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Serum pentadecanoic acid (15:0), a short-term marker of dairy food intake, is inversely associated with incident type 2 diabetes and its underlying disorders[1–3]

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ABSTRACT

Background: Growing evidence suggests that dairy consumption is associated with lower type 2 diabetes risk. However, observational studies have reported inconsistent results, and few have examined dairy's association with the underlying disorders of insulin resistance and β-cell dysfunction.

Objective: We investigated the association of the dairy fatty acid biomarkers pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n$-$7) with type 2 diabetes traits by evaluating *1*) prospective associations with incident diabetes after 5 y of follow-up and *2*) cross-sectional associations with directly measured insulin resistance and β-cell dysfunction.

Design: The study analyzed 659 adults without diabetes at baseline from the triethnic multicenter Insulin Resistance Atherosclerosis Study (IRAS). Diabetes status was assessed by using oral-glucose-tolerance tests. Frequently sampled intravenous-glucose-tolerance tests measured insulin sensitivity (S_I) and β-cell function [disposition index (DI)]. Serum fatty acids were quantified by using gas chromatography. Logistic and linear regression models were adjusted for demographic, lifestyle, and dietary variables.

Results: Serum 15:0 was a significant biomarker for total dairy intake in the IRAS cohort. It was associated with a decreased incident diabetes risk (OR: 0.73, $P = 0.02$) and was positively associated with log S_I (β: 0.84, $P = 0.03$) and log DI (β: 2.21, $P = 0.02$) in fully adjusted models. *trans* 16:1n$-$7 was a marker of total partially hydrogenated dietary fat intake and was not associated with outcomes in fully adjusted models.

Conclusions: Serum 15:0, a marker of short-term intake of this fatty acid, was inversely associated with diabetes risk in this multiethnic cohort. This study may contribute to future recommendations regarding the benefits of dairy products on type 2 diabetes risk. *Am J Clin Nutr* 2014;100:1532–40.

Keywords dairy, epidemiology, fatty acids, nutrition, type 2 diabetes

INTRODUCTION

Type 2 diabetes, a growing global epidemic, arises from both nonmodifiable and modifiable factors (1). Among the many nutritional exposures that have been investigated in type 2 diabetes, dairy consumption is emerging as a potential protective factor. Three meta-analyses of prospective observational studies have reported that high dairy intake was associated with a lower risk of type 2 diabetes (2–4). However, findings have been inconsistent across individual studies (5–15), and only a limited number of investigations have assessed the relation of dairy intake with the main underlying pathophysiologic traits of type 2 diabetes (1)—namely, insulin resistance (14, 16–18) and β-cell dysfunction (14).

Most previous observational studies measured usual dairy consumption by using self-reported intake from food-frequency questionnaires (FFQs),[4] which are susceptible to misclassification error from under- or overreporting (19). A number of dairy bioactives may potentially underlie the associations described in previous studies, including the fatty acid profile, because dairy is a particularly rich source of SFAs and naturally occurring *trans* fatty acids (TFAs) (4, 20). Despite limited data, current literature

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[4] Abbreviations used: DI, disposition index; FFQ, food-frequency questionnaire; FSIGT, frequently sampled intravenous-glucose-tolerance test; IRAS, Insulin Resistance Atherosclerosis Study; S_I, insulin sensitivity index; TFA, *trans* fatty acid.

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suggests that intake of different types of SFAs and TFAs may affect metabolic and cardiovascular disease risk differently (21), and there is growing evidence that certain fatty acids, including those from dairy, may play a role in type 2 diabetes prevention (12). Certain fatty acid biomarkers have been validated as markers for dairy intake, including pentadecanoic acid (15:0) (22–27) and *trans*-palmitoleic acid (*trans* 16:1n−7), with fatty acids measured in serum reflecting short-term dietary intake (28–31). Using dairy-derived fatty acid biomarkers has the potential to provide more objective measures of dairy intake, and thus they may help to elucidate the role of dairy on the risk of type 2 diabetes and its underlying disorders.

The current study aimed to investigate the association between dairy biomarkers and type 2 diabetes traits in a large multiethnic cohort by evaluating *1*) prospective associations of dairy fatty acid biomarkers with incident diabetes after 5 y of follow-up and *2*) cross-sectional associations of dairy fatty acid biomarkers with directly measured insulin resistance and β-cell dysfunction by using frequently sampled intravenous-glucose-tolerance tests (FSIGTs). We hypothesized that pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n−7), independent of covariates, would be inversely associated with insulin resistance, β-cell dysfunction, and incident type 2 diabetes at 5 y.

SUBJECTS AND METHODS

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter epidemiologic study assessing the relation between insulin resistance and subclinical cardiovascular disease. The study consists of a triethnic cohort of 1625 Hispanic, African American, and non-Hispanic white adults aged 40–60 y across a range of glucose tolerance status. Participants were recruited from 4 clinical centers in San Antonio, Texas; San Luis Valley, Colorado; Los Angeles, California; and Oakland, California. Baseline study visits were conducted between October 1992 and April 1994, and 5-y follow-up examinations were conducted from February 1998 to July 1999, with an 81% response rate (32). Participants who did not attend the follow-up examinations were not systematically different in terms of age, sex, ethnicity, or clinic, except for a slightly lower educational attainment compared with those who returned for follow-up (33). Each study center received ethics approval from its institutional review board, and all participants gave informed consent. A comprehensive presentation of the study objectives, design, and recruitment results has been published previously (34).

The present analysis excluded participants with type 2 diabetes at baseline (n = 553) and participants who did not return for follow-up (n = 177). With further exclusions for missing insulin sensitivity and β-cell function measures, as well as 15:0, *trans* 16:1n−7, and total dairy intake values, the final study sample for the current analysis was 659.

Usual dietary intake over the previous year before baseline was assessed by using a 114-item FFQ modified from the National Cancer Institute's Health Habits and History Questionnaire to include ethnic and regional foods relevant to the study population (35). The validity and reproducibility of this FFQ were established in a subsample of 186 women from the IRAS population by using eight 24-h dietary recalls, followed by a second FFQ (35). Food and beverage intake in the FFQ was quantified through interviews in which participants were asked to recall the frequency of consumption of each food, or groups of foods, over the past year. The FFQ contained 9 frequency options, ranging from "never or less than once a month" to "6 or more times per day," and 3 portion sizes: "small, medium, or large compared with other men or women about your age." Servings per day were standardized to the medium serving size for the food intake analyses by multiplying the intake frequency with the portion size after applying a weighting factor (small = 0.5, medium = 1.0, and large = 1.5). One serving, therefore, corresponds to 1 medium-sized portion of the food or food group. Total dairy product intake was calculated by adding 11 dairy food line items from the FFQ: whole milk; 2% milk; skim milk, 1%, or buttermilk; cottage and ricotta cheese; cheese; flavored yogurt (2%, nonfat, or whole); low-fat flavored yogurt (2% or nonfat); ice cream; frozen yogurt or ice milk; milk in coffee or tea; and cream or half-and-half in coffee or tea. Total milk, total cheese, and total yogurt intakes were also calculated. Because *trans* 16:1n−7 may also be found in foods containing partially hydrogenated fats (29), total partially hydrogenated food intake was calculated by summing the following items from the FFQ: french fries and fried potatoes; salty snacks such as crackers, potato chips, corn chips, tortilla chips, and pretzels; margarine on bread or roll; doughnuts; cookies; cakes; pastry; brownies; sopapillas; and pan dulce. A similar approach for summing sources of hydrogenated fats was recently used by Mozaffarian et al. (29). Nutrient and energy intakes were estimated from the FFQ by using a nutrient database (HHHQ-DIETSYS analysis software, version 3.0; National Cancer Institute, 1993), expanded for additional nutrients.

Clinical examinations were conducted at baseline and follow-up during two 4-h visits, which were administered 1 wk to 30 d apart. Before each clinic visit, participants were asked to fast for 12 h and refrain from heavy exercise and alcohol consumption for 24 h, as well as smoking the morning of the visit. All participants received an oral glucose tolerance test to determine glucose tolerance status (normal, impaired glucose tolerance, or diabetes), based on the 2010 American Diabetes Association criteria for fasting or 2-h postload glucose concentrations, and oral hypoglycemic agent or insulin use (36). FSIGTs were administered following a validated modified protocol (34, 37). Insulin resistance was calculated by using minimal-model analysis (MINMOD, version 3.0, 1994) (38) and expressed as the insulin sensitivity index (S_I). Insulin secretion was assessed via acute insulin response, a sensitivity index of β-cell function measured as the mean plasma insulin concentration from 2- to 4-min time points after the initial glucose administration (34). The product of S_I and acute insulin response yields the disposition index (DI), an integrated measure of β-cell function reflecting the ability of β cells to compensate for insulin resistance by up-regulating insulin secretion (39). DI was used as the measure of β-cell function for this investigation.

Waist circumference and height were measured to the nearest 0.5 cm, and body weight was measured to the nearest 0.1 kg, with the average of the duplicate measurements used in all analyses. A validated physical activity recall was used to determine total estimated energy expenditure over the past year (34). Total estimated energy expenditure (kcal/kg per wk) was calculated by summing energy expenditure activities and energy expenditure from sleep. Smoking status was categorized into 3 groups: never,

past, or current. Total usual alcohol intake (g ethanol consumed/d) was evaluated through a separate questionnaire with additional questions about recent use and average lifetime use. Race/ethnicity and age were self-reported. Medical history was assessed by using structured interviews.

Fatty acid analysis

A complete quantitative profile of fatty acids was extracted from participants' serum samples (which were stored at −70°C) by using methods previously described (Lipomics Technologies Inc.) (40). Briefly, total lipids were extracted in the presence of internal standards according to the method of Folch et al. (41). Fatty acid methyl esters were formed by transesterification of total lipid extracts in sulfuric acid/methanol and were then extracted into hexane and prepared for gas chromatography. Capillary gas chromatography (model 6890; Agilent Technologies) equipped with a 30-m HP-88 capillary column (Agilent Technologies) and a flame ionization detector were used to separate and quantify individual fatty acids. The absolute concentration of each fatty acid in the serum sample was measured by comparing its peak area with the internal standard. A total of 35 serum fatty acids were analyzed and quantified by using these methods. For this study, dairy-derived fatty acids pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n−7) are the exposures of interest and are expressed as the mole percentage (mol%) of total fatty acids.

Statistical analysis

Baseline characteristics across quintiles of increasing total dairy intake (servings/wk) are presented. Normally distributed variables are presented as means ± SDs, nonnormally distributed variables are presented as medians (IQRs), and categorical variables are presented as number and percentage of participants in each quintile, with differences across quintiles tested by using ANOVA, Kruskal-Wallis tests, and χ^2 tests, respectively.

Dietary determinants of dairy fatty acids in serum were assessed by analyzing the correlations of 15:0 and *trans* 16:1n−7 with total dairy, total milk, total cheese, total yogurt, and total partially hydrogenated food intakes from the FFQ. Furthermore, multivariable-adjusted linear regressions with 15:0 and *trans* 16:1n−7 as the outcome variable were conducted to assess the contribution of total dairy and total partially hydrogenated food intake to serum concentrations of these dairy fatty acids. The regressions were iteratively adjusted for model 1 (age, sex, and ethnicity), model 2 (physical activity and total energy intake), model 3 (total dairy or total hydrogenated food intake), and model 4 (BMI).

Logistic regression analysis was used to evaluate the prospective associations between dairy fatty acids and incident diabetes at 5 y, with sequential adjustment in 3 models. Model 1 was adjusted for demographic variables: age, sex, ethnicity, and center. Model 2 was also adjusted for lifestyle variables: physical activity, smoking status, alcohol intake, and education. Model 3 was adjusted for dietary variables: total energy, fruit and vegetable, red meat, soft drink, and fiber intakes. On the basis of significant Spearman correlations of BMI and waist circumference with 15:0 and the outcome variables, we included these measures of adiposity in additional mechanistic models because these variables are likely on the etiologic pathway between the fatty acid exposures and outcomes. In the cross-sectional study, univariate analyses between outcomes and exposures were conducted by using Spearman's correlations (r). Multiple linear regression analysis was used to assess the cross-sectional association between the dairy fatty acids with insulin resistance (S_I) and β-cell dysfunction (DI), adjusted for the same covariates in the logistic regressions. Both S_I and DI were skewed, and thus these outcome variables were log transformed to achieve normality. Because some participants had an S_I of 0, a constant of 1 was added to the values before being log transformed. Subgroup analyses on a priori variables of interest, including sex, glucose tolerance status, and ethnicity, were conducted for both logistic and linear regressions. Formal tests of interaction for these variables were carried out by using cross-product terms and considered statistically significant when $P < 0.05$. The assumption of linearity of the associations in the logistic and linear regressions was tested by adding quadratic terms to the models. None of the quadratic terms was statistically significant (all $P > 0.05$), and thus we concluded that the linearity assumption had not been violated.

We conducted additional analyses by using increasing tertiles of 15:0 and *trans* 16:1n−7 as the independent variable. In the prospective analyses, we used logistic regression adjusted for the models previously described. For the cross-sectional analyses, we conducted ANOVA, followed by Tukey's post hoc test, to compare the least squares means of log S_I and log DI in each fatty acid tertile, adjusted for the models mentioned previously.

All analyses were performed by using SAS 9.2 (SAS Institute Inc.). $P < 0.05$ was considered significant for all analyses.

RESULTS

Baseline characteristics of participants across quintiles of increasing total dairy intake (servings/wk) are presented in **Table 1**. There were no differences in age, sex, and educational levels across these categories. Ethnicity differed significantly across quintiles of total dairy intake, with non-Hispanic whites and African Americans having the highest and lowest dairy intakes, respectively. Glucose tolerance status also differed across quintiles, such that the highest percentage of participants with impaired glucose tolerance was in quintile 1, although S_I and DI did not significantly differ across quintiles. Total energy intake, percent energy from SFAs, dietary intake of TFAs, and serum 15:0 and *trans* 16:1n−7 significantly increased across quintiles.

Total intakes of dairy (r = 0.20, $P < 0.0001$), milk (r = 0.13, P = 0.0006), and cheese (r = 0.16, $P < 0.0001$) were positively correlated with 15:0. *trans* 16:1n−7 was not significantly correlated with either 15:0 or reported intakes of dairy foods (data not shown). In contrast, intake of total partially hydrogenated foods, another dietary source of *trans* 16:1n−7, was positively and significantly correlated with serum *trans* 16:1n−7 (r = 0.18, $P < 0.0001$). Linear regression analysis showed that 15:0 in serum was independently and positively associated with total dairy intake, whereas *trans* 16:1n−7 was negatively associated with total dairy intake (**Table 2**). Conversely, 15:0 was negatively and *trans* 16:1n−7 was positively associated with total partially hydrogenated food intake (**Table 3**).

In the prospective analysis, 103 of 659 participants developed diabetes after 5 y of follow-up. In multivariate logistic regression,

TABLE 1
Baseline characteristics of the Insulin Resistance Atherosclerosis Study participants (n = 659) overall and across quintiles of total dairy intake from the food-frequency questionnaire[1]

Characteristic	All	Total dairy intake, servings/wk[2]					P value[3]
		Q1 (0–2.07)	Q2 (2.10–4.10)	Q3 (4.13–6.65)	Q4 (6.69–9.73)	Q5 (9.80–31.08)	
Participants, n (%)	659	132 (20.03)	131 (19.88)	133 (20.18)	132 (20.03)	131 (19.88)	
Age, y	54.68 ± 8.56	54.74 ± 8.84	55.52 ± 8.27	53.69 ± 7.96	55.99 ± 9.24	53.50 ± 8.27	0.07
Sex, n (%)							0.16
Male	297 (45.07)	61 (46.21)	59 (45.04)	60 (45.11)	69 (52.27)	48 (36.64)	
Female	362 (54.93)	71 (53.79)	72 (54.96)	73 (54.89)	63 (47.73)	83 (93.36)	
Ethnicity, n (%)							<0.0001
Non-Hispanic white	277 (42.03)	36 (27.27)	50 (38.17)	56 (42.11)	66 (50.00)	69 (52.67)	
African American	152 (23.07)	55 (41.67)	32 (24.43)	28 (21.05)	16 (12.12)	21 (16.03)	
Hispanic	230 (34.90)	41 (31.06)	49 (37.40)	49 (36.84)	50 (37.88)	41 (31.30)	
Glucose tolerance status, n (%)							0.04
Normal glucose tolerance	447 (67.83)	81 (61.36)	93 (70.99)	103 (77.44)	85 (64.39)	85 (64.89)	
Impaired glucose tolerance	212 (32.17)	51 (38.64)	38 (29.01)	30 (22.56)	47 (35.61)	46 (35.11)	
Smoking status, n (%)							0.0009
Never	306 (46.43)	48 (36.36)	59 (45.04)	65 (48.87)	53 (40.15)	81 (61.83)	
Past	259 (39.30)	55 (41.67)	53 (40.46)	56 (42.11)	58 (43.94)	37 (28.24)	
Current	94 (14.26)	29 (21.97)	19 (14.50)	12 (9.02)	21 (15.91)	13 (9.92)	
Alcohol intake category, g ethanol/wk	3.52 (0–39.54)	2.24 (0–34.35)	7.26 (0–57.82)	0 (0–15.47)	8.86 (0–63.92)	3.52 (0–25.92)	0.007
Highest educational level completed, n (%)							0.48
<12 y	87 (13.20)	21 (15.91)	16 (12.21)	12 (9.02)	20 (15.15)	18 (13.74)	
≥12 y	572 (86.80)	111 (84.09)	115 (87.79)	121 (90.98)	112 (84.85)	113 (86.26)	
BMI, kg/m^2	27.24 (24.83–27.24)	27.24 (24.81–29.99)	26.58 (24.10–29.33)	28.09 (25.56–30.80)	27.20 (24.77–29.55)	27.71 (24.75–33.07)	0.04
Waist circumference, cm	90.28 ± 12.52	90.53 ± 12.74	87.87 ± 11.79	89.92 ± 11.06	91.53 ± 11.57	91.59 ± 14.92	0.10
Total estimated energy expenditure, kcal/kg per wk	272.28 (250.88–305.33)	272.03 (247.84–304.72)	270.05 (251.20–303.87)	267.22 (249.16–295.37)	284.22 (255.35–320.87)	272.96 (251.64–309.56)	0.05
Insulin sensitivity, $\times 10^{-4}$ min^{-1} (μU/mL)$^{-1}$	1.71 (0.95–3.01)	1.73 (0.98–2.80)	1.92 (1.00–3.25)	1.73 (1.00–3.11)	1.53 (0.92–2.50)	1.65 (0.85–3.16)	0.47
Acute insulin response, μU/mL	51.5 (29.5–87.0)	50.50 (30.00–92.25)	51.0 (24.5–93.0)	60.0 (36.0–84.0)	49.5 (30.5–82.0)	49.5 (25.0–81.5)	0.49
Disposition index	85.68 (43.44–154.59)	103.86 (43.10–151.16)	89.70 (48.02–149.49)	98.05 (57.38–179.41)	66.28 (41.22–140.27)	76.44 (36.25–153.00)	0.07
Dietary variables							
Total energy intake, kcal/wk	12,416.84 (8999.48–16,181.69)	9559.99 (7181.22–13,685.13)	11,457.43 (7858.01–13,914.89)	12,257.03 (9288.20–15,780.76)	13,505.43 (10,679.84–17,341.15)	15,005.27 (11,425.64–19,989.34)	<0.0001
% Energy from fat	35.44 ± 8.44	34.68 ± 8.66	35.62 ± 9.51	35.64 ± 8.84	34.94 ± 7.91	36.29 ± 7.14	0.57
% Energy from SFA	12.05 ± 3.39	11.18 ± 3.42	11.74 ± 3.66	12.17 ± 3.31	12.25 ± 3.25	12.88 ± 3.08	0.001
Dietary intake TFA, g/wk	15.05 (7.56–25.90)	11.69 (6.09–21.00)	12.67(6.23–22.19)	14.81 (9.57–22.54)	16.59 (8.62–29.89)	19.75 (11.83–33.32)	<0.0001
Total dairy consumption, servings/wk	5.53 (2.59–8.75)	0.96 (0.35–1.54)	3.05 (2.59–3.54)	5.53 (4.80–6.13)	8.05 (7.28–8.75)	12.46 (11.06–15.23)	<0.0001
Total cheese consumption, servings/wk	1.51 (0.49–3.26)	0.28 (0–0.58)	0.98 (0.32–1.96)	1.58 (0.77–3.01)	2.24 (0.98–3.89)	3.5 (1.96–7.00)	<0.0001
Total milk consumption, servings/wk	0.98 (0–3.50)	0 (0-0.21)	0.56 (0–0.98)	0.98 (0.21–3.50)	2.54 (0.56–4.66)	3.71 (1.75–7.00)	<0.0001

(Continued)

TABLE 1 (*Continued*)

Characteristic	All	Total dairy intake, servings/wk[2]					P value[3]
		Q1 (0–2.07)	Q2 (2.10–4.10)	Q3 (4.13–6.65)	Q4 (6.69–9.73)	Q5 (9.80–31.08)	
Total yogurt consumption, servings/wk	0.210 (0–1.12)	0 (0–0.21)	0.11 (0–1.05)	0.21 (0–1.12)	0.56 (0–2.52)	0.88 (0–3.82)	<0.0001
Serum dairy fatty acid, mol% of total fatty acids							
Pentadecanoic acid (15:0)	0.25 ± 0.06	0.23 ± 0.05	0.24 ± 0.05	0.26 ± 0.05	0.25 ± 0.06	0.26 ± 0.07	<0.0001
Trans-palmitoleic acid (*trans* 16:1n−7)	0.30 ± 0.10	0.30 ± 0.11	0.32 ± 0.10	0.32 ± 0.09	0.30 ± 0.10	0.29 ± 0.09	0.02

[1]Total dairy intake was defined as sum of whole milk; 2% milk; skim milk, 1%, or buttermilk; cottage and ricotta cheese; cheese; flavored yogurt (2%, nonfat, or whole); low-fat flavored yogurt (2% or nonfat); ice cream; frozen yogurt, ice milk; milk in coffee or tea; and cream or half-and-half in coffee or tea. Continuous variables are presented as means ± SDs if normal or medians (IQRs) if distribution is nonnormal. Categorical variables are presented as *n* (%). Q, quintile; TFA, *trans* fatty acid.

[2]Range of total dairy intake per quintile.

[3]For continuous variables, P values are from ANOVA or Kruskal-Wallis comparison across quintiles for normal and skewed variables, respectively. For categorical variables, P values are from χ^2 value for comparison across quintiles.

15:0 was associated with a 27% decreased risk for incident type 2 diabetes in the fully adjusted model, which included demographic, lifestyle, and dietary variables (model 3: OR per SD: 0.73; 95% CI: 0.56, 0.95; P = 0.02) (**Figure 1**). After further adjustment for adiposity variables in a mechanistic model, the associations persisted, with additional adjustment for BMI (OR: 0.76; 95% CI: 0.58, 0.99; P = 0.04) or waist circumference (OR: 0.77; 95% CI: 0.59, 1.00; P = 0.05). None of the interaction terms tested in the effect modification analyses were statistically significant ($P > 0.05$) (Figure 1).

In univariate analyses, 15:0 was positively correlated with S_I (r = 0.14, P = 0.0003) and DI (r = 0.11, P = 0.006), whereas *trans* 16:1n−7 was negatively correlated with S_I (r = −0.12, P = 0.003) and not significantly correlated with DI. Multiple regression analyses showed that 15:0 was positively associated to both log S_I (β: 0.84; SEM: 0.38; P = 0.03) and log DI (β: 2.21; SEM: 0.93; P = 0.02) after adjustment for demographic, lifestyle, and dietary variables (**Table 4**). Further adjustment for BMI (Table 4) and waist circumference (data not shown) in mechanistic models attenuated results to nonsignificance, although the direction and magnitude of these associations were similar. *trans* 16:1n−7 was negatively associated with log S_I after adjustment for age, sex, ethnicity, and study center (β: −0.56; SEM = 0.21; P = 0.0096). Further adjustment with lifestyle and dietary variables attenuated results to nonsignificance. *trans* 16:1n−7 was not associated with log DI. None of the interaction terms tested in the effect modification analyses were statistically significant ($P > 0.05$) (data not shown).

Additional analyses across tertiles of 15:0 and *trans* 16:1n−7 yielded similar results. Briefly, participants in the highest tertile of 15:0 had significantly lower type 2 diabetes risk after 5 y compared with those in the lowest tertile in the fully adjusted model (tertile 3 compared with tertile 1, model 3: OR: 0.47; 95% CI: 0.26, 0.86; P-trend: 0.05). Further adjustment for BMI did not change the association appreciably (tertile 3 compared with tertile 1, model 4: OR: 0.53; 95% CI: 0.29, 0.9; P-trend = 0.12). In cross-sectional analyses, those in the highest tertile of 15:0 had higher least squares mean values of log S_I and log DI than those in the lowest tertile in fully adjusted models (log S_I model 3: P = 0.003; log DI model 3: P = 0.0002). The significant positive associations were maintained after additional adjustment for BMI (data not shown). There was no association with log S_I or log DI across tertiles of *trans* 16:1n−7 in fully adjusted models (data not shown).

DISCUSSION

In the present study, we found that serum 15:0 was a significant biomarker for total dairy intake in this multiethnic cohort. In addition, 15:0 was positively associated with S_I and DI, as well as a decreased incident diabetes risk after 5 y of follow-up, independent of demographic, lifestyle, and dietary variables. Further adjustment for BMI attenuated the cross-sectional results, suggesting that the associations may be partially mediated by adiposity. In contrast, *trans* 16:1n−7 was negatively associated with S_I, although further adjustment with lifestyle and dietary variables attenuated this result to nonsignificance. Although there was some evidence of stronger associations of 15:0 with diabetes and its underlying traits in specific subgroups,

TABLE 2
Regression analysis assessing the contribution of total dairy intake from the food-frequency questionnaire to serum concentrations of pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n−7) in 659 adults in the Insulin Resistance Atherosclerosis Study[1]

Dependent variable	*n*	$\beta \pm$ SEM	*P* value
15:0			
Model 1[2]	659	0.002 ± 0.0005	0.001
Model 2[3]	646	0.002 ± 0.0005	0.002
Model 3[4]	646	0.002 ± 0.0005	0.0002
Model 4[5]	645	0.002 ± 0.0005	0.0001
trans 16:1n−7			
Model 1[2]	659	−0.002 ± 0.0008	0.021
Model 2[3]	646	−0.002 ± 0.0009	0.07
Model 3[4]	646	−0.003 ± 0.0009	0.004
Model 4[5]	645	−0.003 ± 0.0009	0.004

[1]Total dairy intake was defined as sum of whole milk; 2% milk; skim milk, 1%, or buttermilk; cottage and ricotta cheese; cheese; flavored yogurt (2%, nonfat, or whole); low-fat flavored yogurt (2% or nonfat); ice cream; frozen yogurt, ice milk; milk in coffee or tea; and cream or half-and-half in coffee or tea.

[2]Model 1: total dairy intake adjusted for age, sex, and ethnicity.

[3]Model 2: additionally adjusted for physical activity and total energy intake.

[4]Model 3: additionally adjusted for total hydrogenated food intake.

[5]Model 4: additionally adjusted for BMI.

these results should be interpreted cautiously because the interaction terms were nonsignificant.

Few previous studies have examined the associations between 15:0 (42) and *trans* 16:1n−7 (28, 29) biomarkers with type 2 diabetes risk, as well as underlying disorders of insulin resistance and β-cell function in type 2 diabetes. These studies largely demonstrated that higher concentrations of these fatty acids were associated with a lower risk in diabetes. In agreement with the present study, a sex- and age-matched nested case-referent prospective study of 159 Swedish participants without diabetes at baseline showed that a higher proportion of 15:0 in erythrocyte membranes was associated with a 29% decrease in incident diabetes (OR: 0.71; 95% CI: 0.52, 0.97; $P = 0.033$) after 5 y of follow-up with limited adjustment for confounding variables (alcohol intake, BMI, glycated hemoglobin, smoking, and physical activity) (42). On the other hand, the European Prospective Investigation into Cancer and Nutrition cohort did not find significant decreases in diabetes risk with concentrations of 15:0 (43, 44). Furthermore, one previous cross-sectional study found no significant association of 15:0 with insulin resistance or β-cell function (45).

Unlike previous studies, the present study did not find serum *trans* 16:1n−7 to be correlated with total dairy intake or serum 15:0 or to be inversely associated with diabetes risk. In the Cardiovascular Health Study, Mozaffarian et al. (28) found that *trans* 16:1n−7 was highly correlated with other biomarkers of dairy fat intake, including 15:0 ($r = 0.64$), and that higher concentrations of circulating *trans* 16:1n−7 were associated with a lower risk of incident diabetes (quintile 5 compared with quintile 1: HR: 0.38, 95% CI: 0.24, 0.62; P-trend < 0.001). Furthermore, this study found that *trans* 16:1n−7 was associated with a 16.7% lower insulin resistance as measured by HOMA-IR (P-trend < 0.001). A more recent study by Mozaffarian et al. (29) also found *trans* 16:1n−7 to be significantly associated with lower incident diabetes in a multiethnic cohort (Multi-Ethnic Study of Atherosclerosis), with similar findings across different ethnicity subgroup analyses. In contrast, high concentrations of *trans* 16:1n−7 in a European cohort were not significantly associated with a lower diabetes risk (43, 44), and adipose tissue *trans* 16:1n−7 was not associated with diabetes prevalence in a Costa Rican cohort (46). Inconsistencies in the results of these studies are perhaps due to differences in population characteristics, dairy intake behaviors, biological media used to measure fatty acids, and covariates used in the analyses.

Presently, the mechanism underlying the inverse relation of 15:0 with diabetes risk is not known. It is possible that 15:0, given its significant correlation with total dairy intake, may be a marker for other beneficial components of dairy, such as calcium, vitamin D, magnesium, protein, probiotics, or prebiotics (2, 4, 47). Alternatively, because previous studies have shown effects of other types of SFAs on β cells and insulin-sensitive tissues (48), 15:0 may have an as yet to be described direct effect on one or more of the traits underlying diabetes.

Optimal dietary biomarkers for epidemiologic research are those that cannot be endogenously produced in the body. In terms of fatty acids, these include odd-numbered, branch-chained, and *trans* fatty acids (19). A number of fatty acids have been validated as biomarkers of dairy intake, including 15:0 and *trans* 16:1n−7. Studies have shown that 15:0, measured in adipose tissue (22, 24, 26, 27), serum (23–26), plasma (30), and erythrocyte (30), is a valid biomarker for dairy intake with strong correlations between the fatty acid and dairy intake measured through dietary records. Serum fatty acids, in particular, represent short-term dietary intake (31). Our findings are consistent with these aforementioned validation studies on 15:0. In agreement with a previous study (29), partially hydrogenated foods were a source of *trans* 16:1n−7 in this cohort, as demonstrated by the

TABLE 3
Regression analysis assessing the contribution of total partially hydrogenated food intake from the food-frequency questionnaire to serum concentrations of pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n−7) in 659 adults in the Insulin Resistance Atherosclerosis Study[1]

Dependent variable	*n*	$\beta \pm$ SEM	*P* value
15:0			
Model 1[2]	659	−0.0006 ± 0.0004	0.11
Model 2[3]	646	−0.0007 ± 0.0004	0.06
Model 3[4]	646	−0.001 ± 0.0004	0.008
Model 4[5]	645	−0.001 ± 0.0004	0.010
trans 16:1n−7			
Model 1[2]	659	0.003 ± 0.0007	<0.0001
Model 2[3]	646	0.003 ± 0.0007	<0.0001
Model 3[4]	646	0.003 ± 0.0007	<0.0001
Model 4[5]	645	0.003 ± 0.0007	<0.0001

[1]Total partially hydrogenated food was defined as the sum of french fries and fried potatoes; salty snacks such as crackers, potato chips, corn chips, tortilla chips, or pretzels; margarine on bread or roll; doughnuts; cookies; cakes; pastry; brownies; sopapillas; and pan dulce.

[2]Model 1: total partially hydrogenated food intake adjusted for age, sex, and ethnicity.

[3]Model 2: additionally adjusted for physical activity and total energy intake.

[4]Model 3: additionally adjusted for total hydrogenated food intake.

[5]Model 4: additionally adjusted for BMI.

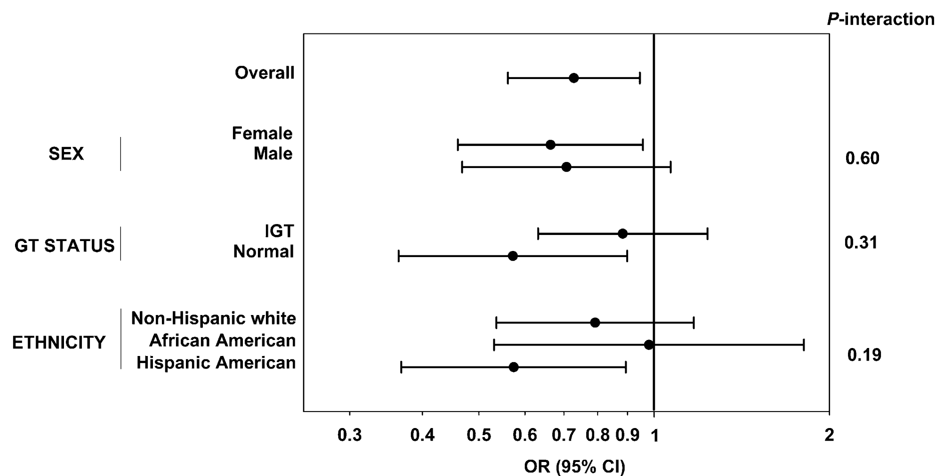

FIGURE 1 Logistic regression analyses, overall and stratified by subgroups, for incident diabetes risk with pentadecanoic acid (15:0) in 659 adults in the Insulin Resistance Atherosclerosis Study. ORs per SD and 95% CIs of each regression are shown, as well as *P*-interaction values from the effect modification analyses. ORs were adjusted for age, sex, ethnicity, center, physical activity, smoking status, alcohol intake, education, and total energy, fruit and vegetable, red meat, soft drink, and fiber intakes. Overall, model 3: OR per SD: 0.73; 95% CI: 0.56, 0.95; $P = 0.02$. GT, glucose tolerance; IGT, impaired glucose tolerance.

significant positive association with total partially hydrogenated food intake in the fully adjusted linear regression. *Trans* 16:1n−7 in plasma (28–30, 49) and erythrocytes (30) has also been shown to be highly correlated with self-reported dairy intake. In contrast, *trans* 16:1n−7 was not significantly correlated with total dairy intake or 15:0 in our study. The samples in which the fatty acid measures were conducted in the IRAS cohort were collected in the early 1990s, before large-scale reformulation of foods to reduce *trans* fat content (29, 50). This may explain the high correlation found in the current study between *trans* 16:1n−7 and total partially hydrogenated food intake and not with total dairy intake or 15:0.

There are several strengths to this study. To our knowledge, this is the first study to simultaneously examine the association of 15:0 and *trans* 16:1n−7 with incident diabetes, as well as its main underlying pathophysiologic traits of insulin resistance and β-cell dysfunction. The design of the IRAS cohort also allowed for the evaluation of these associations across multiple ethnicities and glucose tolerance status. In addition, insulin resistance and β-cell dysfunction were assessed precisely by using FSIGT. Other observational studies on dairy fatty acids and diabetes outcomes did not have measures with this degree of precision (28, 29, 42, 43). As previously mentioned, using biomarkers in our analyses gave us a more objective measure of dairy intake compared with estimates obtained from FFQ data. Moreover, we adjusted for a broad range of potential demographic, lifestyle, and dietary confounders in the analyses. On the other hand, given the observational design, this study is limited in that it cannot infer causal relationships between the exposure and outcomes. Also, although adjustments for several potential confounders were considered in the analyses, other confounding factors may still be unaccounted for.

In conclusion, serum 15:0, a marker of short-term intake of this fatty acid, was a significant and independent biomarker for total dairy intake in the IRAS cohort. This fatty acid was positively associated with insulin sensitivity and β-cell function, as well as a 27% decreased risk of incident diabetes after 5 y. Unlike previous studies, *trans* 16:1n−7 was not correlated with total dairy intake in this cohort but rather with intake of partially hydrogenated fats. Further studies are required to evaluate the association between dairy fatty acid biomarkers and diabetes outcomes and its mechanisms to inform future public health recommendations regarding dairy intake.

TABLE 4
Cross-sectional multiple regression analyses for log S_I and log DI with pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n−7) in 659 adults in the Insulin Resistance Atherosclerosis Study[1]

Independent variable	*n*	Log S_I		Log DI	
		$\beta \pm$ SEM	*P* value	$\beta \pm$ SEM	*P* value
15:0					
Model 1[2]	659	0.689 ± 0.370	0.06	1.763 ± 0.895	0.049
Model 2[3]	645	0.909 ± 0.382	0.018	2.205 ± 0.927	0.018
Model 3[4]	645	0.844 ± 0.381	0.027	2.212 ± 0.931	0.018
Model 4[5]	644	0.504 ± 0.334	0.13	1.628 ± 0.881	0.07
trans 16:1n−7					
Model 1[2]	659	−0.556 ± 0.214	0.010	−0.264 ± 0.520	0.61
Model 2[3]	645	−0.439 ± 0.225	0.05	0.078 ± 0.546	0.89
Model 3[4]	645	−0.415 ± 0.223	0.06	0.096 ± 0.548	0.86
Model 4[5]	644	−0.375 ± 0.195	0.05	0.137 ± 0.516	0.79

[1]DI, disposition index; S_I, insulin sensitivity index.
[2]Model 1 was adjusted for age, sex, ethnicity, and center.
[3]Model 2: additionally adjusted for physical activity, smoking status, alcohol intake, and education.
[4]Model 3: additionally adjusted for total energy, fruit and vegetable, red meat, soft drink, and fiber intakes.
[5]Model 4: additionally adjusted for BMI.

The authors' responsibilities were as follows—LEW and SMH: contributed to the IRAS design; IDS and AJH: designed the current study; SMW: conducted the fatty acid analyses; IDS: wrote the manuscript and analyzed the data with analytical insights from AJH; SMW, ADL, LEW, MJR, SMH, CL, and AJH: revised and provided critical feedback on the manuscript; and AJH: had primary responsibility for the final content. All authors read and approved the final version of the manuscript. SMW is an employee of Metabolon, Inc., which sells diagnostics for the management of metabolic disorders. AJH holds a Tier II Canada Research Chair in Diabetes Epidemiology. None of the funders had any role in the design, analysis,

interpretation, or presentation of the results. None of the other authors reported a conflict of interest related to the study.

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