



Plasma Industrial and Ruminant *Trans* Fatty Acids and Incident Type 2 Diabetes in the EPIC-Potsdam Cohort

Marcela Prada,^{1,2}
 Clemens Wittenbecher,^{1,2,3}
 Fabian Eichelmann,^{1,2}
 Andreas Wernitz,^{1,2} Olga Kuxhaus,^{1,2}
 Janine Kröger,^{1,2} Cornelia Weikert,⁴ and
 Matthias B. Schulze^{1,2,5}

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OBJECTIVE

Although dietary intake of *trans* fatty acid (TFA) is a major public health concern because of the associated increase in the risk of cardiovascular events, it remains unclear whether TFAs also influence risk of type 2 diabetes (T2D) and whether industrial TFAs (iTFA) and ruminant TFAs (rTFA) exert the same effect on health.

RESEARCH DESIGN AND METHODS

To investigate the relationship of 7 rTFA and iTFA, including 2 conjugated linoleic acids (CLAs), plasma phospholipid TFAs were measured in a case-cohort study nested within the European Prospective Investigation Into Cancer and Nutrition–Potsdam cohort. The analytical sample was a random subsample ($n = 1,248$) and incident cases of T2D ($n = 801$) over a median follow-up of 6.5 years. Using multivariable Cox regression models, we examined associations of TFAs with incident T2D.

RESULTS

The TFA subtypes were intercorrelated with each other, with other fatty acids, and with different food sources. After controlling for other TFAs, the iTFA (18:1n-6t, 18:1n-9t, 18:2n-6,9t) were not associated with diabetes risk. Some rTFA subtypes were inversely associated with diabetes risk: vaccenic acid (18:1n-7t; hazard ratio [HR] per SD 0.72; 95% CI 0.58–0.89) and t10c12-CLA (HR per SD 0.81; 95% CI 0.70–0.94), whereas c9t11-CLA was positively associated (HR per SD 1.39; 95% CI 1.19–1.62). *Trans*-palmitoleic acid (16:1n-7t) was not associated with diabetes risk when adjusting for the other TFAs (HR per SD 1.08; 95% CI 0.88–1.31).

CONCLUSIONS

The TFAs' conformation plays an essential role in their relationship to diabetes risk. rTFA subtypes may have opposing relationships to diabetes risk. Previous observations for reduced diabetes risk with higher levels of circulating *trans*-palmitoleic acid are likely due to confounding.

Trans fatty acids (TFAs), which are unsaturated fatty acids (FAs) with at least 1 double bond in *trans* configuration, have been a focus of public health initiatives (1) because their intake has been related to coronary heart disease and all-cause

¹Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany

²German Center for Diabetes Research (DZD), München-Neuherberg, Germany

³Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA

⁴German Federal Institute for Risk Assessment, Department of Food Safety, Berlin, Germany

⁵Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany

Corresponding author: Matthias B. Schulze, mschulze@dife.de

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mortality (2). Although initiatives to reduce TFA intake have focused on TFAs derived industrially by partial hydrogenation of vegetable oils, TFAs can also be found naturally in dairy foods.

Industrial TFAs (iTFA) and ruminant TFAs (rTFA) exist in a range of different FA molecules, differing in carbon-chain length and position of the double bond. The rTFA and iTFA share common isomers; however, the proportions are different (3). For example, *trans*-vaccenic acid (18:1n-7t) is predominant in rTFA, whereas elaidic acid (18:1n-9t) is predominant in iTFA (3). The conjugated linoleic acids (CLAs) are TFAs with only 1 carbon between 2 double bonds, 1 in *trans* configuration and 1 in *cis* configuration. Two common CLA isomers found in dairy fat are *cis*-9,*trans*-11 CLA (c9t11-CLA) and *trans*-10,*cis*-12 CLA (t10c12-CLA) (4).

Whether iTFA and rTFA exert the same effect on health is an ongoing debate. iTFA and rTFA have similar effects on blood lipoprotein concentrations (5), but the epidemiological evidence predominantly links iTFA intake with coronary heart disease risk (2). A higher intake of total TFAs is not clearly linked to higher risk of type 2 diabetes (T2D) in prospective cohort studies (2). However, in cohort studies, researchers have observed lower diabetes risk with higher blood concentrations of the ruminant-derived *trans*-palmitoleic acid (16:1n-7t) (2,6). Still, evidence for the role of iTFA and rTFA in diabetes is limited given that most studies examined only single TFA isomers (6–9) or did not distinguish between specific isomers from industrial and ruminant sources (7,10–13).

Furthermore, the role of CLA for T2D remains largely unknown. Short-term randomized controlled trials usually used a mix of CLA isomers and results suggest an influence on insulin sensitivity (14), which may depend on *PPARG* polymorphism (15). However, we are not aware of prospective studies investigating blood levels of specific CLA isomers with T2D risk. Therefore, in our study, we aimed to examine the relation of individual TFAs from industrial and ruminant sources, including 2 CLAs, measured in plasma phospholipids as a marker of intake, with T2D risk in a large, population-based cohort study.

RESEARCH DESIGN AND METHODS

Study Design and Population

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study is part of the multicenter prospective cohort study EPIC. In Potsdam, Germany, 27,548 participants ($n = 16,644$ women, most aged between 35 and 65 years; $n = 10,904$ men, most aged between 40 and 65 years) from the general population were recruited between 1994 and 1998 (16). The baseline examination included collecting blood samples, anthropometry, a self-administered validated food frequency questionnaire (FFQ), an interview including questions on prevalent diseases, and a questionnaire on sociodemographic and lifestyle characteristics (17,18). Follow-up questionnaires were administered every 2–3 years to identify incident cases of chronic diseases, including diabetes. Response rates for follow-up rounds 1, 2, and 3 were 96%, 95%, and 91%, respectively. We also considered questionnaires within the fourth follow-up round sent out until 31 January 2005, of which 90% were returned by 31 August 2005.

For efficient molecular phenotyping, a case-cohort study within the EPIC-Potsdam study was designed (19). From all participants with blood samples ($n = 26,437$), we randomly selected a subsample ($n = 1,248$) and all case patients with incident diabetes ($n = 801$) (Supplementary Figure 1). By randomly selecting a subcohort, the results are expected to be generalizable to the entire cohort without measuring biomarkers in the whole cohort (19). We excluded participants who had missing TFA analyses ($n = 5$), prevalent diabetes ($n = 58$), and missing follow-up information ($n = 31$). The analytical sample thus included 1,927 participants: 1,159 from the subcohort and 796 incident diabetes case patients, with an overlap of 28 participants. The median follow-up was 6.5 years. Consent was obtained from all participants, and approval was given by the Ethical Committee of the State Brandenburg, Germany.

Ascertainment of Diabetes Cases

Incident diabetes case patients were identified during follow-up via self-reports of diabetes diagnosis, diabetes-

relevant medication, or dietary treatment due to diabetes. Diabetes information was also obtained from death certificates or other sources such as tumor centers, physicians, or clinics. All incident cases were verified by questionnaires mailed to the diagnosing physician asking about the date and type of diagnosis, diagnostic tests, and treatment of diabetes. Only cases with a physician diagnosis of T2D (ICD10 code E11) and a diagnosis date after the baseline examination were considered confirmed incident cases of T2D.

Dietary Assessment

All participants were asked to complete a validated semiquantitative FFQ (20–22), which was used to assess the average intake frequency and portion size of 148 foods consumed during the 12 months before the examination. Additional questions were asked about fat products used for food preparation. Moderate to high correlations were found with repeated administration of the FFQ at a 6-month interval: milk and milk products ($r = 0.55$), cheese ($r = 0.61$), bread ($r = 0.49$), cereals ($r = 0.73$), fruits ($r = 0.61$), vegetables ($r = 0.54$), meat ($r = 0.77$), and processed meat ($r = 0.73$) (22).

We analyzed dairy foods in 2 main groups based on their fat content: Low-fat dairy and full-fat dairy (Supplementary Table 1). In addition, the following dairy subgroups were considered separately: low-fat cheese, full-fat cheese, and butter. Apart from dairy foods, we included other potential dietary sources of TFAs or food that might correlate with TFA intake and thus potentially confound associations: margarine, other vegetable fat (vegetable fat or oil for cooking or salads), other fat (animal cooking fat, various fats and oils), eggs, poultry, meat, processed meat, fish, nuts, fried potatoes, confectionery, cakes, cookies, and desserts, fresh fruit, raw vegetables, cooked vegetables, grains, pasta and rice, bread (whole grain and other), and alcohol from alcoholic beverages.

Plasma Phospholipid TFAs

Venous blood samples were collected at baseline following standardized procedures. Phospholipid TFAs were measured from plasma samples that were stored at -80°C (Supplementary Material). In brief, we measured TFAs using total lipid extraction with *tert*-butyl methyl ether/methanol, solid-phase separation, hydrolysis,

and methylation with trimethyl sulfonium hydroxide, and subsequently analyzed samples by gas chromatography–tandem mass spectrometry. The method was modified for biomarker-relevant FA profile analysis with low abundances in plasma phospholipids. The area count values of multiple reaction monitoring were calibrated against area count values of detection by flame ionization detector (FID) using appropriate standard FAs. This enables relativization between low abundance TFA data with high abundance FA data based on FID detection. A full FA profile, including the odd-chain FAs 15:0 and 17:0, was assessed simultaneously by FID detection, splitting the eluting gas flow for FID and mass spectrometry detection.

We defined 16:1n-7t, 18:1n-7t, c9t11-CLA, and t10c12-CLA as rTFAs. All other TFAs, 18:1n-6t, 18:1n-9t, and 18:2n-6,9t, were defined as iTFAs (Supplementary Table 2).

Other Measurements

Measurements of plasma HDL cholesterol, triglycerides, γ -glutamyltransferase (GGT), hs-CRP, adiponectin, fetuin-A, and red blood cell concentrations of hemoglobin A_{1c} (HbA_{1c}) were performed as described in previous publications (23,24). Participants were genotyped for the Pro-12Ala polymorphism in *PPARG* using the TaqMan technology (Applied Biosystems, Foster City, CA). The reproducibility of the genotyping method was $\geq 99.5\%$.

Statistical Analysis

All TFA variables were log-transformed to stabilize skewed distributions (Supplementary Figure 2) and standardized (mean = 0, SD = 1). We explored intercorrelations of the TFAs and food groups, using Pearson correlation coefficients with adjustment for age, sex, and total energy intake in the subcohort; their 95% CIs were obtained by Fisher z-transformation.

We used Cox proportional hazard regression models stratified by age to study the relationship between FAs and diabetes risk in the case cohort, accounting for the oversampling of cases by Prentice weighting (19). We estimated hazard ratios (HRs) and 95% CIs per 1-SD increase in log-transformed TFA concentrations. The following variables were considered covariables in the models: waist circumference

(continuous), BMI (continuous), smoking status (never, past, current <20 cigarettes/day, current ≥ 20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, ≥ 5 h/week), sports activity (0, 0.1–4.0, >4.0 h/week), occupational activity (light, moderate, heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/day), red meat intake (energy adjusted), coffee intake (energy adjusted), fiber intake (energy adjusted), fasting status, and total energy intake. For energy adjustment, the residual method was used (25). To evaluate the independent associations of each TFA subtype, we further mutually adjusted all TFAs in a separate model. To further explore intercorrelations, a principal component (PC) analysis (PCA) based on the individual TFA was performed on the subcohort's data. We then calculated a PC score for all participants and constructed Cox models adjusting for this score. We also assessed the nonlinear association of TFAs with diabetes, using restricted cubic splines with 3 knots placed at the 5th, 50th, and 95th percentiles.

In sensitivity analyses, we included TFA food sources as covariates in the models to confirm that TFAs associated with T2D risk are not just markers of intake of these food groups. We also considered other FAs for adjustment: the dairy fat–derived 15:0 and 17:0 (expected to correlate with rTFAs), FAs in the de novo lipogenesis pathway as suggested in a previous study (10), and other FAs as potential confounders derived from our correlation analyses. We then examined whether associations between TFAs and diabetes were modified by sex, alcohol intake, BMI, and *PPARG* gene polymorphism (wild type vs. Ala carriers), because it has been reported that CLA isomers influence insulin sensitivity depending on the *PPARG* polymorphism (15). Tests for statistical interactions were conducted by including cross-product terms in the models. Last, we excluded participants with baseline HbA_{1c} >6.5%, thus potentially those with undiagnosed or non-reported diabetes.

We used Pearson correlation analysis adjusted for age and sex to examine the relationship of the TFAs to cardiometabolic risk biomarkers (namely, triglycerides, non-HDL cholesterol, HDL cholesterol, HbA_{1c},

GGT, adiponectin, hs-CRP, and fetuin-A) and the fatty liver index (FLI) (26) in the subcohort. Then, we conducted mediation analyses to assess the extent to which significantly TFA-related biomarkers potentially explained the associations between TFAs and diabetes risk. Cox regression models with and without adjustment for the potential mediator were compared. Differences between the TFA β -coefficients of the reference model and the TFA β -coefficients of the biomarker-fitted model, their stability as well as the corresponding HRs, were estimated as median and dispersion from a bootstrapping procedure ($n = 500$ bootstrap replicates). All statistical analyses were conducted with SAS Software Enterprise Guide 7.1 (SAS Institute, Cary, NC).

RESULTS

Baseline characteristics of the random subcohort were similar to those in the full EPIC-Potsdam cohort (Supplementary Table 3). The characteristics of the 1,159 subcohort participants ($n = 454$ men and 705 women) were considered according to the distribution of ruminant TFAs in plasma phospholipids (Supplementary Table 4). The most abundant of the rTFAs was c9t11-CLA (0.26%), followed by 18:1n-7t (0.19%). Only small portions were 16:1n-7t (0.04%) and t10c12-CLA (0.01%). The most abundant iTFA was 18:1n-9t (0.16%), followed by its isomer 18:1n-6t (0.09%). Study participants with higher rTFA concentrations were more likely to be women and nonsmokers and to have lower waist circumference and less alcohol consumption than participants with lower rTFA concentrations. Age, BMI, and other characteristics were similar across quintiles.

The rTFAs were positively intercorrelated, with the strongest correlation observed between 16:1n-7t and 18:1n-7t ($r = 0.70$) (Supplementary Table 5). Of note is that the rTFAs 16:1n-7t, 18:1n-7t, and t10c12-CLA were moderately positively correlated with some of the iTFAs ($r = 0.29$ – 0.66). Among the iTFAs, the most abundant, 18:1n-9t, was strongly correlated with its isomer, 18:1n-6t ($r = 0.75$); and moderately correlated with the ruminant isomer, 18:1n-7t ($r = 0.50$), and some of the 18:2 isomers (18:2n-6,9t, $r = 0.32$; t10c12-CLA, $r = 0.34$). All rTFAs were positively correlated to 15:0 and 17:0

($r = 0.16\text{--}0.62$), whereas iTFAs were mainly correlated with 17:0 ($r = 0.22\text{--}0.41$). TFAs were also correlated with polyunsaturated fatty acid (PUFA) concentrations, particularly n-6 PUFAs.

The rTFAs 16:1n-7t, 18:1n-7t, and c9t11-CLA were positively correlated with intake of fat-rich dairy foods, particularly with butter (strongest for c9t11-CLA; $r = 0.34$), whereas low-fat dairy foods were not appreciably correlated (Fig. 1). In contrast, these rTFAs were negatively correlated with margarine consumption, especially c9t11-CLA ($r = -0.29$). Noteworthy, c9t11-CLA correlated with dietary variables differently from the other 18:2 isomers. The iTFA 18:1 isomers showed correlations contrasting with those observed for rTFAs: positive for margarine (18:1n-9t; $r = 0.17$) and negative for butter (18:1n-9t; $r = -0.16$). Still, both rTFAs and iTFAs were

positively correlated to a similar degree with confectionery, cakes, cookies, and desserts. Correlations with alcohol intake were inverse and relatively strong compared with most other foods for iTFAs and rTFAs.

In models adjusted for age and sex, inverse associations with diabetes risk were observed for all TFAs except c9t11-CLA (Fig. 2). Among the iTFAs, adjustment for demographic, lifestyle, and anthropometric risk factors rendered the associations of 18:1n-6t and 18:1n-9t with diabetes risk nonsignificant, whereas 18:2n-6,9t remained inversely associated (HR per SD 0.87; 95% CI 0.77–0.98). This association was largely attenuated when adjusted for other TFAs (HR per SD 0.95; 95% CI 0.81–1.12). Among the rTFAs, 18:1n-7t and t10c12-CLA were inversely associated with diabetes risk, and these associations were unaffected or strengthened

by further adjustment for other TFAs (for 18:1n-7t and t10c12-CLA, respectively: HR per SD 0.72 [95% CI 0.58–0.89]; and 0.81 [95% CI 0.70–0.94]). In contrast, c9t11-CLA was positively associated with diabetes risk (in the fully adjusted model, HR 1.39; 95% CI 1.19–1.62). The inverse association of 16:1n-7t with diabetes risk in the age- and sex-adjusted model was attenuated with further adjustment, particularly for other TFAs. Multivariable-adjusted restricted cubic splines did not indicate relevant deviation from linearity (P for linearity > 0.05 for each rTFA) (Supplementary Figure 3).

The first PC from PCA accounted for a large variance proportion and was positively correlated with all TFAs (Supplementary Table 6). Subsequently, we used the PC1 score for adjustment in Cox models, which did not substantially affect our results (Supplementary Figure 4).

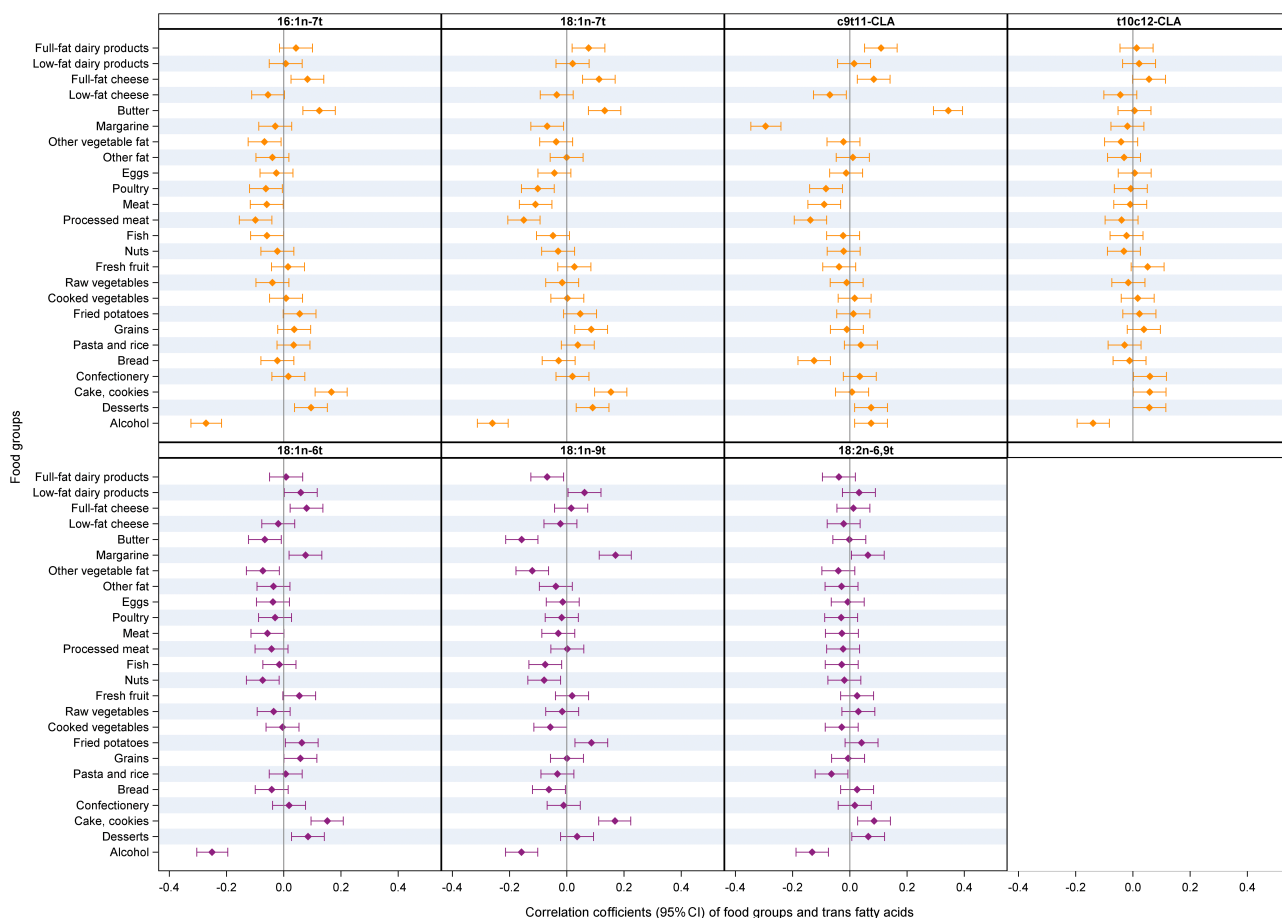


Figure 1—Partial correlations between rTFAs (orange) or iTFAs (purple) and food groups, adjusted for age, sex, and total energy intake, in the EPIC-Potsdam subcohort ($n = 1,159$). Full-fat dairy included full-fat milk and milk beverages ($>1.5\%$ fat), full-fat yogurt ($>1.5\%$ fat), curd cheese ($>5\%$ fat), heavy cream, and full-fat cheese (namely, full-fat cream cheese, Gouda, Emmental, Tilsiter, Camembert, Brie, Gorgonzola, processed cheese). Low-fat dairy included low-fat milk and milk beverages ($\leq 1.5\%$ fat), low-fat yogurt ($\leq 1.5\%$ fat), kefir ($\leq 1.5\%$ fat), curd cheese ($\leq 5\%$ fat), and low-fat cheese (reduced fat or lean: cream cheese, Gouda, Emmental, Tilsiter, Camembert, Brie, Gorgonzola).

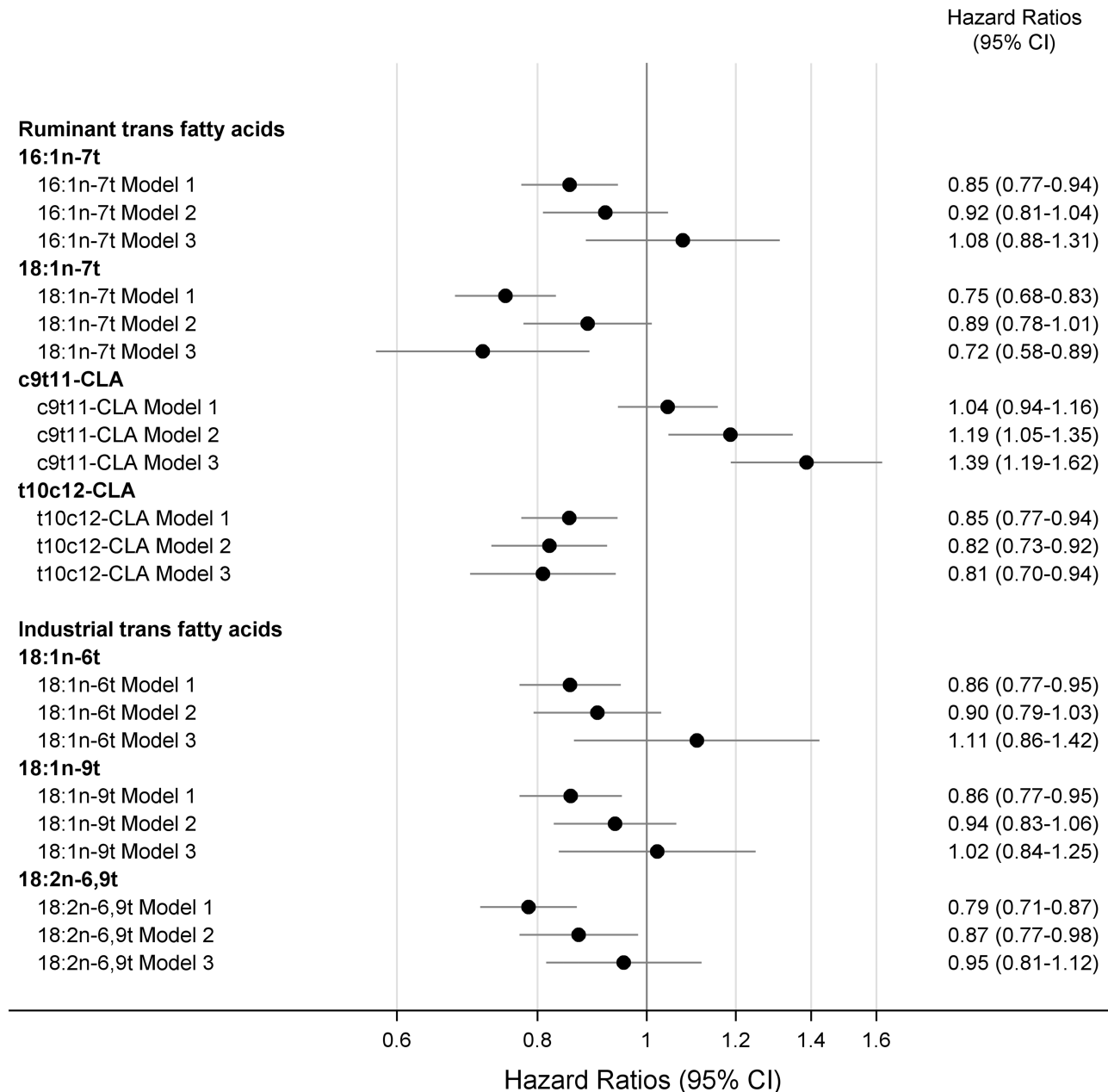


Figure 2—Prospective associations of plasma TFAs with risk of T2D in EPIC-Potsdam ($N = 1,927$; cases, $n = 796$). HR and 95% CI per 1 SD increase of each TFA. Model 1 was adjusted for age (stratum variable) and sex. Model 2 was further adjusted for waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or ≥ 5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, skilled worker, technical school, or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/day), red meat intake (energy adjusted), coffee intake (energy adjusted), fiber intake (energy adjusted), fasting status, and total energy intake. Model 3 was further adjusted for all other TFA subtypes: 16:1n-7t, 18:1n-7t, 18:1n-9t, 18:2n-6,9t, c9t11-CLA, and t10c12-CLA.

In additional sensitivity analyses, we adjusted the models of the 3 significantly diabetes risk-associated rTFAs for other potential confounders (Table 1). Adjustment variables included the strongest food correlates (butter and margarine), dairy-fat biomarkers (15:0 and 17:0), and FAs in the de novo lipogenesis

pathway (16:0, 16:1n-7c, 18:0, and 18:1n-9c). All adjustments had little impact on the rTFA–diabetes risk associations. When adjusting for n-6 PUFA concentrations (18:2n-6c, 20:3n-6c, and 20:4n-6c), the inverse association of t10c12-CLA with diabetes risk remained unchanged. However, the associations for 18:1n-7t

and c9t11-CLA were somewhat attenuated, although directions remained unchanged (for 18:1n-7t and c9t11-CLA, respectively, HR per SD: 0.86 [0.67–1.11] and 1.21 [0.99–1.48]). We did not observe significant interactions of TFAs with alcohol intake, sex, BMI, or the *PPARG* Pro12Ala polymorphism on

Table 1—Prospective association between TFAs and T2D in the EPIC-Potsdam study (N = 1,927; cases, n = 796), with additional models*

	HR per 1 SD (95% CI)			AIC
	18:1n-7t	c9t11-CLA	t10c12-CLA	
Reference model†	0.72 (0.58–0.89)	1.39 (1.19–1.62)	0.81 (0.70–0.94)	4,408
+ 15:0 and 17:0	0.78 (0.61–0.99)	1.41 (1.20–1.66)	0.82 (0.70–0.95)	4,407
+ Butter	0.72 (0.58–0.89)	1.39 (1.18–1.63)	0.81 (0.70–0.94)	4,411
+ Margarine	0.72 (0.58–0.89)	1.41 (1.20–1.65)	0.81 (0.70–0.94)	4,409
+ Total dairy	0.72 (0.58–0.89)	1.38 (1.18–1.61)	0.81 (0.70–0.94)	5,271
+ FAs in the de novo lipogenesis pathway‡	0.68 (0.51–0.90)	1.44 (1.16–1.79)	0.78 (0.67–0.91)	4,374
+ n-6 PUFAs§	0.86 (0.67–1.11)	1.21 (0.99–1.48)	0.81 (0.69–0.94)	4,332

*Adjusted for dairy fat-derived FAs pentadecanoic acid (15:0) and heptadecanoic acid (17:0), butter, margarine, total dairy, FAs in the de novo lipogenesis pathway, and n-6 PUFAs. AIC, Akaike information criterion. †Model adjusted for age (stratum variable), sex, waist circumference, BMI, smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h/week), sports activity (0, 0.1–4.0, or >4.0 h/week), occupational activity (light, moderate, or heavy), education (in or no training, vocational training, technical school, or technical college or university degree), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/day), red meat intake (energy adjusted), coffee intake (energy adjusted), fiber intake (energy adjusted), fasting status, total energy intake, and all other TFA subtypes: 16:1n-7t, 18:1n-6t, 18:1n-7t, 18:1n-9t, 18:2n-6,9t, c9t11-CLA, t10c12-CLA. ‡16:0, 16:1n-7c, 18:0, and 18:1n-9c. §18:2n-6c, 20:3n-6c, and 20:4n-6c.

diabetes risk (Supplementary Tables 7–10). Associations remained unaffected when excluding participants with baseline HbA_{1c} >6.5% (Supplementary Figure 5).

The different cardiometabolic biomarkers generally had comparable correlations with all TFA isomers (Fig. 3) but c9t11-CLA. Most TFA subtypes were negatively correlated with blood lipids (with the strongest correlations observed for triglycerides), negatively with GGT, and positively with adiponectin. The

correlations of c9t11-CLA with biomarkers appeared different in comparison: triglyceride levels (fasted) were positively correlated, whereas other lipids and nonlipid biomarkers showed little correlation overall.

We next evaluated whether adjustment for the correlated biomarkers attenuated the observed associations between rTFAs and diabetes risk (Supplementary Table 11). No appreciable attenuation was observed for the inverse

association of 18:1n-7t with diabetes risk. The associations of c9t11-CLA and t10c12-CLA with T2D risk were attenuated when adjusting for triglycerides (–20% and –35%, respectively) and FLI (–26% and –38%, respectively). Other metabolic markers had no significant impact on the associations.

CONCLUSIONS

In this prospective cohort study, we found opposite diabetes risk associations

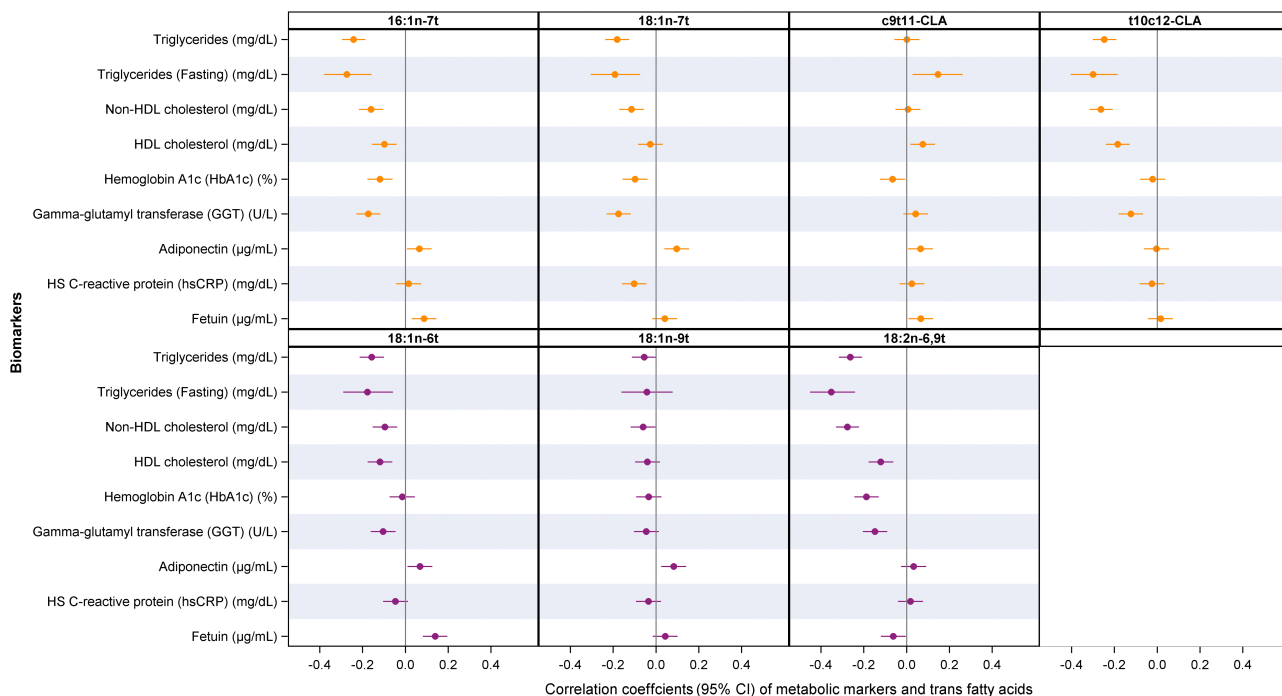


Figure 3—Partial correlations between rTFAs (orange) or iTFAs (purple) and biomarkers, adjusted for age and sex, in the EPIC-Potsdam subcohort. Biomarkers: triglycerides (N = 1,138; fasting, n = 271), non-HDL cholesterol (n = 1,144), HDL cholesterol (n = 1,144), HbA_{1c} (n = 1,109), GGT (n = 1,138), adiponectin (n = 1,134), hs-CRP (n = 1,141), and fetuin (n = 1,146).

among 7 different iTFAs and rTFAs measured in plasma phospholipids. The iTFA subtypes were not associated with diabetes risk independently of other TFAs, and 3 rTFA subtypes were associated with diabetes risk. An inverse association was found for 18:1n-7t and Δ^7 10c12-CLA, whereas c9t11-CLA was associated with higher diabetes risk. The inverse associations of 18:1n-7t and the positive association of c9t11-CLA were essentially unchanged when models were adjusted for correlated foods, FA biomarkers of dairy intake (i.e., 15:0 and 17:0), or FA biomarkers of de novo lipogenesis. However, adjustment for n-6 PUFA concentration attenuated these associations. In contrast, Δ^7 10c12-CLA remained inversely associated with diabetes risk irrespective of such adjustments. The rTFA 16:1n-7t was not associated with diabetes risk when controlling for other correlated TFAs.

We observed that individual TFAs had markedly different associations with diabetes risk, other FAs, cardiometabolic risk markers, and dietary intake. Individual TFAs reflect different food sources (industrial vs. ruminant) and distinct chemical structures, for example, total *trans*-18:1 (7,10,11,13,27), total *trans*-18:2 (10,11,13), and total CLA (12). Our results highlight that collapsing individual TFAs into 1 broad category may obscure potential etiological roles and health relevance. For example, in the Cardiovascular Health Study (CHS), all *trans*-18:1 isomers were summed because of shared food correlations and high intercorrelations (10,28), suggesting that assessing total *trans*-18:1 is sufficient to investigate health outcomes (28). However, although all *trans*-18:1 isomers were similarly associated with confectionery, cake, cookies, desserts, and alcohol in our study, only *trans*-vacenic acid (18:1n-7t) was positively associated with full-fat dairy, full-fat cheese, and butter. Consistently, previous studies reported that 18:1n-7t was more abundant in ruminant fats, and 18:1n-6t and 18:1n-9t were more present in industrial products (3). In addition, 18:1n-7t was not positively associated with margarine in our study, in contrast with the CHS study (28). We found 18:1n-7t more strongly correlated with 16:1n-7t than the other *trans*-18:1 isomers, consistent with their similar dietary sources. The difference in the observed diabetes risk

associations (null for the iTFAs 18:1n-6t and 18:1n-9t; inverse for 18:1n-7t) indicates that analyzing the *trans*-18:1 isomers as a whole group appears oversimplified. Our detailed analyses might also explain the mixed results reported from prior prospective cohort studies on total *trans*-18:1 and diabetes risk (10,13,27).

Considering 18:1n-7t is the predominant TFA in ruminant fat (3), we further adjusted for total dairy intake and odd-chain FAs as markers of dairy intake. The inverse association of 18:1n-7t with T2D risk was largely unaffected. Therefore, 18:1n-7t does not appear as just a biomarker of dairy intake. Potential explanations for a possible antidiabetic role include GLUT2- and PPAR- γ -mediated increase of insulin secretion and enhanced β -cell proliferation through 18:1n-7t, shown in animal models and human islets (29). Other studies suggest that 18:1n-7t may activate insulin signaling genes, including *PPARA* and *PPARG* in adipose tissue (30,31), and 18:1n-7t may improve energy use and fat distribution (30). However, in double-blind, randomized, controlled clinical trials, diets enriched in 18:1n-7t for 4–5 weeks did not modify fasting insulinemia and glycemia (32,33). The supplementation of 18:1n-7t decreased liver fat accumulation in rats (30), but FLI did not explain the 18:1n-7t diabetes risk association in our study.

In several other cohort studies, researchers have reported inverse associations of *trans*-palmitoleic acid (16:1n-7t) with diabetes risk (6,8,9,34). Importantly, our observation that the initial inverse association of 16:1n-7t was completely attenuated when adjusting for other TFAs indicates that previous observations may reflect confounding. In our study, 16:1n-7t was associated with dairy products, as previously reported (28), and was highly correlated with 18:1n-7t, consistent with their common food sources. A small randomized controlled trial revealed that 16:1n-7t increased after supplementation with 18:1n-7t, suggesting that 16:1n-7t may be endogenously synthesized (8). To our knowledge, no previous study has analyzed the independence of associations with diabetes risk of these 2 rTFA subtypes.

We also analyzed several *trans*-18:2 isomers, including 2 CLAs naturally

found in dairy products (4). The weak intercorrelations, variations in the correlations with food groups, and opposing associations with diabetes risk indicate that CLA isomers should be evaluated individually. We are unaware of other epidemiological studies on individual circulating CLA isomers and diabetes risk. Possible heterogeneous effects are particularly relevant because several randomized controlled trials tested the effects of a mix of CLA isomers on diabetes-related traits (14). Some of the few intervention studies focusing on c9t11-CLA reported neutral effects on plasma insulin, glucose, and insulin resistance (measured by HOMA for insulin resistance) (14). However, authors of 1 study reported reduced insulin sensitivity (measured by hyperinsulinemic clamp) (35), which supports our observation of a positive association of this CLA with diabetes risk. Still, c9t11-CLA supplementation did not affect concentrations of circulating triglycerides (15,35). Thus, our observation that triglycerides and hepatic steatosis attenuate the association of c9t11-CLA with diabetes risk must be interpreted with caution. This notion also applies to the isomer Δ^7 10c12-CLA, because supplementation did not affect concentrations of circulating triglycerides in human intervention studies (15,36). Furthermore, in contrast to the reduced diabetes risk we observed for higher circulating concentrations of this CLA, supplementation of Δ^7 10c12-CLA had either neutral or adverse effects on insulin, glucose, and insulin sensitivity (14).

It is worth noting that 18:1n-7t and Δ^7 10c12-CLA (inversely associated with diabetes risk) were inversely associated with alcohol intake, whereas c9t11-CLA (positively associated with diabetes risk) was positively associated with alcohol intake. Associations of TFAs with alcohol intake have been found previously (10). Alcohol intake activates hepatic de novo lipogenesis, an important pathway leading to the development of diabetes (37). However, it is unlikely that alcohol intake and its effect on lipogenesis explain the associations observed for TFAs, because moderate alcohol intake is associated with reduced diabetes risk (38); thus, the correlation of alcohol intake with TFAs instead would result in an underestimation of association. Nevertheless, our models were comprehensively adjusted for alcohol intake.

Consistent with reports from the CHS cohort (10), adjusting for de novo lipogenesis FAs did not appreciably attenuate the associations of 18:1n-7t, c9t11-CLA, and t10c12-CLA with diabetes risk in our study.

Another finding is that the associations for 18:1n-7t and c9t11-CLA were somewhat attenuated when we adjusted for n-6 FA concentrations. Similar to what we found in our study, phospholipid FA levels have shown strong intercorrelation patterns (39). This intercorrelation may introduce confounding when evaluating individual FAs (40). Thus, low TFA blood levels may partly reflect a replacement by other FAs, with n-6 PUFAs being well established to relate to diabetes risk (41). Although we have controlled for intercorrelated FA in our analysis, this has not been the case in most previous studies of TFA biomarkers (7–9,11,12).

This study has important strengths. We have quantified 7 different TFA subtypes, including 2 CLAs—a large panel compared with most previous studies on TFA subtypes, which included 1 to 3 TFAs in their analyses (7–9,11). Individual analyses of several TFAs, without combining in broader groups, uncovered strikingly different associations. Also, to our knowledge, this is the first prospective study of blood concentrations of specific CLA isomers and diabetes risk. Our study includes a comprehensive investigation of intercorrelations and evaluation of potential pathways. Although our study is observational and, therefore, associations may be prone to confounding, we comprehensively adjusted for various diabetes risk factors, including demographic, lifestyle, and dietary variables.

This study also had limitations. First, we did not measure the stability of the TFAs during storage time from the plasma sample collection to the analysis several years after. However, previous evidence suggested sufficient long-term stability of plasma FA concentration when stored at -80°C (42). Second, the association with food groups was investigated using an FFQ that included a limited number of food groups, which probably underestimated TFA sources. Third, TFAs were assessed in single plasma samples at study baseline. Food intake may have changed over time, and iTFA content in foods has been reduced by food reformulation, which is

not reflected in the single baseline measurements. Fourth, we cannot rule out that the TFA concentrations correlate with other unmeasured FAs. Fifth, although several TFAs were significantly associated with diabetes, we might have had limited statistical power to detect weaker associations. Last, identifying cases by self-report could lead to cases remaining undetected. However, case verification ensured no false positives and the false negatives do not bias risk associations if this misclassification is nondifferential to the exposure of interest (43).

In conclusion, our study findings suggest that TFA conformation plays an essential role in TFAs' relationship to diabetes risk. Although iTFAs were not appreciably associated with risk, rTFA isomers may have different relations to diabetes risk, with 18:1n-7t and t10c12-CLA being inversely associated and c9t11-CLA positively associated. Previous observations of reduced diabetes risk with higher circulating *trans*-palmitoleic acid are likely due to confounding.

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to interpretation of results and provided critical revisions to the manuscript. M.B.S. contributed to the study design and interpretation of results and provided critical revisions to the manuscript. All authors revised the manuscript. M.P. takes responsibility for the data integrity and the accuracy of the analyses.

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