7	0.94
Vegetables, servings/day	4.1	4.0	3.9	3.8	0.01
Fish, servings/day	0.30	0.30	0.29	0.27	0.01
Alcohol, servings/day	0.76	0.76	0.69	0.51	<0.001

Table S3. Partial Spearman correlations between plasma fatty acid biomarkers of dairy fat and dietary factors among 3,333 participants in the Nurses' Health Study and Health Professionals Follow-Up Study.*

	Plasma fatty acids			
	15:0	**17:0**	**t-16:1n-7**	**14:0**
Dietary Factors†				
Dairy fat	0.29	0.21	0.22	0.11
Whole-fat dairy	0.26	0.19	0.24	0.06
Low-fat dairy	0.13	0.08	0.06	0.04
Sugar-sweetened beverages	0.07	0.03	0.07	0.04
Refined grains	0.07	0.04	0.01	0.03
Sweets/desserts	0.03	0.02	0.08	-0.00
Alcohol	-0.17	-0.26	-0.10	0.02
French fries	0.00	0.01	0.06	-0.01
Potato (baked, boiled, mashed)	-0.01	0.00	0.00	-0.00
Potato or corn chips	-0.04	-0.05	-0.01	-0.02
Processed meat	0.01	0.07	0.07	-0.01
Unprocessed meat	0.01	0.08	0.08	-0.05
Plasma Fatty Acids‡				
15:0	1.0	0.57	0.50	0.59
17:0		1.0	0.50	0.10
t-16:1n-7			1.0	0.20
14:0				1.0

*Values are adjusted Spearman correlations based on pooling of individual-level data from both cohorts.

†Dietary habits were assessed using the average of self-reported consumption in 1986 and 1990 in the NHS, and 1990 and 1994 in the HPFS. Correlations with diet (N=2,717 due to missing dietary questionnaire data in some participants) were adjusted for age (years), sex, body mass index (kg/m^2), smoking (never, current, former, missing), fasting status at blood draw, consumption of total energy (kcal/day), and each of the other dietary factor in the table simultaneously. Dairy fat was excluded as a covariate when evaluating whole-fat or low-fat dairy foods.

‡Fatty acid intercorrelations (N=3,289) were adjusted for age and sex.

Table S4. Partial Spearman correlations between red blood cell fatty acids and dietary factors among 3,289 women and men in the Nurses' Health Study and Health Professionals Follow-Up Study.*

	Erythrocyte fatty acids			
	15:0	**17:0**	**t-16:1n-7**	**14:0**
Dietary factors†				
Dairy fat	0.16	0.11	0.20	0.10
Whole-fat dairy	0.16	0.13	0.21	0.08
Low-fat dairy	0.06	0.04	0.07	0.02
Sugar-sweetened beverages	0.04	0.02	0.04	0.04
Refined grains	0.03	-0.01	0.00	0.02
Sweets/desserts	0.01	0.01	0.06	0.01
Alcohol	-0.10	-0.17	-0.09	0.02
French fries	0.00	0.03	0.02	-0.01
Potatoes (baked, boiled, mashed)	0.01	0.01	0.04	-0.01
Potato or corn chips	-0.03	-0.04	-0.02	-0.02
Processed meat	0.03	0.07	0.06	0.02
Unprocessed meat	0.01	0.06	0.05	0.00
Erythrocyte fatty acids‡				
15:0	1.0	0.63	0.63	0.68
17:0		1.0	0.69	0.47
t-16:1n-7			1.0	0.48
14:0				1.0

*Values are adjusted Spearman correlations based on pooling of individual-level data from both cohorts. Compared with plasma fatty acids (N=3,333), 44 fewer subjects (N=3,289) had successful measures of erythrocyte fatty acids.

†Dietary habits were assessed using the average of self-reported consumption in 1986 and 1990 in the NHS, and 1990 and 1994 in the HPFS. Correlations with diet (N=2,717 due to missing dietary questionnaire data in some participants) were adjusted for age (years), sex, body mass index (kg/m^2), smoking (never, current, former, missing), fasting status at blood draw, consumption of total energy (kcal/day), and each of the other dietary factor in the table simultaneously. Dairy fat was excluded as a covariate when evaluating whole-fat or low-fat dairy foods.

‡Fatty acid intercorrelations (N=3,289) were adjusted for age and sex.

Table S5. Risk of incident diabetes according to red blood cell fatty acid biomarkers of dairy fat consumption among 3,289 men and women in the NHS (N=179 cases), HPFS (N=97 cases), and both cohorts combined.

	Cohort-specific fatty acid quartiles				
Fatty acid	**1**	**2**	**3**	**4**	**P for trend***
15:0, NHS					
% of total FA, median	0.08	0.11	0.14	0.18	
No. of cases	43	38	53	45	
Person-months	85,143	85,284	89,701	99,253	
Multivariable hazard ratio (95%CI) †	Reference	0.73 (0.46-1.16)	0.93 (0.60-1.43)	0.65 (0.39-1.09)	0.20
15:0, HPFS					
% of total FA, median	0.07	0.09	0.12	0.16	
No. of cases	20	21	26	30	
Person-months	58,160	57,184	60,933	57,792	
Multivariable hazard ratio (95%CI)	Reference	0.87 (0.46-1.65)	1.09 (0.58-2.05)	1.27 (0.65-2.48)	0.35
15:0, pooled	**Reference**	**0.78 (0.53-1.13)**	**0.98 (0.68-1.40)**	**0.83 (0.55-1.25)**	**0.63**
17:0, NHS					
% of total FA, median	0.31	0.37	0.42	0.59	
No. of cases	65	46	23	45	
Person-months	84,278	90,276	85,591	99,236	
Multivariable hazard ratio (95%CI)	Reference	0.66 (0.44-0.98)	0.30 (0.18-0.49)	0.37 (0.20-0.66)	<0.001
17:0, HPFS					
% of total FA, median	0.29	0.34	0.38	0.48	
No. of cases	25	23	19	30	
Person-months	58,563	54,066	61,510	59,930	
Multivariable hazard ratio (95%CI)	Reference	0.88 (0.49-1.61)	0.54 (0.28-1.04)	0.99 (0.47-2.09)	0.90
17:0, pooled	**Reference**	**0.72 (0.52-1.01)**	**0.37 (0.25-0.56)**	**0.54 (0.34-0.87)**	**<0.001**
t-16:1n-7, NHS					
% of total FA, median	0.11	0.14	0.17	0.22	
No. of cases	47	52	35	45	
Person-months	83,002	86,211	89,095	101,073	
Multivariable hazard ratio (95%CI)	Reference	1.03 (0.69-1.54)	0.65 (0.41-1.02)	0.60 (0.36-1.02)	0.02

t-16:1n-7, HPFS					
% of total FA, median	0.09	0.12	0.14	0.18	
No. of cases	20	26	19	32	
Person-months	58,671	57,636	57,138	60,624	
Multivariable hazard ratio (95%CI)	Reference	1.31 (0.72-2.39)	0.82 (0.42-1.59)	1.21 (0.61-2.39)	0.80
t-16:1n-7, pooled	**Reference**	**1.11 (0.80-1.55)**	**0.70 (0.48-1.02)**	**0.78 (0.51-1.18)**	**0.05**
14:0, NHS					
% of total FA, median	0.11	0.20	0.31	0.64	
No. of cases	32	50	37	60	
Person-months	80,336	85,653	92,087	101,305	
Multivariable hazard ratio (95%CI)	Reference	1.18 (0.74-1.87)	0.76 (0.45-1.29)	1.08 (0.61-1.88)	0.82
14:0, HPFS					
% of total FA, median	0.12	0.21	0.31	0.53	
No. of cases	14	20	30	33	
Person-months	57,996	58,816	60,816	56,441	
Multivariable hazard ratio (95%CI)	Reference	1.49 (0.70-3.14)	2.29 (1.10-4.78)	3.43 (1.51-7.77)	0.001
14:0, pooled	**Reference**	**1.26 (0.85-1.87)**	**1.11 (0.72-1.70)**	**1.56 (0.98-2.49)**	**0.13**

*Computed within each cohort by assigning the median level in each quartile to participants and evaluating this as a continuous variable. The pooled P-for-trend was calculated using generalized least squares trend (GLST) meta-analysis.[10]

†Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).

Table S6. Risk of incident diabetes according to red blood cell fatty acid biomarkers, evaluated continuously, among 3,289 men and women in the Nurses' Health Study (N=179 cases), Health Professionals Follow-Up Study (N=97 cases), and both cohorts combined.

Fatty acids	Results standardized to the difference between the midpoints of the highest vs. lowest quartiles (87.5th minus 12.5th percentiles)			
	NHS	**HPFS**	**Pooled**	**P-value**
15:0	range = 0.10*	range = 0.10		
Multivariable HR (95%CI) †	0.75 (0.50-1.12)	1.33 (0.98-1.80)	**1.08 (0.85-1.38)**	**0.53**
+ BMI ‡	0.73 (0.48-1.12)	1.37 (1.01-1.86)	**1.11 (0.86-1.42)**	**0.43**
17:0	range = 0.28	range = 0.19		
Multivariable HR (95%CI)	0.42 (0.26-0.68)	0.66 (0.29-1.53)	**0.47 (0.31-0.71)**	**<0.001**
+ BMI	0.57 (0.31-1.04)	0.88 (0.38-2.06)	**0.66 (0.40-1.08)**	**0.10**
t-16:1n-7	range = 0.11	range = 0.09		
Multivariable HR (95%CI)	0.76 (0.49-1.17)	0.87 (0.50-1.53)	**0.80 (0.57-1.13)**	**0.20**
+ BMI	0.86 (0.55-1.35)	0.95 (0.53-1.70)	**0.89 (0.63-1.27)**	**0.53**
14:0	Range = 0.53	Range = 0.41		
Multivariable HR (95%CI)	1.23 (0.98-1.54)	2.11 (1.31-3.39)	**1.36 (1.11-1.67)**	**0.003**
+ BMI	1.16 (0.91-1.46)	2.11 (1.30-3.42)	**1.30 (1.05-1.61)**	**0.02**

*The difference in % of total fatty acids between the midpoint of the highest vs. lowest quartile.

† Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).

‡ Further adjusted for body mass index (BMI, kg/m^2) as a potential mediator of confounder of the association.

Table S7. Risk of incident diabetes according to plasma fatty acid biomarkers of dairy fat after further adjustment for self-reported consumption of yogurt, cheese, or dairy fat as covariates in the NHS (N=184 cases), HPFS (N=93 cases), and both cohorts combined.

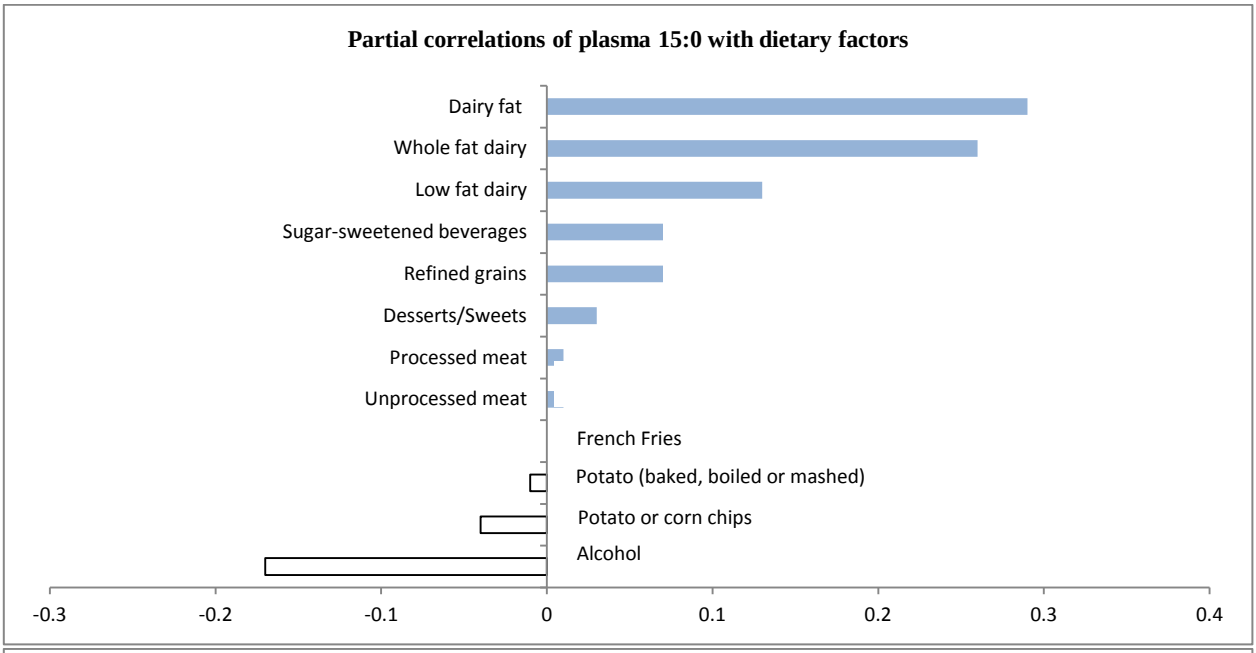
	Multivariable HR (95%CI) per increase in difference of the midpoint of the highest versus lowest quartiles (87.5^{th} minus 12.5^{th} percentiles)			
	Main results	Adding yogurt	Adding cheese	Adding dairy fat
15:0				
NHS	0.63 (0.42-0.92)	0.63 (0.43-0.94)	0.63 (0.42-0.93)	0.63 (0.42-0.94)
HPFS	0.61 (0.36-1.03)	0.62 (0.36-1.06)	0.60 (0.35-1.03)	0.56 (0.32-0.97)
Pooled	**0.62 (0.46-0.85)**	**0.63 (0.46-0.86)**	**0.62 (0.45-0.85)**	**0.61 (0.44-0.84)**
17:0				
NHS	0.71 (0.49-1.04)	0.72 (0.49-1.05)	0.72 (0.49-1.04)	0.72 (0.50-1.05)
HPFS	0.63 (0.39-1.02)	0.64 (0.39-1.03)	0.63 (0.39-1.02)	0.60 (0.37-0.99)
Pooled	**0.68 (0.50-0.91)**	**0.69 (0.51-0.93)**	**0.68 (0.51-0.92)**	**0.67 (0.50-0.91)**
t-16:1n-7				
NHS	0.52 (0.36-0.76)	0.53 (0.36-0.76)	0.52 (0.36-0.76)	0.53 (0.36-0.77)
HPFS	0.58 (0.36-0.93)	0.57 (0.35-0.92)	0.55 (0.34-0.90)	0.49 (0.30-0.82)
Pooled	**0.54 (0.40-0.73)**	**0.55 (0.41-0.73)**	**0.53 (0.40-0.71)**	**0.52 (0.38-0.70)**
14:0				
NHS	0.92 (0.64-1.32)	0.94 (0.65-1.35)	0.92 (0.64-1.33)	0.93 (0.64-1.34)
HPFS	0.61 (0.35-1.09)	0.63 (0.35-1.11)	0.61 (0.34-1.08)	0.56 (0.31-1.01)
Pooled	**0.82 (0.60-1.11)**	**0.84 (0.62-1.14)**	**0.82 (0.60-1.11)**	**0.81 (0.59-1.10)**

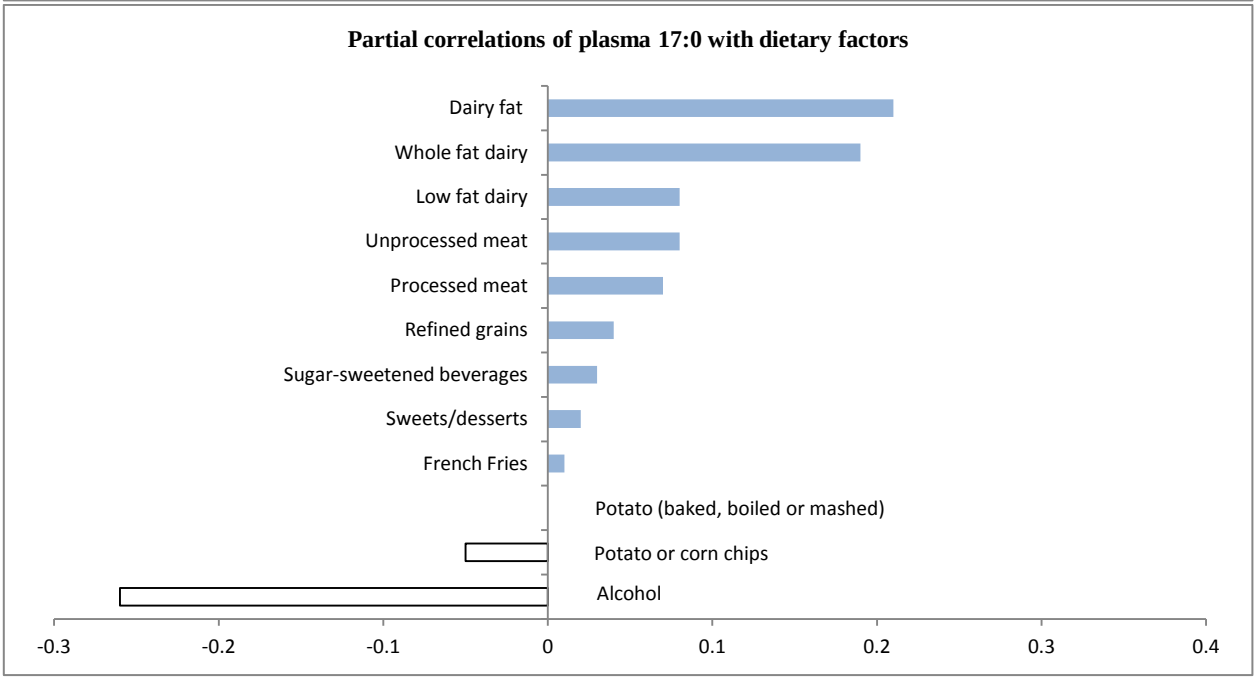
Adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).

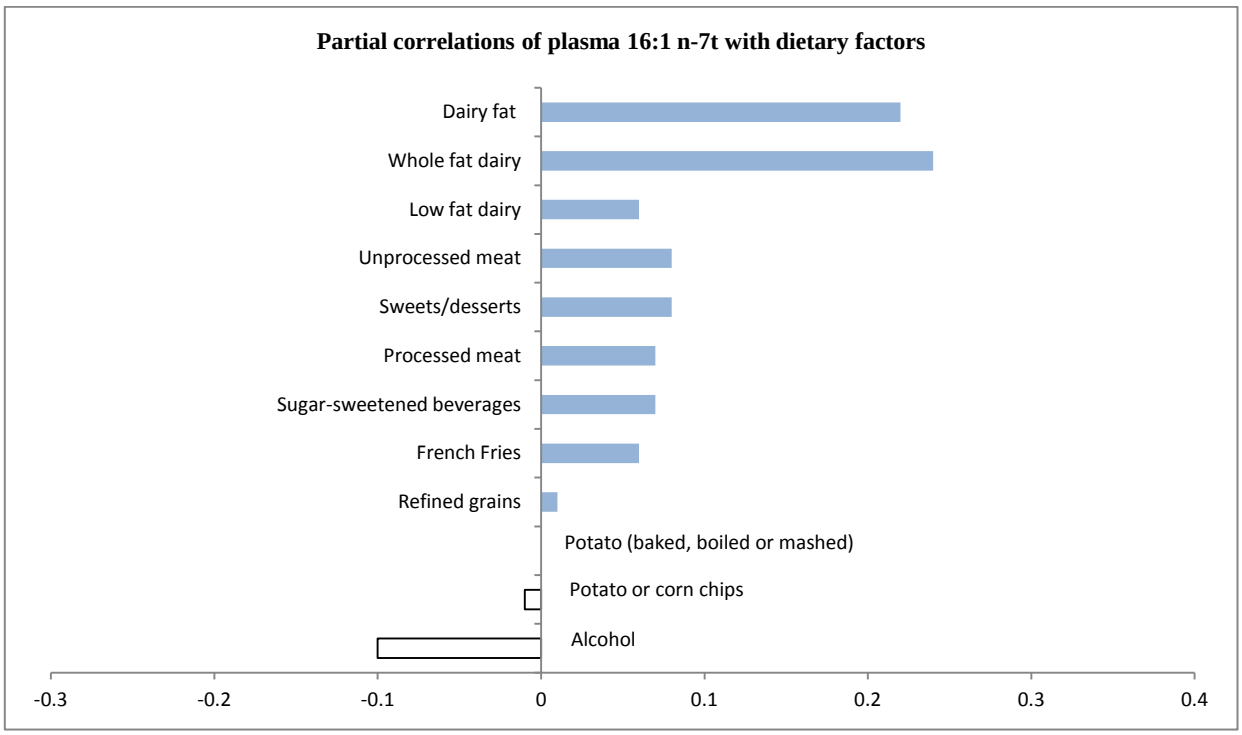
Table S8. Risk of incident diabetes according to plasma fatty acid biomarkers of dairy fat in sensitivity analyses excluding cases in the first 2 years of follow-up and restricting to the first 8 years of follow-up in the NHS (N=172 and 65 cases, respectively), HPFS (N=84 and 50 cases, respectively), and both cohorts combined.

	Results standardized to the difference between the midpoints of the highest vs. lowest quartiles (87.5th minus 12.5th percentiles)		
Fatty Acids	**Full follow-up**	**Excluding cases in the first 2 years**	**Restricting to the first 8 years**
15:0			
NHS	0.63 (0.42-0.92)	0.65 (0.43-0.97)	0.56 (0.29-1.06)
HPFS	0.61 (0.36-1.03)	0.76 (0.45-1.29)	0.71 (0.36-1.40)
Pooled	**0.62 (0.46-0.85)**	**0.69 (0.50-0.95)**	**0.63 (0.39-1.00)**
17:0			
NHS	0.71 (0.49-1.04)	0.75 (0.51-1.10)	0.66 (0.35-1.23)
HPFS	0.63 (0.39-1.02)	0.72 (0.45-1.17)	0.75 (0.40-1.39)
Pooled	**0.68 (0.50-0.91)**	**0.74 (0.55-1.00)**	**0.70 (0.45-1.10)**
t-16:1n-7			
NHS	0.52 (0.36-0.76)	0.58 (0.40-0.84)	0.28 (0.15-0.54)
HPFS	0.58 (0.36-0.93)	0.66 (0.39-1.09)	0.59 (0.31-1.12)
Pooled	**0.54 (0.40-0.73)**	**0.61 (0.45-0.82)**	**0.41 (0.26-0.64)**
14:0			
NHS	0.92 (0.64-1.32)	0.86 (0.59-1.25)	1.08 (0.58-2.00)
HPFS	0.61 (0.35-1.09)	0.67 (0.37-1.20)	0.71 (0.34-1.48)
Pooled	**0.82 (0.60-1.11)**	**0.80 (0.58-1.10)**	**0.91 (0.57-1.46)**

Values are hazard ratio (95%CI) adjusted for age (years), race (white, nonwhite), smoking status (never, former, current, missing), physical activity (METS/week), alcohol (servings/day), family history of diabetes (yes, no, missing), parental history of MI (yes, no, missing), hypercholesterolemia (yes, no), hypertension (yes, no), menopausal status in NHS (pre, post), postmenopausal hormone use in NHS (no, yes, missing), and consumption of fish (servings/day), processed meats (servings/day), unprocessed meats (servings/day), fruits (servings/day), vegetables (servings/day), whole grains (g/day), coffee (servings/day), sugar-sweetened beverages (servings/day), glycemic load (continuous), dietary calcium (mg/day), polyunsaturated fat (g/day), total energy (kcal/day), and plasma trans-18:1, trans-18:2, 16:0, and 18:0 (each as % of total fatty acids).


Partial correlations of plasma 15:0 with dietary factors
Dairy fat
Whole fat dairy
Low fat dairy
Sugar-sweetened beverages
Refined grains
Desserts/Sweets
Processed meat
Unprocessed meat
French Fries
Potato (baked, boiled or mashed)
Potato or corn chips
Alcohol
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4


Partial correlations of plasma 17:0 with dietary factors
Dairy fat
Whole fat dairy
Low fat dairy
Unprocessed meat
Processed meat
Refined grains
Sugar-sweetened beverages
Sweets/desserts
French Fries
Potato (baked, boiled or mashed)
Potato or corn chips
Alcohol
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4



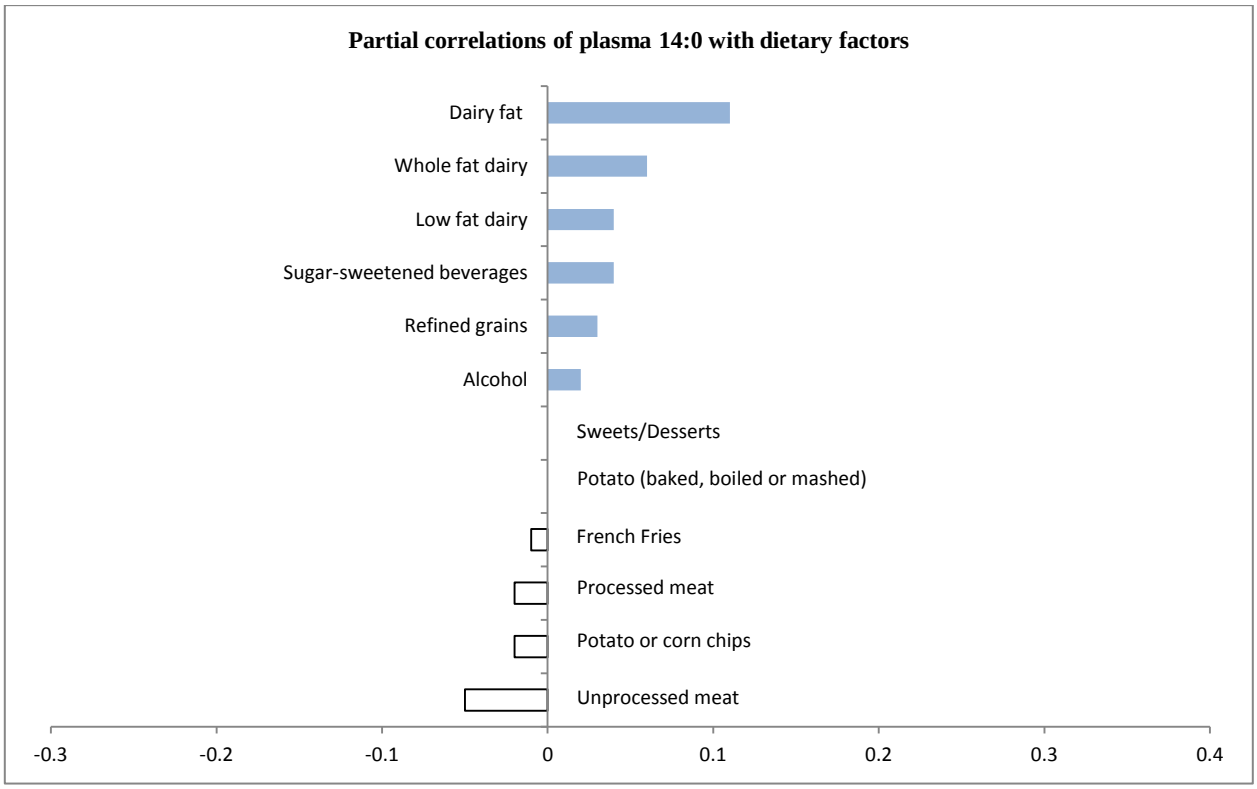


Figure S1. Partial Spearman correlations between plasma fatty acid biomarkers of dairy fat and dietary habits in the Nurses' Health Study and Health Professionals Follow-Up Study. Dietary habits assessed using the average of self-reported intake in 1986 and 1990 in NHS, and 1990 and 1994 in HPFS (total N=2,761). Correlations based on pooled individual-level data, adjusted for age (years), sex, body mass index (kg/m^2), smoking (never, current, former, missing), fasting status at blood draw, consumption of total energy (kcal/day), and each of the other dietary factor in the figure simultaneously. Dairy fat was excluded when evaluating whole-fat and low-fat dairy.

REFERENCES

1. Zhang L, Curhan GC, Hu FB, Rimm EB and Forman JP. Association between passive and active smoking and incident type 2 diabetes in women. *Diabetes Care*. 2011;34:892-7.
2. Wolf AM, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Corsano KA, Rosner B, Kriska A and Willett WC. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol*. 1994;23:991-9.
3. Chasan-Taber S, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E, Ascherio A and Willett WC. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology*. 1996;7:81-6.
4. Zhang X, Smith-Warner SA, Chan AT, Wu K, Spiegelman D, Fuchs CS, Willett WC and Giovannucci EL. Aspirin use, body mass index, physical activity, plasma C-peptide, and colon cancer risk in US health professionals. *Am J Epidemiol*. 2011;174:459-67.
5. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB and Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc*. 1993;93:790-6.
6. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B and Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol*. 1989;18:858-67.
7. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB and Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology*. 1990;1:466-73.
8. Ascherio A, Rimm EB, Giovannucci EL, Colditz GA, Rosner B, Willett WC, Sacks F and Stampfer MJ. A prospective study of nutritional factors and hypertension among US men. *Circulation*. 1992;86:1475-84.
9. Colditz GA, Martin P, Stampfer MJ, Willett WC, Sampson L, Rosner B, Hennekens CH and Speizer FE. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol*. 1986;123:894-900.
10. Orsini N, Bellocco R and Greenland S. Generalized least squares for trend estimation of summarized dose-response data. *Stata J*. 2006;6:40-57.