Evaluation of various biomarkers as potential mediators of the association between Δ5 desaturase, Δ6 desaturase, and stearoyl-CoA desaturase activity and incident type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition–Potsdam Study[1,2]

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ABSTRACT

Background: An association between desaturase activity and risk of type 2 diabetes (T2D) has been found in epidemiologic studies, but little is known about potential mediators of this association.

Objective: We aimed to investigate the potential role of diabetes-related biomarkers as mediators of the association between estimated Δ5 desaturase (D5D), Δ6 desaturase (D6D), and stearoyl-CoA desaturase (SCD) activity and T2D risk.

Design: We analyzed a case-cohort study (subcohort: n = 1533; verified incident T2D cases: n = 400), nested within the European Prospective Investigation into Cancer and Nutrition–Potsdam Study involving 27,548 middle-aged participants. We evaluated the impact of adjustment for several T2D-related biomarkers reflecting liver fat accumulation [reflected by γ-glutamyltransferase (GGT), alanine transaminase (ALT), fetuin-A, and the algorithm-based fatty liver index (FLI)], dyslipidemia (high-density lipoprotein cholesterol, triglycerides), inflammation [C-reactive protein (CRP)], and adiponectin on the association between D5D, D6D, and SCD activity, estimated with fatty acid product-to-precursor ratios derived from erythrocyte membrane proportions, and T2D risk.

Results: Estimated D5D activity was inversely associated with T2D risk, whereas D6D and SCD activities were positively associated with risk of T2D [HRs (95% CIs) (highest vs. lowest tertile): 0.51 (0.36, 0.73), 1.68 (1.18, 2.39), and 1.82 (1.29, 2.58), respectively]. The association between estimated D5D, D6D, and SCD activities and risk of T2D was statistically significantly and markedly attenuated after adjustment for the FLI and, to a lesser extent, after adjustment for triglycerides, whereas adjustment for other desaturase-associated biomarkers (CRP, fetuin-A, ALT, and GGT) did not lead to appreciable attenuations.

Conclusions: Liver fat accumulation, as reflected by the FLI, and dyslipidemia, as reflected by triglycerides, may partly explain the association between estimated D5D, D6D, and SCD activity and T2D risk. *Am J Clin Nutr* 2015;102:155–64.

Keywords: diabetes mellitus type 2, biomarkers, stearoyl-coenzyme-A desaturase, Δ5 desaturase, Δ6 desaturase, mediator analysis, erythrocyte fatty acids

INTRODUCTION

The fatty acid (FA)[7] composition of biological tissues is influenced by dietary intake of FAs as well as the endogenous FA metabolism, in which desaturase enzymes play an important role (1). Desaturase enzymes catalyze the formation of unsaturated FAs by inserting double bonds into the FA carbon chain. Stearoyl-CoA desaturase (SCD) catalyzes the synthesis of MUFAs from SFAs, whereas Δ5 desaturase (D5D) and Δ6 desaturase (D6D) are necessary for the formation of long-chain PUFAs. Desaturase activity can be estimated indirectly as a product-to-precursor FA ratio in human tissue (2). In a prior study in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam, we found an inverse association between estimated D5D activity and type 2 diabetes (T2D) risk and a direct association between estimated D6D and SCD activity and T2D risk (3), mainly in line with other studies (4–6). We corroborated the inverse relation of D5D activity and the direct relation of

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[2] Supplemental Tables 1–6 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[7] Abbreviations used: ALT, alanine transaminase; CRP, C-reactive protein; D5D, Δ5 desaturase; D6D, Δ6 desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; FA, fatty acid; *FADS*, fatty acid desaturase; FLI, fatty liver index; GGT, γ-glutamyltransferase; PPAR, peroxisome proliferator–activated receptor; SCD, stearoyl-CoA desaturase; T2D, type 2 diabetes.

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D6D activity with T2D risk by a Mendelian randomization approach (3).

The biological mechanisms that link estimated desaturase activity with chronic diseases such as T2D are not well understood yet. Given the fact that FAs may act as ligands for transcription factors such as peroxisome proliferator–activated receptors (PPARs), whose expression results in the transcriptional stimulation of several genes, including lipid metabolism (7), hepatic metabolism (8), inflammation, and adiponectin (7), an involvement of these pathways appears plausible in this context.

D6D is the rate-limiting step of the whole PUFA pathway (7), and PUFAs such as arachidonic acid are precursors from eicosanoids, which may act as inflammatory mediators (7). Increased D5D activity has been associated with high plasma concentrations of EPA and DHA (9), which have anti-inflammatory properties and triglyceride-lowering effects (7). An involvement of dyslipidemia, liver fat accumulation, and inflammation in the relation between D5D and D6D activity and T2D risk is supported by associations between genetic variation in fatty acid desaturase genes, which encode the D5D and D6D, and triglycerides (10), liver enzymes (11), and C-reactive protein (CRP) (12, 13). The enzyme SCD is the rate-limiting enzyme in the synthesis of MUFAs (14). Relations of genetic *SCD1* variants with triglycerides (14), CRP (14, 15), and γ-glutamyltransferase (GGT) and alanine transaminase (ALT) activity (16) indicate a role of dyslipidemia, inflammation, and increased liver fat in the relation of SCD activity with T2D risk. Taking together these facts, we hypothesize direct associations of estimated SCD and D6D activity with markers of hepatic fat accumulation, triglycerides, and CRP and inverse relations with adiponectin, whereas we hypothesize estimated D5D activity to be inversely related to liver markers, triglycerides, and CRP and positively related to adiponectin.

To our knowledge, a potential mediating role of various biomarkers on the association between estimated desaturase activity and T2D risk has not been investigated yet. Thus, the aim of this study was to evaluate a potential mediating role of diabetes-related biomarkers reflecting different metabolic pathways, including dyslipidemia, liver fat accumulation, inflammation, and adiponectin, in linking estimated desaturase activity to T2D risk.

METHODS

Study population

The EPIC-Potsdam study is part of the multicenter prospective cohort study EPIC (17). EPIC-Potsdam includes 16,644 women mainly aged 35–64 y and 10,904 men mainly aged 40–64 y recruited from the general population of the city of Potsdam, Germany, and surrounding municipalities from 1994 to 1998. Information on education, smoking, and physical activity was assessed at baseline with a self-administered questionnaire and a personal computer-guided interview (17). Dietary intake was assessed with a self-administered validated food-frequency questionnaire. Energy intake was calibrated to 24-h recall data as described in detail elsewhere (3). The baseline assessment also included the collection of blood samples. Anthropometric measurement procedures followed a standardized protocol (17). Informed consent was obtained from all participants, and approval was given by the ethics committee of the state of Brandenburg, Germany.

A case-cohort study (18) within EPIC-Potsdam was designed. A random sample of 2500 subjects was drawn from all participants of EPIC-Potsdam who provided a blood sample ($n = 26,444$) for a subcohort of which 1533 participants (972 women and 561 men) remained for our analysis after exclusion of prevalent or nonverified cases of T2D ($n = 120$), participants with uncompleted follow-up questionnaire ($n = 58$), participants with missing or implausible data on biomarkers and FAs ($n = 639$), participants taking liver or lipid-lowering medication ($n = 78$), and participants with insufficient filled blood monovettes ($n = 72$). By use of a randomly selected subcohort and the appropriate statistics for this type of research design, the results were expected to be generalizable to the entire cohort without the need of biomarker measurements in the entire cohort. Of the 849 participants with incident diabetes identified in the full cohort during a mean follow-up time of 7 y, 801 provided blood samples; 400 of those participants (229 men and 171 women) remained for analyses after identical exclusion criteria were applied.

Because the subcohort was representative of the full cohort at baseline, it included 37 individuals (18 men, 19 women) who developed T2D during follow-up [so-called internal cases (18)]. Consequently, a total of 363 of the 400 incident cases were identified in the rest of the total cohort and constituted the so-called external cases for analyses (18).

Measurement of FA composition of erythrocytes and estimation of desaturase activities

In total, 30 mL of blood was obtained from each participant during baseline examination, mostly in the nonfasting status. Plasma, serum, red blood cells, and buffy coat were stored at −80°C. The erythrocyte membrane FAs were analyzed between February and June 2008. Thirty-two FAs were determined by gas chromatography and expressed as the percentage of total FAs present in the chromatogram (3). Detailed information with respect to the storage conditions of samples, sample preparation, and analytic procedures was described elsewhere (3). In brief, FA methyl esters were separated on a GC-3900 gas chromatograph (Varian Inc.) equipped with a 100-m × 0.25-mm internal diameter wall-coated open tubular-fused silica capillary column and flame ionization detector with separation of FA methyl ester peaks based on mixed FA methyl ester standards (Sigma-Aldrich). The Galaxie software version 1.9.3.2 (Varian Inc.) was used for quantification and identification of peaks. Intra-assay CVs calculated from a total number of 40 FA measurements in a subset of 20 samples were ≤10% for all FAs used in this analysis, with the exception of 18:3n–6 (18.7%).

The desaturase activities were estimated as product-to-precursor ratios of individual FAs in erythrocyte membranes as follows: 16:1n–7/16:0 to reflect SCD activity, 18:3n–6/18:2n–6 to reflect D6D activity, and 20:4n–6/20:3n–6 to reflect D5D activity (3).

Measurement of biomarkers

For the current analysis, we considered those biomarkers that have been shown to be associated with risk of T2D in the EPIC-Potsdam study (19, 20) liver markers (such as GGT, ALT, and

fetuin-A), biomarkers of dyslipidemia (such as HDL cholesterol and triglycerides), inflammation (such as CRP), and an adipokine (adiponectin). Furthermore, the diabetes-related (21, 22) fatty liver index (FLI) after the definition from Bedogni et al. (23) was used to reflect liver fat accumulation in this analysis, which was calculated based on the following formula:

$$\mathrm{FLI} = \frac{e^{(0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745)}}{1 + e^{(0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745)}} \times 100 \quad (1)$$

(GGT in U/L, triglycerides in mg/dL, waist circumference in cm, and BMI in kg/m^2)

Measurement of plasma HDL cholesterol, triglycerides, GGT, ALT, CRP, and fetuin-A followed standard procedures, which are described in detail elsewhere (16, 19). Plasma total adiponectin concentrations were determined by an ELISA (Linco Research). All assays were performed according to the manufacturer's description. Inter-assay CVs were <2.0% for plasma CRP, <8.5% for adiponectin, <1.5% for triglycerides, <2.1% for GGT, <2.5% for ALT, and 5.4% for fetuin-A (19). Plasma concentrations were multiplied by 1.16 for women and 1.17 for men to obtain concentrations for these citrate plasma samples comparable to concentrations obtainable from EDTA plasma (24).

Definition of cases of T2D

Follow-up questionnaires were sent out every 2–3 y to identify incident cases of T2D with response rates of >90% (25). The verification of self-reports was performed via questionnaires mailed to physicians. For this analysis, we considered the data until the end of the fourth follow-up period (August 2005). Systematic information sources for incident cases were self-reports of a T2D diagnosis, T2D-relevant medication, and dietary treatment due to T2D during follow-up. Furthermore, we obtained additional information from death certificates or from random sources, such as the tumor centers, physicians, or clinics that provided assessments from other diagnoses. Although self-reports of T2D were generally reliable (26), by including other sources of information, we even improved the completeness of case ascertainment. Once a participant was identified as a potential case, disease status was further verified by sending a standard inquiry form to the treating physician. Only physician-verified cases with a diagnosis of T2D (International Classification of Diseases, 10th revision code: E11) and a diagnosis date after the baseline examination were considered confirmed incident cases of T2D.

Statistical methods

A cross-sectional analysis restricted to the subcohort (n = 1533) was performed to investigate associations between estimated desaturase activity and biomarkers by using multiple linear regression. This analysis was stratified by sex because sex-depending associations between desaturase activity and certain biomarkers (such as fetuin-A, GGT, and HDL cholesterol) were detected. The association between estimated D5D, D6D, and SCD activity and biomarkers of dyslipidemia in the EPIC-Potsdam study has been evaluated in a previous analysis (27). Because different exclusion criteria were applied in the prior analysis, the association between estimated desaturase activity and biomarkers of dyslipidemia was recalculated in the present data set to ensure comparability with other biomarkers. We used the $\log_e$ transformation of triglycerides, ALT, GGT, the FLI, CRP, and adiponectin to normalize the right-skewed distributions. We modeled the FA ratios as tertiles to account for nonlinear relations with the outcomes. We estimated geometric means and 95% CIs for triglycerides, ALT, GGT, and the FLI and arithmetic means and 95% CIs for fetuin-A and HDL cholesterol by FA ratio tertiles.

The significance of linear trends for biomarkers across FA ratio tertiles was tested by assigning each participant the median value of the respective FA ratio for the FA ratio tertile and modeling this median value as a value of a continuous variable. The P-trend value indicates whether the biomarker statistically significantly falls or rises across the medians of the FA ratio tertiles. Two different models were calculated, including an age-adjusted model and a model also adjusted for the following variables to account for potential confounding: smoking status (never, past, current smoker <20 units/d, or current smoker ≥20 units/d), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g alcohol/d), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h cycling/wk), leisure-time sports activity (0, 0.1–4.0, or >4.0 h sports activity/wk), education status (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), coffee intake, fiber intake, BMI, and waist circumference. Fiber and coffee intakes were adjusted for the total energy intake by using the residuals from linear regression (28). Categorical variables were entered as binary indicator variables into the models.

Biomarkers associated with estimated desaturase activity in the cross-sectional analysis were subsequently evaluated as potential mediators in the longitudinal analysis if the directions of the relations between the estimated desaturase activity, the biomarkers, and risk of T2D were consistent. Given the direct association between estimated D6D and SCD activity and risk of T2D, those biomarkers that displayed statistically significant associations with the estimated SCD and D6D activity equally directed as the association of these biomarkers with risk of T2D were selected as potential mediators. Given the inverse association between estimated D5D activity and risk of T2D, those biomarkers statistically significantly associated with the estimated D5D activity in the opposite direction as the association of these biomarkers with risk of T2D were further considered.

We used Cox proportional hazards regression analysis stratified by age, adjusted for the same confounders as for the cross-sectional analysis mentioned above, and adapted for the case-cohort design by the weighting method described by Prentice (29), with age as the primary time-dependent variable (entry and exit time of each participant was defined as the age at recruitment and age at first event of T2D diagnosis, death, or return of the last follow-up questionnaire). We estimated multivariable-adjusted HRs and 95% CIs for tertiles of the estimated desaturase activity (tertiles were based on the subcohort distribution) compared with the respective lowest tertile. The use of tertiles was chosen to be able to account for nonlinear associations with a sufficiently high number of incident cases of T2D in each category. Cox regression models were calculated with and without adjustment for potential mediators to study to which extent the adjustment attenuated the association between estimated desaturase activity and T2D risk. Differences in β-coefficients were calculated and tested for statistical significance [according to Hoffmann et al. (30)].

In addition, we performed sensitivity analyses to assess the robustness of our results. Because the fasting status may influence triglyceride concentration, which is also a component of the FLI, we examined only fasted participants (n = 256, including 46 cases) in a first sensitivity analysis. Fasting was defined as time of the last meal or drink >8 h. We further excluded participants with a history of cancer or cardiovascular diseases in a second sensitivity analysis, leaving 1667 participants, including 331 cases, for analyses. Excessive alcohol consumption is known to lead to alcoholic fatty liver disease. The focus of this analysis was, however, on markers associated with nonalcoholic liver fat accumulation. Therefore, in a third sensitivity analysis, we excluded men with a consumption of >30 g alcohol/d and women with a consumption of >20 g alcohol/d, leaving 1585 participants for analysis (including 331 cases) (31). We also investigated the impact of high lifetime alcohol consumption on the results and excluded participants who were former heavy drinkers or consumed alcohol occasionally heavy or always heavy during their lifetime (32) in a fourth sensitivity analysis, leaving 1661 participants for analysis (including 338 cases).

We performed the statistical analyses with the SAS software, release 9.4 (SAS Institute). All statistical tests were 2-tailed except the comparison of the β-coefficients, which was 1-tailed. We considered $P < 0.05$ as being statistically significant.

RESULTS

Baseline characteristics of the participants according to tertiles of estimated desaturase activity are displayed in **Table 1**. Participants in the higher SCD tertile and D6D tertile, as well as the lower D5D tertile, had a higher BMI and waist circumference than others. Participants in the higher D6D tertiles were also more likely to be heavy smokers than others, whereas participants in the higher SCD, D5D, and D6D tertiles were likely to consume more alcohol than participants in the lower tertiles.

Associations between estimated desaturase activity and T2D-related biomarkers

Multivariable-adjusted means and 95% CIs of biomarkers by SCD, D6D, and D5D tertiles stratified by sex are presented in **Tables 2** and **3**. Age-adjusted means are displayed in **Supplemental Tables 1** and **2**. Regarding the multivariable-adjusted model, estimated SCD and D6D activity showed statistically significant positive associations with triglyceride concentrations and the FLI in both sexes. Furthermore, estimated SCD and D6D activity was statistically significantly positively related to ALT and GGT activity in men and to concentrations of HDL cholesterol in women. Estimated SCD activity was statistically significantly positively related to CRP concentrations and GGT activity in women. Estimated D6D and SCD activity was statistically significantly positively related to fetuin-A concentrations

TABLE 1
Characteristics of a subsample from the EPIC—Potsdam cohort by estimated SCD, D5D, and D6D tertiles in women (n = 972) and men (n = 561)[1]

	Estimated SCD activity			Estimated D6D activity			Estimated D5D activity		
	1	3	P value	1	3	P value	1	3	P value
Male sex, %	38.6	35.2	0.26	29.8	48.2	<0.0001	38.4	35.6	0.35
Age, y	47.4 (16.2)	51.5 (15.2)	0.0001	46.2 (15.7)	51.8 (14.9)	<0.0001	49.8 (17.1)	47.4 (14.9)	0.008
BMI, kg/m^2	24.7 (4.64)	26.6 (5.86)	<0.0001	24.3 (4.63)	27.0 (5.39)	<0.0001	26.1 (5.60)	24.6 (4.61)	<0.0001
Waist circumference, cm									
Women	76.0 (13.0)	81.0 (18.0)	<0.0001	75.5 (11.5)	84.0 (16.5)	<0.0001	80.5 (16.5)	75.5 (12.0)	0.0004
Men	90.0 (11.0)	96.5 (14.5)	<0.0001	90.0 (13.0)	94.5 (13.5)	<0.0001	94.0 (14.0)	90.5 (13.0)	<0.0001
Sport activity, h/wk	0.00 (2.00)	0.00 (1.00)	0.001	0.00 (1.50)	0.00 (1.00)	0.08	0.00 (1.00)	0.00 (2.00)	0.005
Biking, h/wk	0.50 (2.50)	0.50 (2.50)	0.06	1.00 (2.50)	0.50 (2.50)	0.05	0.50 (2.50)	1.00 (2.50)	0.98
Alcohol intake, g/d	6.63 (11.9)	9.41 (20.4)	<0.0001	6.87 (12.9)	10.3 (20.7)	<0.0001	7.77 (16.3)	9.10 (17.2)	0.03
Current smoker, ≥20 units/d,[2] %	5.88	7.83	0.15	4.31	7.62	0.01	6.08	6.46	0.76
Education: technical college/university,[3] %	39.4	37.2	0.16	36.6	37.7	0.72	36.9	36.2	0.41
Heavy occupational activity,[4] %	6.67	6.85	0.02	6.46	7.42	0.05	5.88	6.46	0.81
Coffee consumption, cups/d	3.00 (2.00)	3.00 (2.00)	0.998	2.28 (2.50)	3.00 (2.00)	0.13	2.32 (2.00)	3.00 (2.00)	0.35
Fiber intake, g/d	20.0 (8.83)	19.3 (7.45)	0.02	19.8 (8.16)	19.8 (7.71)	0.83	19.6 (8.20)	19.1 (8.13)	0.19

[1]Values are medians; IQRs in parentheses for continuous variables and percentages for categorical variables. P value reflects whether the values of the variables significantly differ between extreme erythrocyte fatty acid ratio tertiles (Wilcoxon's test for continuous variables and χ^2 test for categorical variables). D5D, Δ5 desaturase; D6D, Δ6 desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; SCD, stearoyl-CoA desaturase.
[2]Smoking status (never, past, current smoker <20 units/d, or current smoker ≥20 units/d).
[3]Education status (in training or no training, vocational training, technical school, or technical college or university degree).
[4]Occupational activity (light, moderate, or heavy).

TABLE 2
Multivariable-adjusted means of biomarkers associated with type 2 diabetes by estimated desaturase tertiles of a subsample of the EPIC-Potsdam cohort in men (n = 561)[1]

	Estimated SCD activity				Estimated D6D activity				Estimated D5D activity			
	1	2	3	P-trend	1	2	3	P-trend	1	2	3	P-trend
Fetuin-A,[2] μg/mL	265 (257, 273)	258 (251, 266)	254 (245, 262)	0.06	257 (249, 265)	260 (252, 268)	260 (252, 268)	0.61	264 (256, 272)	263 (255, 271)	250 (242, 258)	0.02
ALT,[3] μkat/L	0.45 (0.42, 0.48)	0.44 (0.41, 0.47)	0.51 (0.48, 0.55)	0.006	0.46 (0.43, 0.49)	0.44 (0.41, 0.47)	0.50 (0.47, 0.54)	0.04	0.49 (0.45, 0.52)	0.47 (0.44, 0.50)	0.45 (0.42, 0.48)	0.10
GGT,[3] μkat/L	0.43 (0.39, 0.48)	0.42 (0.38, 0.47)	0.65 (0.59, 0.72)	<0.0001	0.44 (0.39, 0.48)	0.46 (0.41, 0.51)	0.60 (0.54, 0.66)	<0.0001	0.58 (0.52, 0.64)	0.48 (0.43, 0.53)	0.43 (0.39, 0.48)	0.0002
FLI[3] (score points)	34.2 (31.8, 36.8)	38.5 (35.8, 41.3)	47.0 (43.7, 50.6)	<0.0001	33.9 (31.6, 36.5)	39.0 (36.3, 41.9)	46.8 (43.5, 50.3)	<0.0001	43.4 (40.4, 46.7)	40.1 (37.3, 43.1)	35.6 (33.1, 38.3)	0.0002
HDL cholesterol,[2] mmol/L	1.30 (1.25, 1.34)	1.33 (1.28, 1.37)	1.36 (1.32, 1.41)	0.06	1.32 (1.27, 1.37)	1.33 (1.28, 1.37)	1.34 (1.29, 1.39)	0.61	1.32 (1.27, 1.36)	1.32 (1.28, 1.37)	1.35 (1.30, 1.40)	0.31
Triglycerides,[3] mmol/L	1.33 (1.22, 1.44)	1.48 (1.37, 1.60)	1.90 (1.75, 2.05)	<0.0001	1.34 (1.24, 1.45)	1.49 (1.38, 1.61)	1.87 (1.72, 2.02)	<0.0001	1.67 (1.55, 1.81)	1.59 (1.47, 1.72)	1.40 (1.29, 1.52)	0.002
CRP,[3] mg/L	0.62 (0.52, 0.75)	0.64 (0.54, 0.76)	0.73 (0.61, 0.88)	0.23	0.69 (0.58, 0.82)	0.61 (0.52, 0.73)	0.69 (0.57, 0.82)	0.99	0.76 (0.64, 0.90)	0.65 (0.55, 0.78)	0.59 (0.50, 0.71)	0.06
Adiponectin,[3] μg/mL	6.04 (5.65, 6.44)	5.83 (5.47, 6.21)	6.16 (5.76, 6.58)	0.63	6.16 (5.77, 6.57)	6.00 (5.63, 6.40)	5.86 (5.49, 6.25)	0.31	5.90 (5.53, 6.29)	6.02 (5.65, 6.41)	6.10 (5.72, 6.51)	0.48

[1]The model was adjusted for age at recruitment, smoking status (never, past, current smoker < 20 units/d, or current smoker ≥ 20 units/d), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or > 40.0 g alcohol/d), cycling (0, 0.1–2.4, 2.5–4.9, or ≥ 5 h cycling/wk), leisure-time sports activity (0, 0.1–4.0, or > 4.0 h sports activity/wk), education status (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), coffee intake (energy adjusted), fiber intake (energy adjusted), BMI (kg/m^2), and waist circumference (cm). P-trend value reflects whether the biomarker significantly falls or rises across the medians of the fatty acid ratio tertiles. ALT, alanine transaminase; CRP, C-reactive protein; D5D, Δ5 desaturase; D6D, Δ6 desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; FLI, fatty liver index; GGT, γ-glutamyltransferase; SCD, stearoyl-CoA desaturase.
[2]Values are arithmetic means; 95% CIs in parentheses.
[3]Values are geometric means; 95% CIs in parentheses.

in women, whereas estimated SCD activity also showed an inverse trend with fetuin-A in men (P-trend: 0.06). No association was detected between estimated D6D and SCD activity and adiponectin concentrations. In contrast, estimated D5D activity was inversely associated with CRP concentrations in women (P-trend: <0.0001) and men (P-trend: 0.06), inversely associated with fetuin-A and triglyceride concentrations in both sexes (all P-trends at least 0.02), and inversely associated with GGT activity in men (P-trend = 0.0002) and women (P-trend = 0.09) and with the FLI in both sexes (both P-trends at least 0.0002). No associations were detected between estimated D5D activity and adiponectin or ALT concentrations.

Associations between estimated SCD and D6D activity and HDL cholesterol in women and between estimated SCD activity and fetuin-A in men were not equally directed as their associations with risk of T2D. Therefore, HDL cholesterol was not evaluated as a potential mediator in the subsequent longitudinal analysis including estimated SCD and D6D activity, and fetuin-A was not evaluated in longitudinal analyses including estimated SCD activity.

Mediator analyses

Estimated SCD and D6D activity was directly related to risk of T2D in both sexes, whereas estimated D5D activity showed an inverse association with T2D risk (**Table 4**). We next evaluated single biomarkers and their combination as potential mediators by additional adjustment for these markers in Cox models.

Statistically significant and strongest attenuations regarding magnitude were seen after adjustment for the FLI in the association between estimated SCD, D6D, and D5D activity and T2D risk. Still significant but weaker attenuations were seen after adjustment for triglycerides, followed by adjustment for GGT activity. Attenuations after adjustment for other biomarkers were only very modest in magnitude, although a significant attenuation was detected after adjustment for CRP in the association between estimated SCD activity and T2D risk. The associations between estimated SCD activity and D6D activity and risk of T2D were attenuated and lost significance after adjustment for desaturase-related biomarkers, whereas the association between estimated D5D and risk of T2D was also attenuated but still remained significant after simultaneous adjustment for desaturase-associated biomarkers.

Sensitivity analyses

After restriction of analyses to fasted participants, associations were in general qualitatively comparable, but only a few reached statistical significance because of the loss of power (**Supplemental Table 3**). The exclusion of prevalent cases of cardiovascular disease and cancer (**Supplemental Table 4**), participants exceeding alcohol consumption at baseline (**Supplemental Table 5**), or those exceeding lifetime alcohol consumption (**Supplemental Table 6**) had no major influence on the results.

DISCUSSION

In this prospective study of middle-aged men and women, estimated SCD and D6D activity were positively related to T2D risk, whereas estimated D5D activity was inversely associated with

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TABLE 3
Multivariable-adjusted means of biomarkers associated with type 2 diabetes by estimated desaturase tertiles of a subsample of the EPIC-Potsdam cohort in women (n = 972)[1]

	Estimated SCD activity				Estimated D6D activity				Estimated D5D activity			
	1	2	3	P-trend	1	2	3	P-trend	1	2	3	P-trend
Fetuin-A,[2] μg/mL	259 (252, 266)	268 (262, 275)	271 (264, 277)	0.03	259 (252, 266)	265 (258, 272)	274 (267, 281)	0.005	275 (268, 281)	268 (262, 275)	255 (248, 262)	0.0001
ALT,[3] μkat/L	0.29 (0.27, 0.30)	0.28 (0.27, 0.29)	0.30 (0.29, 0.32)	0.07	0.29 (0.27, 0.30)	0.29 (0.27, 0.30)	0.30 (0.28, 0.31)	0.30	0.28 (0.27, 0.30)	0.30 (0.29, 0.31)	0.29 (0.28, 0.30)	0.66
GGT,[3] μkat/L	0.22 (0.21, 0.24)	0.23 (0.21, 0.25)	0.27 (0.25, 0.29)	0.0005	0.23 (0.21, 0.25)	0.23 (0.22, 0.25)	0.25 (0.23, 0.27)	0.10	0.25 (0.23, 0.27)	0.24 (0.23, 0.26)	0.22 (0.21, 0.24)	0.09
FLI[3] (score points)	11.0 (10.3, 11.8)	12.0 (11.3, 12.9)	14.6 (13.6, 15.6)	<0.0001	11.2 (10.5, 12.0)	12.3 (11.5, 13.1)	14.1 (13.1, 15.1)	<0.0001	13.5 (12.6, 14.5)	13.0 (12.1, 13.9)	11.0 (10.3, 11.8)	<0.0001
HDL cholesterol,[2] mmol/L	1.52 (1.48, 1.56)	1.55 (1.51, 1.59)	1.61 (1.58, 1.65)	0.001	1.52 (1.49, 1.56)	1.57 (1.53, 1.61)	1.60 (1.56, 1.64)	0.02	1.55 (1.51, 1.59)	1.59 (1.55, 1.62)	1.55 (1.51, 1.59)	0.95
Triglycerides,[3] mmol/L	0.95 (0.91, 1.00)	1.07 (1.02, 1.12)	1.26 (1.20, 1.32)	<0.0001	0.98 (0.93, 1.03)	1.08 (1.03, 1.14)	1.21 (1.15, 1.27)	<0.0001	1.19 (1.13, 1.25)	1.10 (1.05, 1.16)	0.97 (0.93, 1.02)	<0.0001
CRP,[3] mg/L	0.79 (0.69, 0.91)	0.94 (0.82, 1.07)	1.31 (1.14, 1.50)	<0.0001	1.02 (0.89, 1.18)	0.96 (0.83, 1.10)	0.99 (0.86, 1.14)	0.80	1.35 (1.17, 1.54)	0.93 (0.82, 1.07)	0.77 (0.67, 0.89)	<0.0001
Adiponectin,[3] μg/mL	9.09 (8.68, 9.51)	8.89 (8.50, 9.30)	8.85 (8.45, 9.27)	0.44	9.04 (8.63, 9.46)	9.14 (8.73, 9.56)	8.66 (8.27, 9.07)	0.19	8.78 (8.39, 9.19)	8.91 (8.52, 9.33)	9.14 (8.73, 9.57)	0.24

[1]The model was adjusted for age at recruitment, smoking status (never, past, current smoker <20 units/d, or current smoker ≥20 units/d), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g alcohol/d), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h cycling/wk), leisure-time sports activity (0, 0.1–4.0, or >4.0 h leisure-time sports activity/wk), education status (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), coffee intake (energy adjusted), fiber intake (energy adjusted), BMI (kg/m^2), and waist circumference (cm). P-trend value reflects whether the biomarker significantly falls or rises across the medians of the fatty acid ratio tertiles. ALT, alanine transaminase; CRP, C-reactive protein; D5D, Δ5 desaturase; D6D, Δ6 desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; FLI, fatty liver index; GGT, γ-glutamyltransferase; SCD, stearoyl-CoA desaturase.
[2]Values are arithmetic means; 95% CIs in parentheses.
[3]Values are geometric means; 95% CIs in parentheses.

T2D risk. These associations were considerably attenuated after adjustment for the FLI and, to a lesser extent, after adjustment for triglycerides, whereas adjustment for other desaturase-related biomarkers did not lead to appreciable attenuations.

In our study, adjustment for the FLI led to a substantial and significant attenuation of the relation of estimated SCD, D6D, and D5D activity with T2D risk, and adjustment for GGT activity led to significant but weaker attenuation, whereas adjustment for ALT or fetuin-A did not have substantial effects. Potential mechanisms of the association of D5D and D6D activity with liver markers have not been clarified yet; however, the effects are likely to be mediated by changes in FA composition. Long-chain PUFAs may act as ligands for transcription factors such as PPARs and nuclear transcription factor κB, which are involved in controlling hepatic metabolism (e.g., modulating hepatic β-oxidation and inflammation) (8). We found strong positive associations of estimated SCD activity with the FLI, ALT, and GGT. In prior studies, the role of SCD in liver fat accumulation has led to inconclusive results. An elevated SCD activity, estimated as the FA ratio from total hepatic FAs (33) and serum cholesterol esters (34), was positively related to histologically determined liver fat (33) and ALT activity (34). In line with this, 2 *SCD1* tag–single-nucleotide polymorphisms and several inferred haplotypes were associated with GGT and ALT activity in the EPIC-Potsdam subcohort (16). In contrast, hepatic SCD messenger RNA expression did not correlate with liver fat in participants undergoing liver surgery (35), and SCD activity based on serum VLDL triglycerides correlated inversely with liver fat in obese participants (36). Discrepancies in measurements of liver fat, study populations, and lipid fractions used to estimate the FA ratio complicate the comparison of results from these studies and ours. In this context, depending on the lipid fraction, FA ratios might rather represent SCD activity in adipose or hepatic tissue, which may differ in metabolic effects (1). The SCD index should preferably be based on defined lipid fractions, whereas SCD derived from total hepatic FAs should be cautiously interpreted (35). FA ratios based on plasma cholesterol ester and VLDL triglycerides should both reflect hepatic SCD activity, but only the latter has been validated (37). The erythrocyte phospholipid composition is in exchange with plasma lipid pools (2) and, thus, does not reflect tissue-specific SCD activity. Our results suggest that a favorable liver marker profile reflecting less liver fat could partly explain the inverse relation of D5D with T2D risk, whereas a less favorable liver marker profile reflecting higher liver fat may partly explain the direct relations of SCD and D6D with T2D risk.

Triglycerides are a well-established risk marker for T2D (38). We observed substantial positive associations between estimated D6D and SCD activity and triglycerides and a strong inverse association between estimated D5D activity and triglycerides. As a potential biological mechanism of these relations, FAs may act as ligands for transcription factors such as PPARα and PPARγ, whose expression results in the transcriptional stimulation of genes involved in triglyceride clearance and secretion of triglycerides and VLDL (7). The strength of the positive relation of SCD and D6D activity with T2D risk and of the inverse relation of D5D activity with T2D risk in our study was significantly and markedly reduced after adjustment for triglycerides. Our findings are supported by associations of genetic variants in *SCD1* and *FADS*, encoding D5D and D6D, gene regions with triglycerides

TABLE 4
RRs (95% CIs) for T2D by estimated desaturase tertiles in a case-cohort study embedded in the EPIC-Potsdam study in women and men (n = 1896; cases = 400)[1]

	Estimated SCD activity			Estimated D6D activity			Estimated D5D activity		
	1 (n = 79)	2 (n = 107)	3 (n = 214)	1 (n = 66)	2 (n = 113)	3 (n = 221)	1 (n = 194)	2 (n = 144)	3 (n = 62)
Multivariable-adjusted (referent)	1	1.28 (0.87, 1.88)	1.82 (1.29, 2.58)	1	1.51 (1.03, 2.21)	1.68 (1.18, 2.39)	1	0.80 (0.60, 1.08)	0.51 (0.36, 0.73)
+Fetuin-A				1	1.51 (1.03, 2.22)	1.67 (1.17, 2.38)	1	0.80 (0.60, 1.07)	0.52 (0.36, 0.74)
+ALT	1	1.34 (0.91, 1.98)	1.81 (1.27, 2.59)	1	1.54 (1.04, 2.28)	1.68 (1.17, 2.42)			
+GGT	1	1.28 (0.87, 1.89)	1.78 (1.25, 2.52)*	1	1.49 (1.02, 2.19)	1.64 (1.15, 2.34)*	1	0.80 (0.60, 1.08)	0.52 (0.36, 0.75)*
+FLI	1	1.14 (0.77, 1.69)*	1.27 (0.88, 1.83)*	1	1.38 (0.92, 2.08)	1.33 (0.92, 1.94)*	1	0.90 (0.67, 1.21)*	0.63 (0.43, 0.92)*
+All liver markers simultaneously	1	1.18 (0.79, 1.75)[2]	1.29 (0.89, 1.87)[2]*	1	1.41 (0.94, 2.13)[3]	1.35 (0.92, 1.96)[3]*	1	0.89 (0.66, 1.20)[4]*	0.63 (0.43, 0.93)[4]*
+Triglycerides	1	1.20 (0.82, 1.77)*	1.48 (1.03, 2.12)*	1	1.48 (1.01, 2.18)	1.46 (1.02, 2.09)*	1	0.85 (0.64, 1.14)*	0.56 (0.39, 0.81)*
+CRP	1	1.22 (0.83, 1.80)*	1.76 (1.24, 2.49)*				1	0.82 (0.61, 1.09)	0.52 (0.36, 0.74)
+All desaturase-associated markers simultaneously	1	1.11 (0.75, 1.66)[5]*	1.19 (0.82, 1.73)[5]*	1	1.43 (0.95, 2.15)[6]	1.32 (0.91, 1.92)[6]*	1	0.92 (0.68, 1.24)[7]*	0.66 (0.45, 0.96)[7]*

[1]Multivariable-adjusted HRs and 95% CIs (in parentheses) of type 2 diabetes for participants in the highest and middle SCD, D5D, and D6D compared with the lowest tertiles are presented. The model was stratified by age at recruitment and adjusted for smoking status (never, past, current smoker <20 units/d, or current smoker ≥20 units/d), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g alcohol/d), cycling (0, 0.1–2.4, 2.5–4.9, or ≥5 h cycling/wk), leisure-time sports activity (0, 0.1–4.0, or >4.0 h leisure-time sports activity/wk), education status (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), coffee intake (energy adjusted), fiber intake (energy adjusted), BMI (kg/m^2), and waist circumference (cm). Only those biomarkers that displayed a significant association with the respective estimated desaturase activity in the cross-sectional analyses, at least in men or women and did not show a trend ($P < 0.1$) in the opposite direction in the other sex, consequently fulfilling the second mediation criterion, were investigated on a potential mediating role and presented in this table. For this reason, we did not evaluate the impact of adjustment for fetuin-A on the association between estimated SCD activity and risk of T2D, we did not evaluate the impact of adjustment for CRP on the association between estimated D6D activity and risk of T2D, and we did not evaluate the impact of adjustment for ALT on the association between estimated D5D activity and risk of T2D in this table. *Significant difference of β-coefficients obtained from the multivariable-adjusted model (reference) and the multivariable-adjusted model additionally adjusted for desaturase-associated biomarkers. ALT, alanine transaminase; CRP, C-reactive protein; D5D, Δ5 desaturase; D6D, Δ6 desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; FLI, fatty liver index; GGT, γ-glutamyltransferase; SCD, stearoyl-CoA desaturase; T2D, type 2 diabetes.

[2]Adjustment for ALT, GGT, and the FLI simultaneously.

[3]Adjustment for fetuin-A, ALT, GGT, and the FLI simultaneously.

[4]Adjustment for fetuin-A, GGT, and the FLI simultaneously.

[5]Adjustment for ALT, GGT, the FLI, triglycerides, and CRP simultaneously.

[6]Adjustment for fetuin-A, ALT, GGT, the FLI, and triglycerides simultaneously.

[7]Adjustment for fetuin-A, GGT, the FLI, triglycerides, and CRP simultaneously.

(10, 14). Our results thus indicate that dyslipidemia may be a pathway linking desaturase activity to T2D risk.

Estimated SCD activity was positively related to CRP and not related to adiponectin in our study, and estimated D6D activity was related to neither CRP nor adiponectin, whereas estimated D5D activity was inversely related to CRP and not related to adiponectin. Results from prior studies are inconsistent (15, 39–43), suggesting either a positive (15, 41, 42) or no relation (39, 43) of estimated SCD activity with CRP and an inverse (40) or no relation (39) with adiponectin. Estimated D6D activity was overall positively (39, 41) or not (43) related to CRP and inversely related to adiponectin in prior studies (39), whereas D5D was inversely (39, 43) or not related (41) to CRP and not related to adiponectin (39). Comparison of findings from these studies and our study is difficult because of the use of different lipid fractions to calculate the FA ratio, adjustment sets, and study populations with respect to age range, sex, and ethnicity. Several pathways of how blood FAs may modulate CRP and adiponectin have been proposed. PUFAs and their metabolites may lower concentrations of proinflammatory cytokines by inhibiting nuclear transcription factor κB–dependent gene expression (7). FAs are also biological ligands for PPARγ (7), the activation of which regulates the transcription of several genes, including upregulation of adiponectin and anti-inflammatory effects (7). In this context, a prior study in EPIC-Potsdam conducted a stratified analysis to investigate whether relations of erythrocyte PUFAs with CRP and adiponectin vary by PPARγ2 genotype (44). The relation of 20:4n–6 (D5D product) with CRP varied by PPARγ2 genotype in women, suggesting that PPARγ2 indeed modulates this relation (44). However, 18:2n–6 (D6D substrate), 18:3n–6 (D6D product), and 20:3n–6 (D5D substrate) did not differ by PPARγ2 genotype regarding their relations with CRP or adiponectin among women, and the relations in men were largely independent of the polymorphism (44). Genome-wide association studies of CRP (45) and adiponectin (46) did not detect relations with *FADS*, encoding D5D and D6D, and *SCD*, whereas candidate gene studies found associations of genetic variation in the *FADS* (12, 13) and the *SCD1* (14, 15) genes with CRP. In sum, our results do not suggest that relations of estimated D5D, D6D, or SCD activity with T2D risk are importantly mediated by adiponectin or CRP.

Reverse causation cannot be ruled out in our cross-sectional analysis of estimated desaturase activity with biomarkers. Still, our findings are supported by associations between genetic variation in the *FADS* and *SCD1* genes and biomarkers of dyslipidemia and liver fat (10, 11, 14, 16). Unmeasured confounding between mediator or exposure and outcome may lead to biased effect estimates (47). To take this into account, we incorporated various confounders for the association between mediators or exposures and outcome. Desaturase activities were measured indirectly as FA ratios. However, direct measures are difficult to obtain in large epidemiologic studies. In an intervention study of isotope-labeled α-linolenic acid, acceptable correlations of labeled α-linolenic acid and EPA as direct indicators of D5D and D6D activity with the plasma FA ratios were observed (48). Two studies indicate an acceptable correlation between the gene expression and calculated FA ratio for SCD, derived from plasma VLDL triglycerides, and adipose tissue FA composition, whereas the correlations for D5D and D6D of the latter were moderate to low (37, 49). However, the extent of transferability of these findings on FA ratios derived from erythrocytes remains unclear. Still, we have previously reported a relatively strong association of genetic variation in the *FADS* genes, encoding D5D and D6D, with desaturase activity estimated from erythrocyte FA ratios (50). We used data of nonfasted and fasted participants for our analyses. The fasting status affects neither the T2D risk nor the erythrocyte FA profile used to estimate desaturase activity that reflects the dietary fat intake of several weeks (2); still, plasma triglycerides are known to depend on food intake (51). On the population level, triglycerides, however, increased only modestly in response to normal food intake (51). A higher imprecision in triglycerides might lead to an underestimation of the percentage of mediation in models with triglycerides or the FLI. In our analysis restricted to fasted participants, associations were generally qualitatively comparable, although only a few reached statistical significance owing to the loss of power.

In this prospective study of middle-aged men and women, significant and considerable attenuations were seen after adjustment for the FLI and, to a lesser extent, after adjustment for triglycerides on the association between estimated SCD, D6D, and D5D activity and T2D risk. Liver fat accumulation and dyslipidemia could explain the association of SCD, D6D, and D5D activity, estimated as FA ratios in erythrocyte membranes, with T2D risk to an extent.

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