Trans Fatty Acid Biomarkers and Incident Type 2 Diabetes: Pooled Analysis of 12 Prospective Cohort Studies in the Fatty Acids and Outcomes Research Consortium (FORCE)

Diabetes Care 2022;45:854–863 | https://doi.org/10.2337/dc21-1756

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OBJECTIVE

Trans fatty acids (TFAs) have harmful biologic effects that could increase the risk of type 2 diabetes (T2D), but evidence remains uncertain. We aimed to investigate the prospective associations of TFA biomarkers and T2D by conducting an individual participant-level pooled analysis.

RESEARCH DESIGN AND METHODS

We included data from an international consortium of 12 prospective cohorts and nested case-control studies from six nations. TFA biomarkers were measured in blood collected between 1990 and 2008 from 25,126 participants aged ≥18 years without prevalent diabetes. Each cohort conducted de novo harmonized analyses using a prespecified protocol, and findings were pooled using inverse-variance weighted meta-analysis. Heterogeneity was explored by prespecified between-study and within-study characteristics.

RESULTS

During a mean follow-up of 13.5 years, 2,843 cases of incident T2D were identified. In multivariable-adjusted pooled analyses, no significant associations with T2D were identified for *trans/trans*-18:2, relative risk (RR) 1.09 (95% CI 0.94–1.25); *cis/trans*-18:2, 0.89 (0.73–1.07); and *trans/cis*-18:2, 0.87 (0.73–1.03). *Trans*-16:1n-9, total *trans*-18:1, and total *trans*-18:2 were inversely associated with T2D (RR 0.81 [95% CI 0.67–0.99], 0.86 [0.75–0.99], and 0.84 [0.74–0.96], respectively). Findings were not significantly different according to prespecified sources of potential heterogeneity (each $P \geq 0.1$).

CONCLUSIONS

Circulating individual *trans*-18:2 TFA biomarkers were not associated with risk of T2D, while *trans*-16:1n-9, total *trans*-18:1, and total *trans*-18:2 were inversely associated. Findings may reflect the influence of mixed TFA sources (industrial vs. natural ruminant), a general decline in TFA exposure due to policy changes during this period, or the relatively limited range of TFA levels.

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Type 2 diabetes (T2D) is a rising global epidemic, with a nearly fivefold increase to 463 million global cases between 1980 and 2019 (1). As T2D is a major contributor to morbidity, mortality, and economic costs, including from cardiovascular disease, blindness, amputations, and kidney diseases (2), identification of modifiable risk factors to reduce T2D is critically important.

Trans fatty acids (TFAs), unsaturated fatty acids with at least one double bond in a *trans* configuration, include TFAs of differing chain lengths and saturation such as *trans*-18:1, *trans*-18:2n-6, *trans*-16:1n-9, and *trans*-16:1n-7 (3). In Western nations prior to 2000, most TFA exposure was from foods cooked with or containing partially hydrogenated vegetable oils (PHVO), predominantly containing *trans*-16:1n-9 and *trans*-18:1 isomers (4). After 2003, when Denmark placed limits on the amount of TFA in fats and oils, a series of legislative and policy efforts reduced exposure to industrially produced TFAs in most Western nations (5–9), with continuing exposure to smaller amounts of naturally occurring TFAs (such as *trans*-16:1n-7) from dairy and other ruminant products, and possibly *trans*-18:2 from the process of oil deodorization. Yet, industrial TFA exposure remains higher in many low- and middle-income nations (8), where inexpensive partially hydrogenated fats and shortening continue to be used in homes, restaurants, and packaged foods.

While TFAs have established adverse effects on cardiovascular disease (10), effects on T2D are less well-established. Mechanistic studies suggest adverse effects on insulin resistance, hepatic lipogenesis, inflammation, and obesity through alterations in gene expression of several proteins (11,12), but findings from rodent models were mixed and inconclusive (13,14). In small, short-term human trials, adverse effects were seen on insulin sensitivity among overweight or obese adults with diabetes or hyperlipidemia (15,16). However, a meta-analysis of randomized controlled trials found no association between reducing levels of dietary TFA and glucose homeostasis (17).

Few studies of T2D have assessed circulating biomarker levels of TFA, which reflect both diet and metabolism, provide an objective measurement free from reporting bias or memory error, and allow assessment of long-term effects of specific individual TFAs on health outcomes. One U.S. study reported a positive association between circulating *trans*-18:1n-9 (18) and T2D, but did not assess relationships between all TFAs and T2D; while a community-based U.S. cohort that explored associations between all circulating TFAs reported positive associations between total *trans*-18:1 and *trans*-16:1n-9 (19) and T2D. To date, no pooling effort has been undertaken to assess how circulating individual TFA biomarkers relate to T2D that can avoid publication bias. Thus, the relationship between different TFAs and incidence of T2D remains unclear.

To address this key question, we harmonized data from the Fatty Acids and Outcomes Research Consortium (FORCE), bringing together de novo, standardized, individual-level analyses from 12 cohorts and nested case-control studies with prospectively collected information on circulating TFA biomarkers and incident T2D.

RESEARCH DESIGN AND METHODS

Study Design and Population

We conducted this investigation in FORCE (https://force.nutrition.tufts.edu), a consortium that brings together longitudinal cohorts established around the world to understand relationships between circulating fatty acid biomarkers and chronic disease outcomes. Briefly, studies were identified and invited based on participation in prior projects (3,20–22) and availability of both measured TFA biomarker levels and ascertainment of incident T2D. From among 23 cohorts contacted, 12 studies (including 8 cohorts, 2 nested case-control studies, and 2 nested case-cohorts) were eligible and agreed to participate (Table 1). Eight studies were excluded due to absence of TFA measures and four due to insufficient resources or response. Participants in each study were included if aged ≥18 years and without prevalent T2D at baseline. All studies

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Received 20 August 2021 and accepted 10 January 2022

This article contains supplementary material online at https://doi.org/10.2337/figshare.18396443.

Table 1—Baseline characteristics from 12 prospective studies in the pooled analysis for TFA biomarker measures and follow-up data for incident T2D

Study	Country	Study design	Baseline year(s) of blood sampling	Incident T2D cases/total participants (*n*/*N*)	Maximum follow-up (years)	Women, *n* (%)	Age, years (SD)	BMI, kg/m^2 (SD)	Biomarker compartment assessed
AGES-R	Iceland	PC	2002–2006	28/753	7.8	451 (60)	76 (5)	27 (4)	PL
CCCC	Taiwan	PC	1992–1995	302/1,443	10.3	623 (43)	60 (10)	23 (3)	TP
CHS	U.S.	PC	1992–1993	291/3,007	22.1	1,804 (60)	75 (5)	26 (5)	PL
EPIC-Norfolk	U.K.	PCC	1993–1998	199/383	12.1	199 (52)	64 (8)	26 (4)	PL, RBC
EPIC-Potsdam	Germany	PNC	1994–1998	488/2,165	10.1	1,342 (62)	49 (9)	26 (4)	RBC
FHS	U.S.	PC	2005–2008	95/1,870	5.8	1,075 (58)	64 (8)	28 (5)	RBC
HPFS	U.S.	PC	1994	108/1,471	20.2	0 (0)	65 (9)	26 (3)	RBC, TP
MCCS	Australia	PNC	1990–1994	333/3,711	9.9	2,057 (55.4)	55 (9)	27 (4)	Plasma PL
MESA	U.S.	PC	2000–2002	297/2,234	11.2	1,214 (53.9)	61 (10)	28 (5)	PL
NHS	U.S.	PC	1990	152/1,482	24.8	1,482 (100)	60 (6)	25 (4)	RBC, TP
PHS	U.S.	PCC	1995–2001	60/939	13.9	0 (0)	69 (9)	25 (3)	RBC
WHIMS	U.S.	PC	1995	490/5,668	14.1	6,349 (100)	70 (4)	28 (5)	RBC

Characteristics reported are at the time of fatty acid biomarker measurement. AGES-R, Age, Gene/Environment Susceptibility Study (Reykjavik); CCCC, Chin-Shan Community Cardiovascular Cohort Study; CHS, Cardiovascular Health Study; EPIC-Norfolk, European Prospective Investigation into Cancer (Norfolk); EPIC-Potsdam, European Prospective Investigation into Cancer (Potsdam); FHS, Framingham Heart Study; HPFS, Health Professionals Follow-up Study; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses' Health Study; PC, prospective cohort; PCC, prospective nested-case-control; PHS, Physician's Health Study; PNC, prospective nested case-cohort; WHIMS, Women's Health Initiative Memory Study.

obtained institutional ethical approval and written informed consent from all participants.

Standardized Analysis Protocol

A standardized analysis protocol was developed and issued to participating cohorts, including harmonized definitions for population inclusion, exposures, outcomes, model specifications, covariates, effect modifiers, analysis, and specifications for pooling. Individual-level data were analyzed by an experienced data analyst from each cohort, and results were returned to the lead investigator in standardized electronic forms.

Fatty Acid Measurements

Fatty acid biomarkers were assessed in plasma phospholipids (PL), red blood cell membrane PL (RBC), or total plasma (TP) in each study, each expressed as a percentage of total fatty acids. Primary exposures of this investigation were *trans*-16:1n-9, total *trans*-18:1 (sum of 18-carbon isomers with the *trans* bond in the 6th to 12th position, as these isomers are highly correlated), total *trans*-18:2, and the individual *trans*-18:2 isomers (*trans*/*trans*-18:2, *trans*/*cis*-18:2, and *cis*/*trans*-18:2). Associations of *trans*-16:1n-7, primarily a ruminant rather than industrial TFA, with T2D in FORCE have been previously reported (3). In the Cardiovascular Health Study, TFA at baseline (1992–1993) was moderately correlated with its later measures over time (1998–1999 and 2005–2006) (ρ = 0.35–0.60). These correlations are generally similar to other well established cardiometabolic factors (e.g., blood pressure [ρ = 0.57–0.69]), when measured at health examinations just 1 year apart (23), suggesting that even with some changes in diet and metabolism, a level at one time reasonably predicts levels many years later. All studies used established gas chromatography methods to measure TFAs, with coefficients of variation $<$10% (see Supplementary Methods for detailed laboratory information in each study).

Ascertainment of Type 2 Diabetes

Incident T2D was ascertained by each cohort based on established combinations of self-report together with physician's diagnosis using medical records, fasting glucose levels $\geq$126 mg/dL, oral glucose tolerance test ($>$200 mg/dL), HbA_{1c} $\geq$6.5%, or use of insulin or oral

hypoglycemic medication (see Supplementary Methods for ascertainment in each study).

Model Covariates

Model covariates were prespecified based on biologic plausibility and established associations with T2D (24–28). Levels of missingness were generally low at <5% across cohorts, except for triglyceride levels in the Melbourne Collaborative Cohort Study (36%). In general, single imputations were conducted to address missing covariate data as previously established in each cohort. If imputations were not performed, participants with missing data were excluded. For categorical variables, participants with missing data were assigned to a "missing category." Our first model adjusted for age (years), sex (male, female), field site (if applicable, cohort-specific), ethnicity (White as reference, cohort-specific), education (less than high school, high school graduate, college or higher, as available, cohort-specific), occupation (as available, cohort-specific), physical activity (kcal/week, METS/week, or h/day, or categorical, cohort-specific), smoking (never, former, current), alcohol use (drinks or servings/day, g/day or mL/day), prevalent hypertension (treated or self-reported, yes/no), prevalent dyslipidemia (treated or self-reported, yes/no), and prevalent coronary heart disease (treated or self-reported, yes/no). A second model further adjusted for adiposity, including BMI (kg/m^2) and waist circumference (cm), as available. Lastly, circulating levels of palmitic acid, stearic acid, linoleic acid (% total fatty acids), and triglycerides (mg/dL) were adjusted in a third model, as palmitic acid, stearic acid, and triglycerides are products of de novo lipogenesis resulting from excess carbohydrate intake, a prominent risk pathway for the development of T2D, and insulin resistance, while linoleic acid can be consumed with TFA and has been inversely associated with T2D (21).

Interactions and Heterogeneity

We prespecified potential sources of heterogeneity based on demographic, anthropometric, and biological relevance. We hypothesized a priori that factors associated with insulin resistance—obesity (BMI ≥30 kg/m^2) and hypertriglyceridemia (≥150 mg/dL [1.7 mmol/L])—would be associated with a stronger TFA-T2D association. We also explored interactions by age, sex, and race/ethnicity. All within-cohort interactions were evaluated using within-cohort stratification, with statistical significance evaluated using the Wald test for a multiplicative interaction term in the multivariable model. Potential sources of between-study heterogeneity were also explored in pooled analyses, described below, including by study design (cohort vs. nested case-control), calendar year of blood sampling (<2000 vs. ≥2000), world region (U.S. vs. non-U.S.), and lipid compartment (PL, RBC, TP).

Individual-Level Analysis

Each study assessed population characteristics, TFA summary statistics, and inter-TFA Pearson correlations. Cox proportional hazards models were used for cohorts and nested case-cohorts with time-to-event data to obtain hazard ratios and robust SEs. Time at risk was from fatty acid measurement to incident T2D, death from other causes, loss to follow-up, or censoring at end follow-up. In nested case-control studies with risk-set sampling, conditional logistic regression was used to obtain the odds ratio and SE. The exception being the Melbourne Collaborative Cohort Study (nested case-cohort), which reported odds ratios and SE using logistic regression. Both hazard ratios and odds ratios are henceforth referred to as relative risks, or RRs. To facilitate comparisons across studies and lipid compartments, TFAs were evaluated per the interquintile range (IQR), defined as the difference between the midpoint of the first and fifth quintiles, and also by study-specific quintiles in indicator categories.

Pooling and Meta-analysis

Study-specific risk coefficients were pooled using inverse-variance weighted meta-analysis, both overall and stratified by lipid compartment. For studies that measured TFA levels in multiple compartments, one lipid compartment was used, prioritized in the overall analysis (RBC > PL > TP) for sensitivity to relatively longer-term intake. Sensitivity analyses explored whether findings varied by prioritizing PL over RBC measures. The continuous (per IQR) and categorical (quintiles) findings were pooled across cohorts. Potential nonlinear relationships were further explored using multivariable meta-regression, modeling-restricted cubic splines in each compartment. Potential interactions were assessed by pooling each study-specific stratified analysis, with statistical significance tested by pooling each study-specific coefficient of the multiplicative interaction term using inverse-variance weighted meta-analysis, with Bonferroni correction for the exploratory interactions by age, sex, and race/ethnicity (two-tailed α = 0.05/18 = 0.003 based on six TFA exposures and three exploratory effect modifiers). Pooled meta-regression and stratified meta-analyses also explored the four between-study sources of heterogeneity (Bonferroni-corrected two tailed α = 0.05/24 = 0.002). Heterogeneity was assessed using the I^2 statistic. Sensitivity analyses evaluated the effects of removing a single cohort from each pooled analysis. All analyses were performed using Stata 14.2 software (StataCorp, College Station, TX), with two-tailed α = 0.05 unless stated otherwise.

RESULTS

The investigation included 25,126 participants from 12 studies across six nations. including the U.S., U.K., Taiwan, Iceland, Germany, and Australia (Table 1). Four cohorts included only men or women; the rest included both sexes. Mean age at baseline in each cohort ranged from 49 to 76 years, and mean BMI from 23 to 28 kg/m^2. Most participants were White, with larger numbers of non-Whites in the Women's Health Initiative Memory Study (11.6%), Cardiovascular Health Study (11.7%), and Multi-Ethnic Study of Atherosclerosis (71.7%). A range of risk factors was present, including prevalent smoking, hypertension, dyslipidemia, and coronary heart disease (Supplementary Table 1).

Fatty acid biomarkers were measured as RBC PL (n = 7 cohorts), plasma PL (n = 5), or TP (n = 3); three cohorts assessed more than one lipid compartment (Table 1). Total *trans*-18:1 was most commonly assessed (n = 12 cohorts), followed by total *trans*-18:2 (n = 10), *trans*-16:1n-9 (n = 7), and individual *trans*-18:2 isomers (*trans/trans*, *cis/trans*, *trans/cis*) (n = 5). Midpoint (median) and IQRs (10th and 90th percentile) for each TFA and lipid compartment are reported in Fig. 1. Median levels for *trans*-16:1n-9, total *trans*-18:1, and total *trans*-18:2 ranged from 0.05% to 1.34%, 0.51% to 2.51%, and 0.09% to 4.83%, respectively. Correspondingly, the 10th percentile for *trans*-16:1n-9, total *trans*-18:1, and total *trans*-18:2 ranged from 0.03% to 0.92%, 0.04% to 1.71%, and 0.05% to 3.45%, while the 90th percentile ranged from 0.07% to 1.87%, 0.21% to 3.41%, and 0.14% to

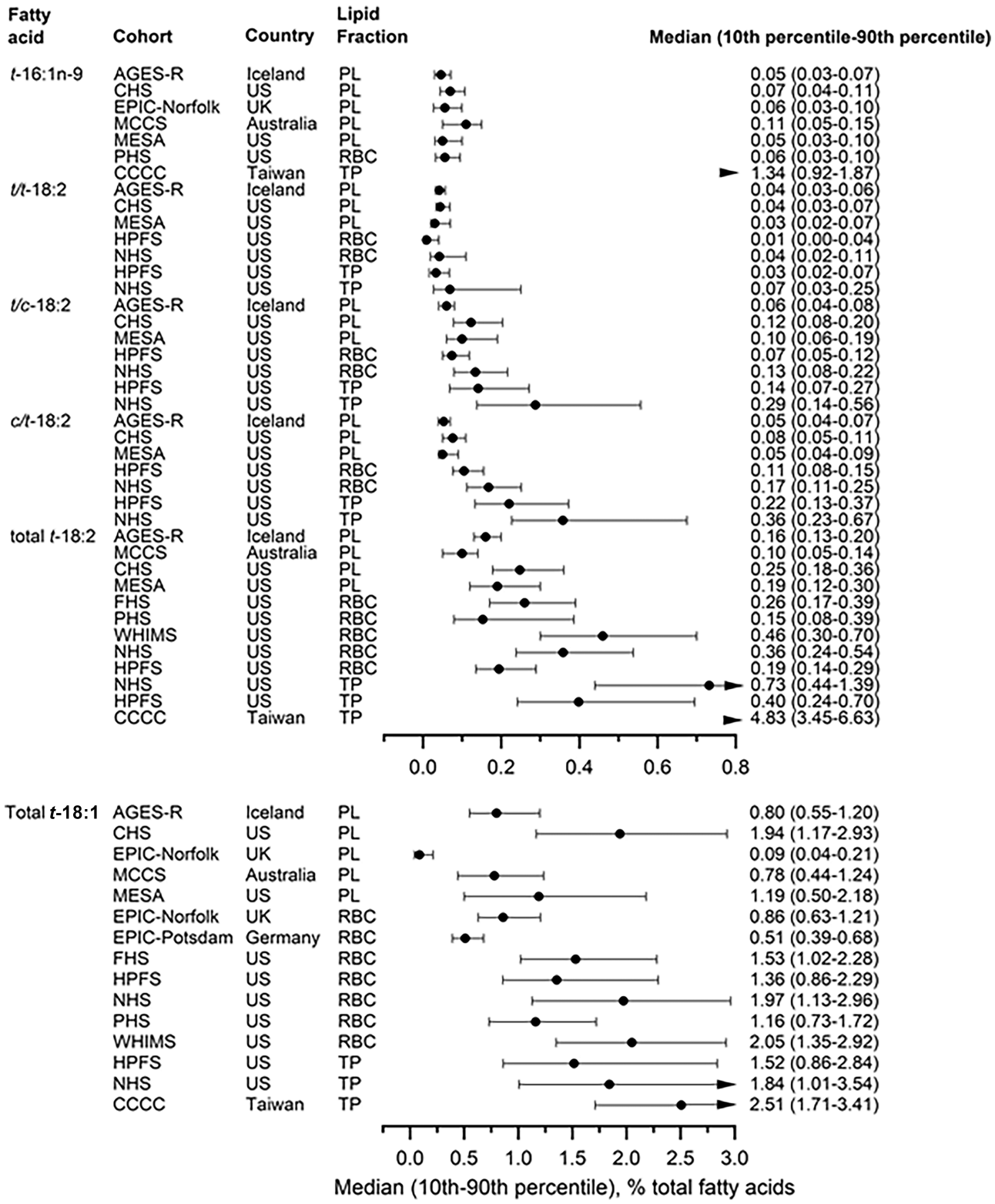

Figure 1—Median (circle) and IQR (horizontal bar) of each TFA by lipid fraction. AGES-R, Age, Gene/Environment Susceptibility Study (Reykjavik); *c*, *cis*; CCCC, Chin-Shan Community Cohort; CHS, Cardiovascular Health Study; EPIC-Norfolk, European Prospective Investigation into Cancer (Norfolk); EPIC-Potsdam, European Prospective Investigation into Cancer (Potsdam); FHS, Framingham Heart Study; HPFS, Health Professionals Follow-up Study; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses' Health Study; PHS, Physician's Health Study; *t*, trans; WHIMS, Women's Health Initiative memory Study.

6.63%, respectively. Median levels for *trans*-18:2 isomers were low, ranging from 0.01% to 0.07%, 0.06% to 0.14%, and 0.05% to 0.22% for *trans/trans*-18:2, *trans/cis*-18:2, and *cis/trans*-18:2 respectively. Similarly, the 10th percentile ranged from 0.00% to 0.03%, 0.04% to 0.08%, and 0.05% to 0.36%, while the 90th percentile ranged from 0.04% to 0.25%, 0.08% to 0.56%, and 0.07% to 0.67% for *trans/trans*-18:2, *trans/cis*-18:2, and *cis/trans*-18:2, respectively (Fig. 1). Intercorrelations between individual TFAs within each compartment varied greatly (Spearman r = 0.05–0.76) (Supplementary Table 2). Study-level covariates are summarized in Supplementary Tables 3 and 4.

During a mean maximum follow-up of 13.5 years, 2,843 cases of incident T2D were identified across the studies. In multivariable-adjusted pooled analyses, no significant associations were identified for individual *trans*-18:2 isomers, with RRs per IQR (95% CIs) for *trans/trans*-18:2 of 1.09 (0.94–1.25); for *cis/trans*-18:2, 0.89 (0.73–1.07); and for *trans/cis*-18:2, 0.87 (0.73–1.03). An inverse risk for incident T2D was seen for *trans*-16:1n-9 (RR, 95% CI 0.81, 0.67–0.99), total *trans*-18:1 (0.86, 0.75–0.99), and total *trans*-18:2 (0.84, 0.74–0.96) (Fig. 2). Between-study heterogeneity for these findings ranged from 0% to 76%. Findings were generally similar in sensitivity analysis prioritizing plasma PLs over RBC PLs. Excluding one cohort that relied only on self-report for diagnosis of T2D also did not appreciably alter the findings (data not shown).

Pooled analyses across quintiles of TFA were generally consistent with the continuous findings (Fig. 3). In the fully adjusted multivariable model, participants in the highest quintile for *trans*-16:1n-9 (0.72, 95% CI 0.56–0.92, P = 0.008), total *trans*-18:1 (0.82, 0.69–0.97, P = 0.023), and total *trans*-18:2 (0.82, 0.68–0.99, P = 0.031) had a lower risk of incident T2D.

When lipid compartments were pooled separately, results were also generally consistent with continuous findings, except for the nonsignificant association between *trans*-16:1n-9 (0.80, 0.50–1.27) and total *trans*-18:2 (1.03, 0.85–1.24) in the TP compartment, and *trans*-16:1n-9 (0.82, 0.66–1.01) and total *trans*-18:1 (0.87, 0.75–1.01) in the PL compartment (Supplementary Figs. 1–6). Restricted cubic splines meta-regression identified little evidence for nonlinearity (Supplementary Figures 7–12), although in most cases, the numbers of studies per compartment were too few (n = 2–3) to derive meaningful interpretation.

Pooled meta-regression did not identify any significant sources of between-study heterogeneity by study design, calendar year of blood sampling, world region, or lipid compartments ($P_{\text{heterogeneity}}$ by each factor $>$0.1). Significant interaction was also not identified by within-study characteristics including age, sex, and race/ethnicity ($P_{\text{interaction}}$ $>$0.3 each after Bonferroni correction), or by BMI or blood triglycerides ($P_{\text{interaction}}$ $>$0.08 each) (Supple-mentary Tables 5 and 6).

CONCLUSIONS

In this international pooling project including $>$25,000 participants in 12 cohorts from six nations, objectively measured circulating biomarkers of TFA intake were not associated with higher risk of T2D. *Trans*-16:1n-9, total *trans*-18:1, and total *trans*-18:2 were inversely associated with T2D, although with high between-study heterogeneity (especially for *trans*-18:2). Findings were generally similar by study design, time period of blood sampling, world region, lipid compartment, BMI, blood triglyceride levels, age, sex, and race/ethnicity. To our knowledge, this is the largest and most comprehensive analysis of individual TFA biomarkers and incident T2D.

In experimental studies, TFAs exhibit several harmful biological activities that could increase the risk of T2D, including increased expression of proinflammatory genes such as interleukin-15 and tumor necrosis factor-α, and lipogenic genes such as fatty acid synthase, stearoyl-CoA desaturase-1, and SREBP-1 (11,12). Many of these mechanistic effects have been demonstrated with *trans*-18:1n-9, the predominant industrial TFA; while some additional limited evidence supports potential adverse effects of *trans/trans*-18:2 on apoptosis and inflammation in endothelial cells (29). The observed inverse associations of *trans*-16:1n-9, total *trans*-18:1, and total *trans*-18:2 with T2D were unexpected and not consistent with known biological mechanisms. Individual *trans*-18:2 isomers were also not associated with T2D. One possible explanation for these inconsistent results is residual confounding by linoleic acid (the fatty acid precursor of *trans*-18:2), which is associated with lower risk of T2D (21). For example, health consciousness could increase dietary intakes of plant oils containing both linoleic acid and industrial TFA content (with the latter declining over time as discussed below) (8). However, these inverse associations were not altered by adjustment for biomarker levels of linoleic acid and should be interpreted cautiously as potentially due to chance.

Our results do not provide support for adverse effects of TFA exposure on risk of T2D in the high-income nations represented by these 12 cohorts. Several explanations are possible. First, despite evidence of potential harm in mechanistic studies, TFA exposure at the level seen in these studies may have little net effect on T2D risk among generally healthy populations. We cannot exclude, for example, that higher levels of certain TFAs may produce different effects.

Second, in these Western nations, exposure to industrially produced TFAs meaningfully declined due to policy changes after 2000 (8), a time period that overlaps with the follow-up in all these studies. Such systemic changes in TFA exposure over time would cause misclassification of exposure, which could be sizeable and would attenuate findings toward the null. However, the majority of follow-up in most of our studies was initiated before any major policy actions to reduce TFA in these countries. Furthermore, PHVO still exist in our food chain, and have yet to be eradicated. Our findings therefore carry public health significance.

Finally, TFA biomarkers reflect exposure to both industrially produced and natural ruminant TFA. A typical major source of total *trans*-18:1 is PHVO, which can be found in margarine spreads, bakery foods, and fried foods (4). Sources of some *trans*-18:2 isomers are less well established but may include the deodorization of plant oils (30). Dairy foods are also a natural source of multiple TFAs, and this ruminant source can predominate in populations where PHVO has been reduced or eliminated through policy actions. Although we adjusted for a range of major risk factors for diabetes, certain nutritional factors in some of the dietary sources could offset any potential harms of low levels of TFA exposure (31). For example, dairy foods (and dairy fat biomarkers) are associated with lower risk of T2D (32,33), potentially related to menaquinone content from

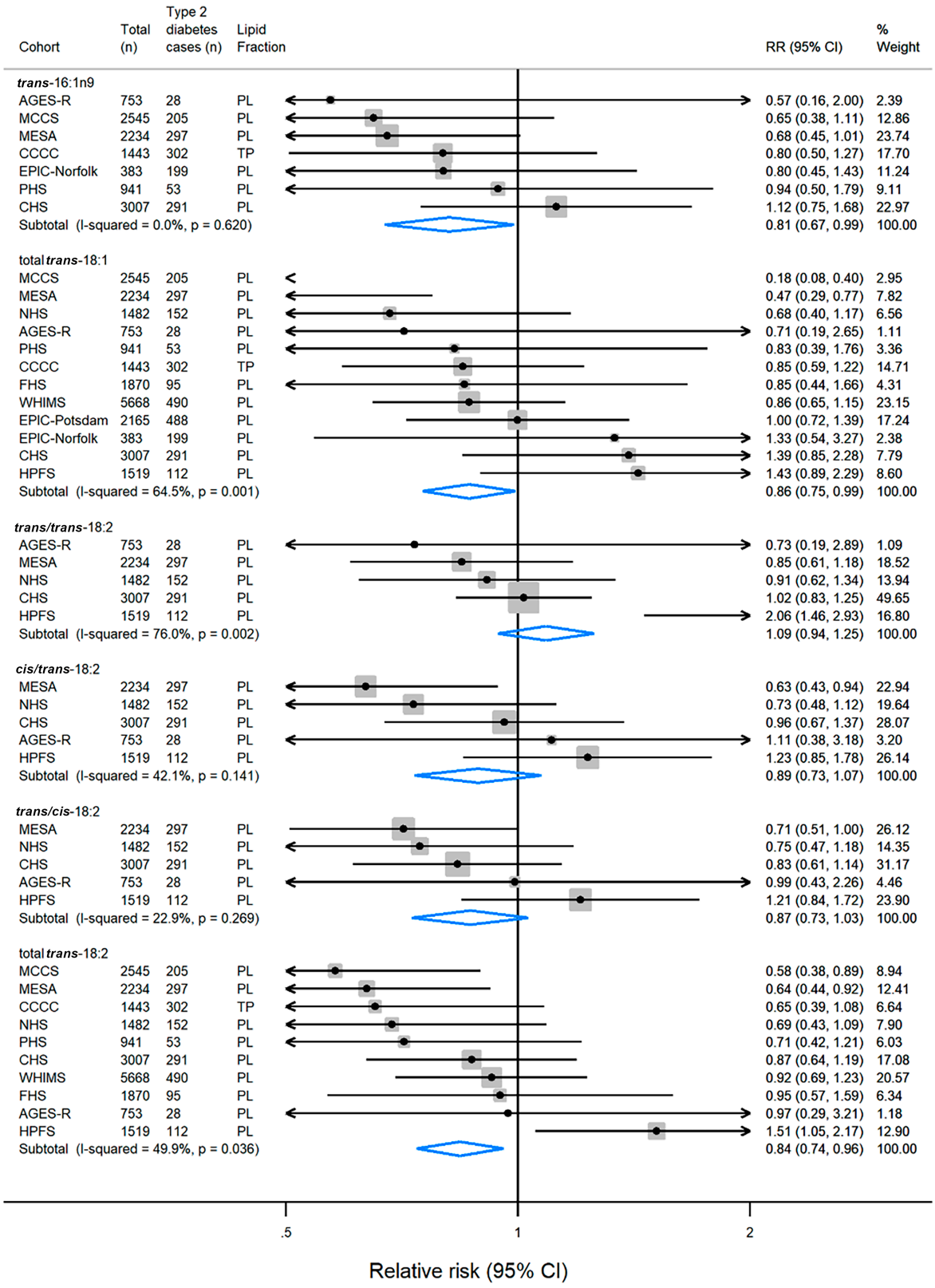

Figure 2—Pooled RRs of T2D by IQR (difference between the midpoint of the 1st and 5th quintile) of TFAs. The association between all TFAs and T2D was assessed in multivariable models in each cohort at an individual level, adjusting for age, sex, race, field site (if applicable), education, occupation, physical activity, smoking, alcohol use, prevalent hypertension, prevalent dyslipidemia, prevalent coronary heart disease, BMI, waist circumference, circulating palmitic acid, circulating stearic acid, circulating linoleic acid, and triglycerides. Results were pooled using inverse-variance weighted fixed-effects meta-analysis. AGES-R, Age, Gene/Environment Susceptibility Study (Reykjavik); CCCC, Chin-Shan Community Cohort; CHS, Cardiovascular Health Study; EPIC-Norfolk, European Prospective Investigation into Cancer (Norfolk); EPIC-Potsdam, European Prospective Investigation into Cancer (Potsdam); FHS, Framingham Heart Study; HPFS, Health Professionals Follow-up Study; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses' Health Study; PHS, Physician's Health Study; WHIMS, Women's Health Initiative Memory Study.

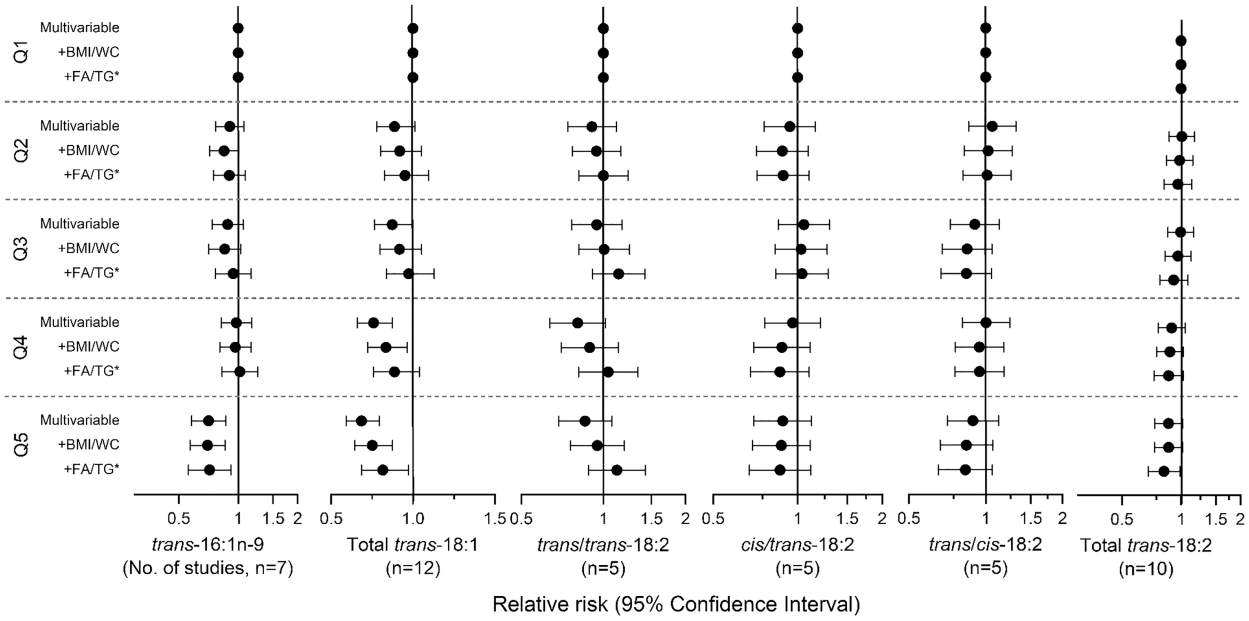

Figure 3—Pooled RRs of T2D per quintile of TFA biomarker. The association between TFA biomarkers and T2D was assessed in multivariable models adjusting for age, sex, race, field site (if applicable), education, occupation, physical activity, smoking, alcohol use, prevalent hypertension, prevalent dyslipidemia, and prevalent coronary heart disease; additionally adjusted for BMI and waist circumference (WC); and further adjusted for circulating palmitic acid, circulating stearic acid, circulating linoleic acid, and triglycerides (TG). Results were pooled using inverse-variance weighted meta-analysis. If multiple biomarkers are available in the study, one was chosen for the overall analysis based on its ability to reflect long-term dietary intake (in the order of preference): RBC, PL, and TP.

fermentation of cheese, probiotics in yogurt, or effects of other nutrients, such as vitamin D and calcium. This could partly explain the null findings in populations where TFA exposure is largely from dairy rather than industrial sources.

Two prior cohorts reported positive associations between circulating *trans*-16:1n-9, total *trans*-18:1 isomers and T2D (19), and circulating *trans*-18:1n-9 and T2D (18). These studies have potentially limited generalizability due to their demographics (only U.S.), and had limited statistical power to address heterogeneity. Furthermore, the cross-sectional study has significant limitations related to temporality of associations (18). It is important to note that our findings identifying no significant association are in line with published meta-analyses of cohort studies evaluating incidence of T2D associated with estimated dietary intake of TFA from self-reported questionnaires (34,35) as well as meta-analyses of randomized controlled clinical trials of TFA intake and glucose homeostasis (17). The two former meta-analyses using mostly self-reported dietary estimates reported no significant association between total dietary TFAs and T2D (RR 1.10, 95% CI 0.95–1.27, I^2 = 66% [35]; and 1.00, 0.95–1.06, I^2 = 67% [34]), and an inverse association between ruminant TFAs and T2D (RR 0.58, 95% CI 0.46–0.74, I^2 = 30%) (17); findings for industrial TFAs were not reported in both studies (34,35). Such studies estimating self-reported dietary TFA can be limited by imprecise food databases and changes in food TFA content over time.

Short-term trials have suggested that industrial TFAs may reduce insulin sensitivity among small groups of overweight or obese adults with diabetes or hyperlipidemia (15,16). However, a meta-analysis of randomized controlled trials pooling healthy and overweight populations with longer intervention times did not echo these findings (17). Thus, while the overall evidence remains inconsistent, the results of this meta-analysis together with our new findings suggest no major detrimental effect of modest exposure to TFAs on T2D risk among generally healthy populations. More importantly, prior dietary policy recommendations related to TFAs are based primarily upon dietary estimates of total TFA intake from food frequency questionnaires. Prior studies of circulating TFAs as biomarkers have raised the hypothesis that different TFAs may differ in their health effects (36). As a result, it is possible that not all TFAs or their food sources are harmful. Our findings therefore also support the need for additional studies to better understand the associations of specific circulating TFAs with other health outcomes. However, as TFAs are associated with higher risk of CHD and exert adverse effects on blood lipids, policies should continue to limit levels of TFAs in foods (10).

Our study has several strengths. The studies in our investigation included a range with varying follow-up times (maximum of 6–25 years), timing of TFA measurements (1990–2008), and region, including European, Australian, and Asian studies (12 studies across six nations), which increased generalizability. Selection bias was minimized by including prospective cohorts. Reporting bias typical of subjective dietary assessment which estimates intake levels from self-reported dietary questionnaires and estimates of food composition was also absent by using fatty acid biomarkers directly measured from circulation. Our findings therefore reflect potential biologic effects of individual TFA isomers. Furthermore, a standardized protocol and harmonized de novo analyses pooling several international cohorts reduced methodological heterogeneity and increased

statistical power, facilitating exploration of sources of heterogeneity across these cohorts. Most importantly, we also avoided publication bias by identifying and including cohorts before the results from any of them were known, especially as null or inverse findings in individual cohorts could have discouraged publication.

Several limitations warrant consideration. Different TFA isomers can be derived from both industrial and ruminant sources (4), which may lead to different overall health associations based on other characteristics of these foods. TFA levels were only assessed at baseline, and like most other biologic measures, plasma and/or RBC fatty acid levels can in theory change relatively rapidly over time. However, as habitual diets and other lifestyle do not tend to change dramatically over time, long-term reliability of circulating TFA measures is reasonable, similar to many other commonly assessed biologic risk factors such as blood pressure (23). While the long-term reliability of a single TFA measure at baseline is reasonable, within-individual changes or national actions to reduce TFA over time may have attenuated modest risk relationships toward the null. Our findings may not be generalizable to TFA exposure and risk of T2D in countries with higher exposures than in most high-income nations, such as Iran (8). Our observational analysis cannot fully exclude the possibility of residual confounding from known sources such as n-3 polyunsaturated fatty acids (37), which may be relevant for future analyses, as well as unknown sources, despite adjusting for major potential confounders.

In conclusion, among 12 major cohorts from six nations, circulating TFA biomarkers were not associated with higher incidence of T2D, while *trans*-16:1n-9, total *trans*-18:1 and total *trans*-18:2 were inversely associated. Findings may reflect a lack of biological harms of circulating TFA on T2D, the influence of mixed food sources of TFA (industrial vs. natural ruminant), or a general decline in TFA exposure due to policy efforts during this time period.

Acknowledgments. Acknowledgments are included in the Supplementary Material.

Funding. D.M. reports research funding from the National Institutes of Health (2R01HL115189-06A1), the Gates Foundation (OPP1176682), and the Rockefeller Foundation (2020 FOD 036). Additional individual funding and cohort-specific funding are included in the Supplementary Material. The funders of the study had no role in study design, data collection, data analysis, writing of the report, or the decision to submit for publication.

Duality of Interest. D.M. has received personal fees from Cleveland Clinic Foundation, Acasti Pharma, America's Test Kitchen, Barilla, Danone, GOED, and Motif FoodWorks; has served on scientific advisory boards for Beren Therapeutics, Brightseed, Calibrate, DayTwo (ended 6/20), Elysium Health, Filtricine, Foodome, HumanCo, January Inc, Perfect Day, Season, and Tiny Organics; and has received chapter royalties from UpToDate, all outside the submitted work. R.A.M. has consulted for and received compensation from Pharmavite LLC. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. H.T.M.L. and F.I. verified the underlying data. H.T.M.L., F.I., A.V.A.K., R.A.M., N.T., J.K.B., J.C., J.K., K.-L.C., and M.S. conducted the data analysis. All authors interpreted the data. H.T.M.L. and D.M. wrote the first draft of the article. All authors contributed to study conception and design. All authors reviewed and edited the manuscript. All authors had final responsibility for the decision to submit for publication. H.T.M.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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