Associations of circulating very-long-chain saturated fatty acids and incident type 2 diabetes: a pooled analysis of prospective cohort studies

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ABSTRACT

Background: Saturated fatty acids (SFAs) of different chain lengths have unique metabolic and biological effects, and a small number of recent studies suggest that higher circulating concentrations of the very-long-chain SFAs (VLSFAs) arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0) are associated with a lower risk of diabetes. Confirmation of these findings in a large and diverse population is needed.

Objective: We investigated the associations of circulating VLSFAs 20:0, 22:0, and 24:0 with incident type 2 diabetes in prospective studies.

Methods: Twelve studies that are part of the Fatty Acids and Outcomes Research Consortium participated in the analysis. Using Cox or logistic regression within studies and an inverse-variance-weighted meta-analysis across studies, we examined the associations of VLSFAs 20:0, 22:0, and 24:0 with incident diabetes among 51,431 participants.

Results: There were 14,276 cases of incident diabetes across participating studies. Higher circulating concentrations of 20:0, 22:0, and 24:0 were each associated with a lower risk of incident diabetes. Pooling across cohorts, the RR (95% CI) for incident diabetes comparing the 90th percentile to the 10th percentile was 0.78 (0.70, 0.87) for 20:0, 0.84 (0.77, 0.91) for 22:0, and 0.75 (0.69, 0.83) for 24:0 after adjustment for demographic, lifestyle, adiposity, and other health factors. Results were fully attenuated in exploratory models that adjusted for circulating 16:0 and triglycerides.

Conclusions: Results from this pooled analysis indicate that higher concentrations of circulating VLSFAs 20:0, 22:0, and 24:0 are each associated with a lower risk of diabetes. *Am J Clin Nutr* 2019;109:1216–1223.

Keywords: saturated fatty acids, very-long-chain saturated fatty acids, diabetes, meta-analysis, Fatty Acids and Outcomes Research

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Introduction

Type 2 diabetes is a major cause of morbidity and mortality, and the global burden of the disease has reached epidemic proportions. In 2014, the WHO estimated that 8.5% of adults 18 y of age or older have type 2 diabetes worldwide (1), and this estimate is expected to rise with an increase in life expectancy, global urbanization, and the adoption of Western lifestyles (2, 3). Identification of risk factors associated with the development of diabetes is therefore of considerable public health interest.

Circulating SFAs, which can be derived from both endogenous metabolic processes as well as diet, provide biomarkers of specific SFAs of different chain lengths. The very-long-chain SFAs (VLSFAs) arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0) are found in foods, such as peanuts, peanut butter, and macadamia nuts, and are also produced endogenously from the elongation of shorter-chain SFAs [e.g., palmitic acid (16:0) to stearic acid (18:0) and 20:0, and then of 20:0 to 22:0 and 24:0] (4). SFAs 16:0 and 18:0 may originate from dietary and metabolic sources; dietary sources of SFAs 16:0 and 18:0 include red meats, hard cheeses, and tropical oil (5, 6), whereas de novo lipogenesis in the presence of low-fat and high-carbohydrate diets (7–10) is a major metabolic pathway for synthesis of SFAs 16:0 and 18:0.

Recent studies suggest that associations of circulating SFAs with diabetes risk may vary by SFA chain length, likely due to the unique metabolic and biological effects of different SFAs (11–13). Particularly, these studies suggest that higher concentrations of circulating VLSFAs 20:0, 22:0, and 24:0 are associated with a lower risk of diabetes than lower concentrations of circulating VLSFAs 20:0, 22:0, and 24:0. Although these findings are important because circulating concentrations of VLSFAs are at least in part modifiable through diet (e.g., intake of peanut butter), the study designs, covariates of interest, measurement of VLSFAs, and ascertainment of incident diabetes differed across studies, and the generalizability of the study results is unknown because the studies were performed among primarily Caucasian adults in Europe (12, 13) and the United States (11). To address this gap, we investigated the associations of circulating VLSFAs 20:0, 22:0, and 24:0 with incident type 2 diabetes among 12 prospective cohort studies as part of the Fatty Acids and Outcomes Research Consortium (FORCE) (14).

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Supplemental Tables 1–3 and Supplemental Figures 1–9 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CCCC, Chin-Shan Community Cardiovascular Cohort Study; CHS, Cardiovascular Health Study; EPIC-Interact, European Prospective Investigation into Cancer-InterAct; FORCE, Fatty Acids and Outcomes Research Consortium; IRAS, Insulin Resistance Atherosclerosis Study; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; MCCS, Melbourne Collaborative Cohort Study; MESA, Multi-Ethnic Study of Atherosclerosis; METSIM, Metabolic Syndrome in Men Study; SFA, saturated fatty acids; TG, triglyceride; VLSFA, very-long-chain SFA; WHIMS, Women's Health Initiative Memory Study; 3C Study, Three City Study.

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We hypothesized that higher concentrations of VLSFAs 20:0, 22:0, and 24:0 are associated with a lower risk of type 2 diabetes.

Methods

Study sample

The study sample comprised participants from 12 prospective cohort studies that are part of the FORCE: a consortium derived from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) to examine the associations of circulating fatty acids of interest with nongenetic outcomes. Details on FORCE have been described previously (15, 16). For the present analysis, we included all cohorts that are part of FORCE who were interested in the project and who had available data on VLSFAs, and whose cohorts included participants 18 y of age or older who were free of prevalent type 2 diabetes (as defined by self-reported diabetes, fasting glucose ≥126 mg/dL, or use of diabetes drugs) at the time of fatty acid measurement (**Supplemental Figure 1**). Standardized analysis plans were developed and provided to each of the 12 participating cohorts, including inclusion and exclusion criteria; definitions for exposures, outcomes, and covariates of interest; and a detailed statistical analysis protocol. Contributing studies included: the Age, Gene, Environmental Susceptibility-Reykjavik Study (17); the Chin-Shan Community Cardiovascular Cohort Study (CCCC) (18); the Cardiovascular Health Study (CHS) (19); the Framingham Heart Study (20); the European Prospective Investigation into Cancer-InterAct (EPIC-Interact) (21); the Insulin Resistance Atherosclerosis Study (IRAS) (22); the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) (23); the Melbourne Collaborative Cohort Study (MCCS) (24); the Multi-Ethnic Study of Atherosclerosis (MESA) (25); the Metabolic Syndrome in Men Study (METSIM) (26); the Women's Health Initiative Memory Study (WHIMS) (27); and the Three City Study (3C-Study) (28). Of the 12 participating studies, 2 (CHS and EPIC-Interact) have previously assessed associations of VLSFAs and incident diabetes (11, 12). All procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. Each participating study had local institutional review board approval and written informed consent was obtained from all participants.

VLSFA assessment

Details on the measurement of circulating fatty acid biomarkers for each participating cohort are described in **Supplemental Table 1**. In brief, gas chromatography was used to assess individual fatty acid concentrations in each cohort in ≥1 lipid compartment including plasma phospholipids (the Age, Gene, Environmental Susceptibility-Reykjavik Study, CHS, EPIC-Interact, MCCS, MESA, METSIM), total plasma (CCCC, IRAS, KIHD, 3C Study), or red blood cells (Framingham Heart Study, WHIMS, 3C Study, METSIM). Fatty acid levels in each cohort were expressed as a percentage of total measured fatty acids. A list of the VLSFAs of interest available in each study is given in **Table 1**; all studies had each of VLSFA 20:0, 22:0, and 24:0 available, except KIHD did not measure VLSFA 20:0, and MESA and 3C Study did not measure VLSFA 24:0.

Ascertainment of incident diabetes

Cohort-specific methods for assessing development of diabetes are described in detail in Supplemental Table 1. Briefly, for most participating cohorts, incident diabetes was defined based on ≥1 criterion: fasting glucose concentrations ≥126 mg/dL, nonfasting or 2-h postchallenge glucose concentrations ≥200 mg/dL, glycated hemoglobin ≥6.5%, use of insulin or oral hypoglycemic medications, or self-report. For 3 European studies (EPIC-InterAct, KIHD, and METSIM), diabetes was ascertained by linkage to registries of primary care, secondary care, medication use, hospital admissions, or mortality.

Measurement of covariates

The standardized analysis plan included detailed definitions and categorizations for the major risk factors of interest, including physical activity, smoking, alcohol use, prevalent hypertension, prevalent dyslipidemia, prevalent coronary artery disease, and self-reported health status. The standardized definitions of risk factors were adopted to minimize heterogeneity across cohorts (29). Details on data collection methods for covariates for each cohort are described in Supplemental Table 1.

Cohort statistical analyses

Each cohort performed new individual-level analyses and provided results to the lead author (AMF) using a standardized electronic form. In 10 of the participating cohorts, Cox regression models were used to examine the associations of each VLSFA of interest with incident type 2 diabetes. For these study participants, follow-up time was assessed from baseline (i.e., time of fatty acid measurement) to date of development of incident diabetes, death from any cause, or loss to follow-up. The MCCS and the IRAS did not have detailed time-to-event data available for participants, and therefore used logistic regression. For each study, each VLSFA was incorporated in models as a continuous linear variable in units of the study-specific interquintile range (i.e., the difference between the 90th and 10th percentiles) and, in separate models, as quintiles in indicator (i.e., dummy) categorical variables with the referent group as the lowest quintile of each circulating VLSFA: 20:0, 22:0, or 24:0. Each cohort estimated coefficients and SEs for the associations from 3 prespecified multivariable models. The first model adjusted for major potential confounders including age, sex, clinic, race, education, physical activity, smoking, alcohol use, prevalent hypertension, prevalent dyslipidemia, prevalent coronary artery disease, and self-reported health status. A second model also adjusted for BMI and waist circumference to better understand if these factors influence the associations of each VLSFA with incident diabetes (primary model). A third model (exploratory model) further adjusted for circulating SFA 16:0 and triglycerides (TGs) (model 3), biomarkers of hepatic de novo lipogenesis in the presence of low-fat and high-carbohydrate diets (30, 31). In the CHS, SFA 16:0 and TGs were shown to potentially mediate or confound the association of VLSFAs and incident diabetes (11).

We examined the potential interactions of age, sex, and BMI with each VLSFA of interest (modeled linearly) on risk of incident diabetes. Participating cohorts provided coefficients and SEs for multiplicative interaction terms for each factor of interest

with each VLSFA of interest, after adjustment for the covariates included in the primary model described above.

Meta-analyses

Results from each cohort were compiled and combined using inverse-variance-weighted meta-analysis in STATA version 13.1 (Stata Corporation). Inverse-variance-weighted fixed-effects meta-analysis approximates results that would be obtained if the data from all studies could be analyzed together with adjustment for study (32). Heterogeneity between studies was assessed using the I^2 index derived from the Cochran Q statistic (33). In preliminary meta-analyses, IRAS contributed 65–71% of the sample weight in each model despite a small total sample size ($n = 719$) and few cases of diabetes ($n = 146$) owing to influential outliers in concentrations of VLSFAs for some participants. As the cohort was unable to provide updated results excluding influential outliers, it was subsequently excluded from primary analyses. In sensitivity analyses, we repeated each meta-analysis omitting 1 cohort at a time to confirm that individual cohorts were not overly influencing the observed levels of association. We also performed additional exploratory meta-regression according to lipid compartment (i.e., plasma phospholipid, total plasma, or red blood cell measures) and region (i.e., cohorts based in the United States, Europe, Asia, or Australia).

Results

Descriptions and baseline characteristics for each of the 12 participating cohorts are given in **Tables** 1 and **2**. The mean cohort age ranged from 52.3 y to 76.0 y, and BMI (in kg/m^2) from 23.2 to 28.1. Mean cohort fasting glucose ranged from 86.4 to 104.8 mg/dL. Most cohorts included primarily participants of European descent, although several included significant proportions of other races/ethnicities including the CCCC study (100% Chinese), the Cardiovascular Health Study (11% African American), IRAS (33.2% Hispanic, 24.5% African American), MESA (23.9% Hispanic, 22.2% African American, 25.5% Asian), and WHIMS (6.0% African American, 2.1% Hispanic, 1.7% Asian). Mean cohort levels of each VLSFA ranged from 0.13% to 0.62% for VLSFA 20:0, 0.23% to 1.67% for VLSFA 22:0, and 0.20% to 4.0% for VLSFA 24:0. For most cohorts, VLSFA 20:0, 22:0, and 24:0 were moderately-to-highly correlated, and each VLSFA was negatively correlated with circulating 16:0 (**Supplemental Table 2**). Circulating concentrations of fatty acids were generally similar across region (i.e., cohorts based in the United States, Europe, Asia, or Australia) or year of blood sampling (data not shown).

Higher circulating concentrations of 20:0, 22:0, and 24:0 were each associated with a lower risk of incident diabetes. Across the 11 studies, there were 14,276 cases of incident diabetes. Pooling across cohorts, comparing the 90th percentile to the 10th percentile, the RR of incident diabetes was 0.78 (95% CI: 0.70, 0.87) for 20:0, 0.84 (95% CI: 0.77, 0.91) for 22:0, and 0.75 (95% CI: 0.69, 0.83) for 24:0 after adjustment for age, sex, site, race, education, occupation, physical activity, smoking, alcohol use, hypertension, dyslipidemia, coronary heart disease, self-reported health status, BMI, and waist circumference (**Figure 1**). The model without BMI and waist circumference did not produce materially different results (**Supplemental Figure 2**). In contrast, further adjustment for TGs and circulating SFA 16:0 fully attenuated observed associations with RRs across the interquintile range of 0.93 (95% CI: 0.83, 1.04) for 20:0, 1.04 (95% CI: 0.94, 1.14) for 22:0, and 0.97 (95% CI: 0.88, 1.08) for 24:0 (**Supplemental Figure 3**). Omitting 1 cohort at a time did not materially alter RR estimates (data not shown). Results were similar in: *1)* analyses that included IRAS (**Supplemental Figures 4–6**) and *2)* pooled analyses that assessed each VLSFA in quintiles as indicator categories (**Supplemental Figures 7–9**).

We observed little evidence of effect modification for each VLSFA with age, sex, or BMI on risk of incident diabetes (**Supplemental Table 3**). Although we observed notable het-

TABLE 1 Description of 12 studies that participated in analyses of circulating very-long-chain SFAs and incident diabetes[1]

Study	Country	Study design[2]	Biomarker compartment	Year of blood sampling	Fatty acids assessed	Year follow-up ended
AGES-Reykjavik	Iceland	PC	Plasma phospholipid	2002–2006	20:0, 22:0, 24:0	2007–2011
CCCC	Taiwan	PC	Total plasma	1992	20:0, 22:0, 24:0	2000
CHS	United States	PC	Plasma phospholipid	1992–1993	20:0, 22:0, 24:0	2011
EPIC-InterAct	Europe	PCC	Plasma phospholipid	1993–1997	20:0, 22:0, 24:0	2007
FHS	United States	PC	Red blood cells	2005–2008	20:0, 22:0, 24:0	2015
IRAS	United States	PCC	Total plasma	1992–1994	20:0, 22:0, 24:0	1999
KIHD	Finland	PC	Total plasma	1998–2001	22:0, 24:0	2010
MCCS	Australia	PC	Plasma phospholipid	1992	20:0, 22:0, 24:0	2002
MESA	United States	PC	Plasma phospholipid	2000–2002	20:0, 22:0	2010–2012
METSIM	Finland	PC	Plasma phospholipid	2006–2010	20:0, 22:0, 24:0	2014
WHIMS	United States	PC	Red blood cells	1995	20:0, 22:0, 24:0	2009
3C Study	France	PC	Red blood cells, total plasma	1999–2000	20:0, 22:0	2011–2012

[1] AGES-Reykjavik, Age, Gene, Environmental Susceptibility-Reykjavik Study (17); CCCC, Chin-Shan Community Cardiovascular Cohort Study (18); CHS, Cardiovascular Health Study (19); EPIC-Interact, European Prospective Investigation into Cancer-InterAct (21); FHS, Framingham Heart Study (20); IRAS, Insulin Resistance Atherosclerosis Study (22); KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study (23); MCCS, Melbourne Collaborative Cohort Study (24); MESA, Multi-Ethnic Study of Atherosclerosis (25); METSIM, Metabolic Syndrome in Men Study (26); PC, prospective cohort; PCC, prospective nested case-control; WHIMS, Women's Health Initiative Memory Study (27); 3C Study, Three City Study (28).

[2] Details on the design of each study are described in Supplemental Table 1.

TABLE 2 Characteristics of participating cohorts at time of fatty acid biomarker measurement[1]

Study	*n* (incident cases of diabetes)	Age, y	Sex (% female)	BMI (kg/m^2)	20:0[2]	22:0[2]	24:0[2]	Baseline fasting glucose, mg/dL
AGES-Reykjavik	753 (28)	75.5 ± 5.2	59.5	27.0 ± 4.0	0.62 ± 0.10	1.67 ± 0.27	1.33 ± 0.22	99.0 ± 8.9
CCCC	616 (128)	58.7 ± 9.7	40.0	23.2 ± 2.9	0.48 ± 0.29	0.18 ± 0.31	0.80 ± 0.33	104.8 ± 13.8
CHS	3107 (282)	75.1 ± 5.3	61.5	26.4 ± 4.5	0.50 ± 0.08	1.70 ± 0.32	1.40 ± 0.28	97.8 ± 9.8
EPIC-InterAct	27,296 (12,132)	52.3 ± 9.2	62.3	26.0 ± 4.2	0.13 ± 0.04	0.24 ± 0.08	0.23 ± 0.07	89.3 ± 23.2
FHS	1870 (95)	64.4 ± 8.3	57.2	27.8 ± 5.0	NA	NA	0.42 ± 0.16	100.1 ± 9.1
IRAS	719 (146)	55.1 ± 8.5	55.8	28.4 ± 5.6	0.11 ± 0.03	0.23 ± 0.08	0.20 ± 0.08	98.1 ± 11.1
KIHD	1543 (205)	62.7 ± 6.5	52.7	27.6 ± 4.4	NA	0.48 ± 0.09	0.48 ± 0.12	86.4 ± 8.1
MCCS	5617 (485)	56.3 ± 8.6	53.9	27.0 ± 4.4	0.25 ± 0.07	0.71 ± 0.17	0.59 ± 0.15	99.6 ± 9.2
MESA	2252 (309)	60.9 ± 9.7	53.9	27.6 ± 5.4	0.25 ± 0.09	0.56 ± 0.29	NA	89.7 ± 10.7
METSIM	1302 (71)	55.0 ± 7.1	0	26.4 ± 3.5	0.38 ± 0.07	0.74 ± 0.16	0.62 ± 0.14	102.8 ± 8.3
WHIMS	6510 (502)	70.1 ± 3.8	100.0	28.1 ± 5.5	0.13 ± 0.06	0.16 ± 0.10	0.35 ± 0.24	94.7 ± 9.8
3C Study	565 (39)	76.0 ± 4.0	64.3	25.0 ± 4.0	0.46 ± 0.08	1.00 ± 0.30	NA	88.0 ± 10.0

[1] Values are means ± SDs unless otherwise indicated. AGES-Reykjavik, Age, Gene, Environmental Susceptibility-Reykjavik Study (17); CCCC, Chin-Shan Community Cardiovascular Cohort Study (18); CHS, Cardiovascular Health Study (19); EPIC-Interact, European Prospective Investigation into Cancer-InterAct (21); FHS, Framingham Heart Study (20); IRAS, Insulin Resistance Atherosclerosis Study (22); KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study (23); MCCS, Melbourne Collaborative Cohort Study (24); MESA, Multi-Ethnic Study of Atherosclerosis (25); METSIM, Metabolic Syndrome in Men Study (26); WHIMS, Women's Health Initiative Memory Study (27); 3C Study, Three City Study (28).

[2] Measured as percentage of total fatty acids.

erogeneity between studies (i.e., the I^2 index for each primary analysis of VLSFA 20:0, 22:0, and 24:0 was 78.3%, 60.5%, and 57.2%, respectively), the heterogeneity was not explained by region (i.e., cohorts in Europe, the United States, Australia, or Asia) or fatty acid compartment (i.e., plasma phospholipids, total plasma, or red blood cells) in post hoc meta-regression analyses (data not shown). Although fixed-effects meta-analyses have been shown to produce valid estimates of risk across heterogeneous studies (34), in sensitivity analyses, we reran all analyses using a random-effects meta-analysis, and results were similar (data not shown).

Discussion

The results from this pooled analysis of new, harmonized, individual-level analyses in 12 prospective cohort studies globally indicate that higher concentrations of circulating VLSFAs 20:0, 22:0, and 24:0 are each associated with a lower risk of diabetes. Results were robust to adjustment for major diabetes risk factors, including measures of adiposity. In comparison, results were fully attenuated after adjustment for circulating 16:0 and TGs (30).

The relative contributions of metabolism and diet on circulating concentrations of VLSFAs are unknown, but studies provide evidence that VLSFAs, as well as other SFAs, are derived from both endogenous and dietary sources. For example, these fatty acids can be synthesized from the elongation of 18:0 to 20:0, 22:0, and then 24:0 (4, 35, 36). In the diet, VLSFAs 20:0, 22:0, and 24:0 are contained in meaningful amounts only in selected foods, including peanuts, peanut butter, and Macadamia nuts (37, 38). A previous study indicated that consumption of peanuts and peanut butter is inversely associated with diabetes risk (39). Although the authors attributed these findings to the high amounts of monounsaturated fat, polyunsaturated fat, fiber, and magnesium found in peanuts and peanut butter (39), the findings reported herein suggest that VLSFAs 20:0, 22:0, and 24:0 contained in these foods may also partly explain these associations. In other words, circulating VLSFAs may be a marker of peanut, peanut butter, or Macadamia nut consumption—which is associated with diabetes risk.

Compared to other long-chain SFAs, VLSFAs possess properties that appear to have distinct effects on specific biological processes, although these processes are complex and not completely understood (40–44). For example, circulating VLSFAs are major components of ceramides and sphingomyelins, and it is possible that the inverse associations of VLSFAs and incident diabetes reported herein may be explained at least in part by the impact of ceramides and sphingomyelins on diabetes-related pathways. Both animal and in vitro studies suggest that *1*) ceramides play a role in insulin resistance and glucose homeostasis (40, 41) and *2*) effects of ceramides and sphingomyelins on cardiometabolic outcomes may be dependent on the chain length of the incorporated fatty acids. For instance, ceramides of different chain lengths differentially permeabilize mitochondria (43), and studies in animal and in vitro models have indicated that ceramides containing SFA 16:0 induce apoptosis in β-cells (42, 44), whereas ceramides containing fatty acids 20:0 and 22:0 inhibit apoptosis in β-cells (42, 45–47). Apoptosis may influence type 2 diabetes by means of β-cell death and reduced insulin secretion (48–51).

The findings in the present investigation were fully attenuated after adjustment for SFA 16:0 and TGs in exploratory models. This attenuation may be due to potential mediation by SFA 16:0 and TGs of observed associations of VLSFAs with diabetes risk (11). Alternatively, as SFA 16:0 is inversely associated with VLSFAs 20:0, 22:0, and 24:0, it has been proposed that this attenuation may be a result of residual confounding due to an unhealthy lifestyle. This theory is based on the premise that SFA 16:0 may be a marker of poor diet quality (i.e., diet high in red meats, processed meats), and thus low

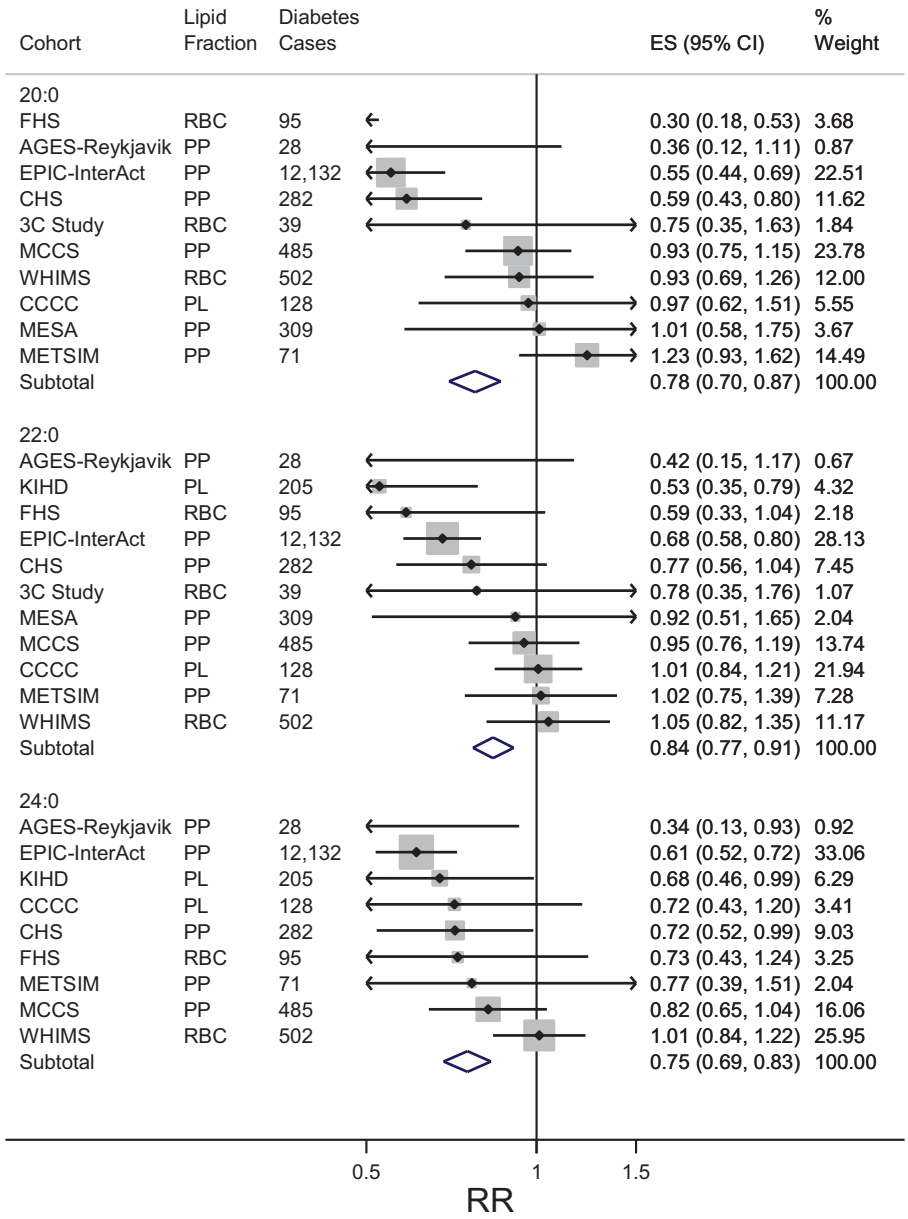

FIGURE 1 Forest plots of prospective associations of circulating very-long-chain SFAs with incident diabetes in 11 studies. RR and 95% CI per interquintile range (medians of the first and fifth quintile in each cohort) are represented by a filled circle and horizontal line for each cohort, and by a diamond for the overall pooled results. Cohort-specific associations were assessed in multivariable models adjusted for age, sex, clinic, race, education, physical activity, smoking, alcohol use, prevalent hypertension, prevalent dyslipidemia, prevalent coronary heart disease, self-reported health status, BMI, and waist circumference. The size of the shaded square is a marker of study weight in the inverse-variance-weighted meta-analysis. AGES-Reykjavik, Age, Gene, Environmental Susceptibility-Reykjavik Study (17); CCCC, Chin-Shan Community Cardiovascular Cohort Study (18); CHS, Cardiovascular Health Study (19); EPIC-Interact, European Prospective Investigation into Cancer-InterAct (21); ES, effect size; FHS, Framingham Heart Study (20); IRAS, Insulin Resistance Atherosclerosis Study (22); KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study (23); MCCS, Melbourne Collaborative Cohort Study (24); MESA, Multi-Ethnic Study of Atherosclerosis (25); METSIM, Metabolic Syndrome in Men Study (26); PC, prospective cohort; PCC, prospective nested case-control; PL, total plasma; PP, plasma phospholipid; RBC, red blood cell; WHIMS, Women's Health Initiative Memory Study (27); 3C Study, Three City Study (28).

concentrations of circulating VLSFAs may reflect metabolic dysfunction associated with poor diet quality (31). In addition, high TGs are a major risk factor for insulin resistance [and a marker of an unhealthy diet high in simple carbohydrates and processed meats, and low levels of physical activity (52)], and VLSFAs have been shown to be associated with lower concentrations of fasting TGs in previous studies (11, 53). These theories are hypothesis-generating, and more studies are needed to better understand the interplay of VLSFAs, 16:0, TGs, and diabetes risk.

To date, only a handful of studies have assessed the associations of VLSFAs 20:0, 22:0, and 24:0 with diabetes risk (11–13). In the CHS and EPIC-Interact, both included in the present study, 20:0, 22:0, and 24:0 were each associated with a lower risk of

diabetes (11, 12). Our analysis builds upon and greatly extends these prior findings by pooling data from 12 prospective studies from 13 countries and 4 continents—and incorporates data on an additional 22,000 participants not previously included in previous reports (11, 12).

Our study has several strengths. To our knowledge, this is the largest and most complete analysis to date to examine the associations of VLSFAs with incident diabetes. Owing to the richness of the data available for each participating study, we were able to employ a standard analysis plan to perform de novo individual-level analyses and adjust for major potential confounders and mediators. The 12 participating studies also represent a broad range of ages, geographical regions, and background diets, increasing generalizability. Compared to reports of individual studies, for which positive results are much more likely to be published, our methods for identifying and including studies reduce the possibility of publication bias.

This study also has potential limitations. Circulating fatty acids were only measured at a single time, and we were unable to adjust for changes in VLSFA concentrations over time in this meta-analysis. Given the prospective design, changes in VLSFA concentrations over time would likely attenuate results toward the null. Our study sample comprised primarily participants of European descent, although several of the cohorts included significant numbers of other races/ethnicities. Although we adjusted for several factors that may be associated with SFAs and diabetes, residual confounding by imprecisely measured or unknown factors is possible. In addition, the intercorrelations of VLSFAs make it challenging to interpret the independent associations of each individual VLSFA with risk of diabetes. Finally, because our primary interest was in circulating concentrations of VLSFAs, analyses of the relations of foods that contain VLSFAs with incident diabetes were beyond the scope of this project.

In conclusion, the results of this study suggest that higher concentrations of circulating VLSFAs 20:0, 22:0, and 24:0 are each associated with a lower risk of diabetes, perhaps because of their association with lower de novo lipogenesis. This study adds to the growing body of evidence that supports positive health outcomes with higher concentrations of VLSFAs (11, 12, 37, 54) and highlights the need for additional research studies to identify relevant biological mechanisms and pathways that may contribute to observed associations.

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