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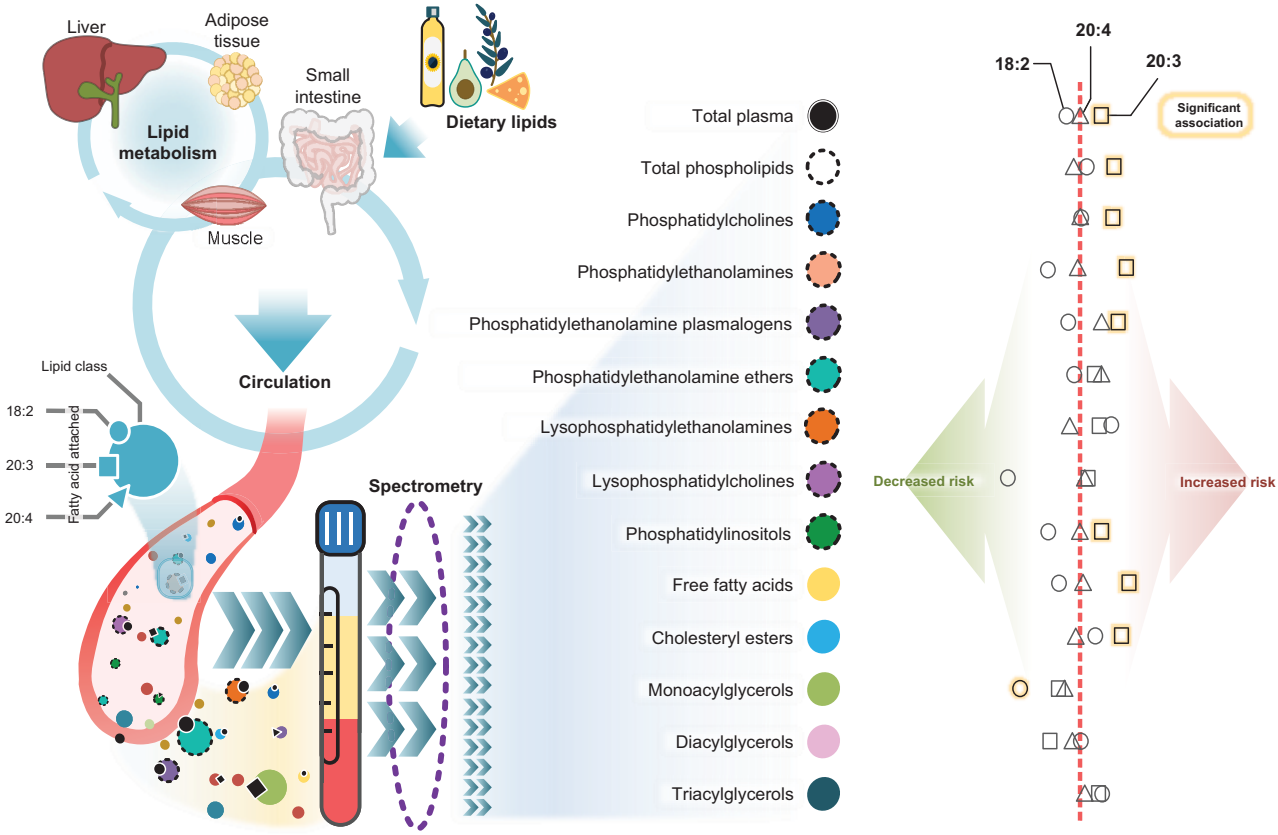

ARTICLE HIGHLIGHTS

- Studies examining the type 2 diabetes risk associations of n-6 polyunsaturated fatty acids (PUFAs) in a large panel of lipid classes are lacking.
- We investigated whether associations of plasma n-6 PUFA concentrations with type 2 diabetes risk are consistent across lipid classes.
- PUFAs were associated differently with diabetes incidence depending on the specific PUFA and lipid class; higher estimated Δ-5 desaturase activity was associated with reduced diabetes risk when determined in phospholipids and cholesteryl esters.
- The identified class-specific associations of PUFAs with type 2 diabetes provide new insights into the role of n-6 PUFAs in diet and metabolism.

Plasma Lipidomic n-6 Polyunsaturated Fatty Acids and Type 2 Diabetes Risk in the EPIC-Potsdam Prospective Cohort Study

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ORIGINAL ARTICLE

OBJECTIVE

Evidence on plasma n-6 polyunsaturated fatty acids (PUFAs) and type 2 diabetes risk is inconsistent. We examined the associations of lipid class–specific PUFA concentrations with type 2 diabetes risk.

RESEARCH DESIGN AND METHODS

In the prospective European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort (nested case-cohort study: subcohort 1,084 participants, 536 participants with type 2 diabetes, median follow-up 6.5 years), we measured plasma 18:2, 20:3, and 20:4 concentrations in 12 lipid (sub)classes, likely reflecting the plasma concentrations of linoleic acid (18:2n-6), dihomo-γ-linolenic acid (20:3n-6), and arachidonic acid (20:4n-6). The Δ-5 desaturase (D5D) activity was estimated as the 20:4/20:3 ratio. Associations with diabetes were estimated with Cox proportional hazards models.

RESULTS

Higher concentrations of 18:2 were inversely associated with type 2 diabetes risk, particularly in lysophosphatidylcholines (hazard ratio [HR] per 1 SD 0.53; 95% CI 0.23–1.26) and monoacylglycerols (HR 0.59; 0.38–0.92). Higher concentrations of 20:3 in phospholipid classes phosphatidylcholines (HR 1.63; 1.23–2.14), phosphatidylethanolamines (HR 1.87; 1.32–2.65), and phosphatidylinositol (HR 1.40; 1.05–1.87); free fatty acids (HR 1.44; 1.10–1.90); and cholesteryl esters (HR 1.47; 1.09–1.98) were linked to higher type 2 diabetes incidence, and these associations remained statistically significant after correction for multiple testing. Higher 20:4 concentrations were not associated with risk. The estimated D5D activity in phospholipids and cholesteryl esters was associated with lower type 2 diabetes risk. Single nucleotide polymorphisms in the D5D-encoding *FADS* genes explained relatively high proportions of variation of estimated D5D activity in those lipid classes.

CONCLUSIONS

Plasma n-6 PUFAs were associated differently with type 2 diabetes, depending on fatty acid and the lipid class.

Current dietary recommendations to reduce coronary heart disease risk suggest consuming polyunsaturated fatty acids (PUFAs) in exchange for saturated fatty acids (FAs) (1). However, recommendations for type 2 diabetes prevention do not clearly emphasize PUFAs as a component of diet quality (2). In recent years,

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prospective studies have evaluated the association of the most abundant dietary and circulating PUFA, linoleic acid (18:2n-6), with type 2 diabetes incidence. According to a recent meta-analysis of prospective cohorts, higher 18:2n-6 intake and higher concentrations in adipose tissue and different blood compartments are associated with a decreased diabetes risk (3).

Besides the dietary intake of 18:2n-6, its downstream metabolism to arachidonic acid (20:4n-6) may be relevant for diabetes risk (4). Phospholipid 20:4n-6 was not related to type 2 diabetes risk in the two largest cohort investigations: European Prospective Investigation into Cancer (EPIC)-InterAct and the Fatty Acids and Outcomes Research Consortium (FORCE) (5,6). However, associations vary substantially across individual cohorts and may also differ for other blood fractions (6). In contrast, dihomo-γ-linolenic acid (20:3n-6) has been consistently related to an increased risk of type 2 diabetes in prospective cohort studies (5,7–9). Critical enzymes of PUFA conversion are Δ-5 desaturase (D5D) and Δ-6 desaturase (D6D) (4). Product-to-precursor FA ratios in phospholipids to estimate the activity of these desaturases were associated with diabetes risk in prospective studies, but in opposite directions: D5D activity with lower risk and D6D activity with higher risk (5,10). A Mendelian randomization analysis using variants in *FADS1* (encoding D5D) and *FADS2* (encoding D6D) supported a causal role of desaturase activity in type 2 diabetes (11).

We have previously observed that associations of FAs with type 2 diabetes risk vary across plasma lipid classes, including PUFAs (12,13). However, very few studies have simultaneously compared risk associations of n-6 PUFAs in different lipid compartments. Plasma 20:3n-6 levels were positively associated with type 2 diabetes risk in phospholipids and cholesteryl esters (CEs) but not in triacylglycerols (TGs) in a study of Finnish men (7), whereas another study observed that 20:3n-6 was positively associated in CEs but not in phospholipids (8). Apart from phospholipids, CEs, and TGs, other n-6 PUFA–containing lipid classes in plasma include, for example, free fatty acids (FFAs) and other glycerolipids (monoacylglycerols [MGs] and diacylglycerols [DGs]). In addition, among the major phospholipid subclasses are phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), lysophosphatidylethanolamines (LPEs), lysophosphatidylcholines (LPCs), and phosphatidylinositols (PIs). The diabetes risk associations of n-6 PUFAs across these lipid classes have not been systematically examined. Furthermore, whether the estimated desaturase activity is similar in all lipid classes is still largely unexplored, and to our knowledge, lipid class–specific associations of estimated desaturase activities with diabetes risk have not been comprehensively reported. Moreover, while *FADS* variants have been associated with desaturase activities in phospholipids (11,14) and CEs (15), other plasma lipid classes have been underexplored.

We aimed to evaluate associations of n-6 PUFA concentrations and estimated desaturase activities with type 2 diabetes risk across 12 lipid (sub)classes from plasma lipidomics profiles in a prospective cohort study. In addition, we aimed to evaluate the influence of *FADS* variation on estimated D5D activity across the lipid classes.

RESEARCH DESIGN AND METHODS

Design and Population

The EPIC-Potsdam cohort recruited 27,548 participants (16,644 women aged mainly 35–65 years and 10,904 men aged 40–65 years) from the general population in Potsdam, Germany, between 1994 and 1998 (16). Consent was obtained from all participants, and approval was given by the ethics committee of the State of Brandenburg, Germany.

The baseline examination included blood sampling, anthropometry, blood pressure measurements, and collection of information on prevalent diseases and sociodemographic and lifestyle characteristics. Blood plasma was stored in liquid nitrogen tanks at −196°C or deep freezers at −80°C until analysis. Follow-up questionnaires were administered every 2–3 years to identify cases of incident diabetes. Response rates for follow-up rounds 1, 2, 3, and 4 were 96%, 95%, 91%, and 90%, respectively. The final censoring date was 31 August 2005.

A case-cohort study nested within the prospective EPIC-Potsdam study was designed for efficient molecular phenotyping (13,17). From all participants who provided blood samples (N = 26,437), we randomly selected a subcohort (n = 1,248) and considered all participants with incident type 2 diabetes (n = 801) identified during follow-up (median 6.5 years, interquartile range 6.0–8.7 years) (Supplementary Fig. 1). After excluding participants with missing follow-up information, prevalent diabetes at recruitment, and insufficient blood, the analytical sample involved 1,886 participants, including 775 with incident diabetes of whom 26 were part of the random subcohort. However, our analyses were further restricted to 1,602 participants (including 536 cases) with baseline HbA_{1c} <6.5% to exclude potentially undiagnosed cases.

Type 2 Diabetes Ascertainment

Participants with incident diabetes were detected by self-report of the diagnosis, antidiabetic medication use, or diabetes dietary treatment and information from death certificates or sources such as tumor centers, physicians, and clinics. All potential cases were verified by the treating physician. Only cases diagnosed after the baseline examination and verified by a physician as type 2 diabetes (ICD-10 code E11) were included as incident cases.

FA and Lipidomic Profiling

To compare with the concentrations of PUFAs derived from the lipidomics analyses by mass spectrometry (MS), we measured PUFAs in total plasma phospholipids by gas chromatography (GC) with a flame ionization detector, as previously described (12). The proportion of the FA was expressed as a percentage of the total FAs present in the chromatogram (13 FAs, including 18:2n-6, 20:3n-6, and 20:4n-6).

The lipidomics analysis was performed by Metabolon using the Metabolon Complex Lipid Panel, as previously described (13,17). Briefly, lipids were extracted from plasma samples in the presence of deuterated internal standards using an automated butanol-methanol extraction. Extracts were infused and MS analyzed using Shimadzu liquid chromatography with nano polyether ether ketone tubing and a SCIEX 5500 QTRAP with Selexion mass spectrometer. Upon ionization, the lipids passed through Selexion differential mobility spectrometry, which separated lipids by class. After differential mobility spectrometry filtering, lipids entered multiple reaction monitoring in which both the mass of lipid and the mass of its characteristic fragments were measured. Individual lipid species were quantified by taking the ratio of the signal intensity of each target compound to that of its assigned internal standard, then multiplying

by the concentration of internal standard added to the sample. This platform quantified FA composition across 15 lipid classes (PCs, PEs, LPEs, LPCs, PIs, CEs, FFAs, MGs, DGs, TGs, ceramides, dihydroceramides, lactosylceramides, hexosylceramides, and sphingomyelins) and 2 subclasses of PEs (phosphatidylethanolamine ethers [PEOs] and phosphatidylethanolamine plasmalogen [PEPs]) presented separately from other PEs. However, relevant PUFAs were not detected in ceramides, dihydroceramides, lactosylceramides, hexosylceramides, and sphingomyelins.

From all available PUFAs (18:2, 18:3, 20:2, 20:3, 20:4, 22:2, 22:4, and 22:5), total 18:2 was the most abundant (46.8 μmol/L); the second most abundant was total 20:4 (13.5 μmol/L), followed by total 20:3 (3.3 μmol/L) (Supplementary Table 1). We compared n-6 PUFA concentrations assessed in total plasma phospholipids by traditional GC, with the PUFA relative concentration in all the phospholipid classes measured by MS. The high correlations (18:2n-6[GC]-18:2[MS] r = 0.96; 20:3n-6[GC]-20:3[MS] r = 0.93; and 20:4n-6[GC]-20:4[MS] r = 0.91) indicated n-6 conformation. Besides the sum in all phospholipid classes, class-specific correlations are presented in Supplementary Fig. 2. Of note, lipids containing 18:3 were also relatively abundant (2.7 μmol/L); however, we did not consider them for our analysis because it remained unclear whether they reflected γ-linolenic acid (18:3n-6) or α-linolenic acid (18:3n-3). For 18:2, 20:3, and 20:4, we considered their total concentration in each lipid (sub)class. We also calculated total phospholipid concentration by summing all phospholipid species with that FA and total plasma by summing all lipids with that FA. D5D activity was estimated as the product-to-precursor ratio (20:4/20:3) calculated for each lipid class.

Genotyping

We genotyped rs174546, a single nucleotide polymorphism (SNP) strongly associated with estimated D5D activity (11) and in strong linkage disequilibrium with other SNPs in that region. We therefore used rs174546 to reflect genomic influence on estimated lipid class–specific D5D activity. Detailed information on array types, quality control, and software packages are reported elsewhere (11).

Statistical Analyses

Because of the few missing values for covariates, the imputation was model based (BMI: n = 2). There were no missing values of GC plasma phospholipid FA concentrations. We assumed that missing values in lipidomics-based lipid species concentrations were below the limit of quantification. Lipids with >70% values missing were excluded. The remaining were imputed using the quantile regression imputation of left-censored data approach from the R package imputeLCMD (18). We used log-transformation to stabilize skewed distributions and z scaled (mean 0, SD 1) the lipid species concentrations and ratios.

All descriptive analyses were based on the random subcohort. We evaluated and visualized intercorrelations among the lipidomics-measured lipids using Gaussian graphical models (19). In this network model, the edges represent covariance between two lipids that could not be explained by adjustment for any subset of other lipids. The reported correlation coefficients were adjusted for all other lipids.

The longitudinal associations between lipids and diabetes risk were evaluated in the case-cohort with Cox proportional hazards models stratified by age, accounting for the oversampling of cases by Prentice weighting. We estimated multivariable-adjusted hazard ratios (HRs) and 95% CIs, considering lipid concentrations as continuous variables standardized to 1-SD increments in the log-scale. Potential confounders were selected based on prior knowledge of relationships between covariables and diabetes (Supplementary Fig. 3). Model 1 was adjusted for age, sex, waist circumference, height, leisure time physical activity, education level, smoking status, alcohol intake, fasting status, medication (antihypertensive, lipid lowering, acetylsalicylic acid), and respective class sum (to separate the association from the total of the class). Model 2 was further adjusted for standard clinical blood lipid markers: total cholesterol, HDL cholesterol, and TGs (except in models using TG class as exposure). Model 3 was further adjusted for other FAs associated with type 2 diabetes, including odd-chain FAs (OCFAs) 15:0 and 17:0 (12) and FAs in the de novo lipogenesis pathway (DNLFAs) 16:0, 18:0, 16:1, and 18:1 (20), within each class. To account for multiple comparisons, the P values were controlled for false discovery rate (FDR) separately for each FA and the 20:4/20:3 ratio (21). Additionally, to examine whether presentation of stratified results was necessary, we performed multiplicative interaction analyses for sex by including the cross product in the most adjusted models.

Nonlinear associations were studied using restricted cubic splines (knots located at the 5th, 50th, and 95th percentiles). The likelihood ratio test was used to compare the fit between the linear and cubic spline models. For those FAs with $P < 0.05$, i.e., indication for a nonlinear association, we estimated the HRs and 95% CIs for quintiles of PUFA concentration (calculated based on subcohort distributions), taking the lowest quintile as reference. Association of rs174546 with estimated D5D activity was assessed assuming an additive genetic model adjusted for age at recruitment and sex. All analyses were performed with SAS Enterprise Guide 7.1 and R version 4.1.0 software.

Data and Resource Availability

The data sets analyzed during the current study are not publicly available due to data protection regulations. In accordance with German federal and state data protection regulations, epidemiological data analyses of EPIC-Potsdam may be initiated upon an informal inquiry addressed to the secretariat of the Human Study Center (office.hsz@dife.de). Each request will then have to pass a formal process of application and review by the respective principal investigator and a scientific board.

RESULTS

Baseline characteristics of subcohort participants of the EPIC-Potsdam cohort are presented in Table 1. Women made up 61% of the subcohort, and the median age was 49 years. Median BMI was 25.3 kg/m^2. Almost two-thirds had secondary education or higher, most were never smokers, and one-half reported a diagnosis of hypertension.

PUFA Concentrations by Lipid Class

The distribution of PUFAs (18:2, 20:3, and 20:4) among the lipid classes was heterogeneous (Fig. 1). In the phospholipid classes, 18:2 accounted from 10% (PEOs) to 46% (PCs) of the total FA concentration (sum of all compounds containing 18:2 within the lipid class). TGs

Table 1—Baseline characteristics of the EPIC-Potsdam subcohort (n = 1,084)

Variable	Median (IQR) or %
Women	61.1
Age (years)	49.2 (41.8–57.5)
BMI (kg/m^2)	25.3 (22.9–27.9)
Waist circumference (cm)	84.5 (75.0–93.5)
Leisure time activity: sport, biking, gardening (h/week)	5 (2–8)
Education	
Primary school	37.5
Secondary/high school	24.2
College/higher	38.3
Smoking	
Never	49.2
Former	31.2
Current smoker (<20 units/day)	14.9
Current smoker (≥20 units/day)	4.8
Medication	
Antihypertensive	19.6
Lipid-lowering	5.0
Acetylsalicylic acid	9.9
Alcohol intake (g/day)	
0	2.7
1–6	39.5
6.1–12	19.6
12.1–24	19.3
24.1–60	16.7
60.1–96	2.0
≥96	0.2
Total energy intake (kJ/day)	8,447 (6,790–10,295)
Blood pressure (mmHg)	
Systolic	127.5 (116.5–140.0)
Diastolic	83.0 (76.0–90.5)
Total cholesterol (mg/dL)	203 (176–230)
TG (mg/dL)	106 (74–161)
HDL cholesterol (mg/dL)	55 (46–65)
Prevalent hypertension	49.3
Prevalent cardiovascular disease	3.0
Prevalent cancer	6.2

There were no missing data for each variable.

and CEs had a median proportion of 46% and 39% of 18:2, respectively, while other classes had considerably lower proportions. In all classes, 20:3 had <8% abundance, and 20:4 was particularly abundant in PI (48%) and PE subclasses (40% of PEPs, 32% of PEOs, and 26% of other PEs), whereas lower concentrations were found in CEs (8%) and FFAs (0.4%).

Correlation of PUFA Concentrations Across Lipid Classes

Correlations among PUFAs within a class appeared to be class dependent (Supplementary Fig. 4*A*). For example, 20:3 and 20:4 (biochemically connected through a desaturation step) were most strongly positively correlated in CEs (r = 0.83) and PI (r = 0.78), but less so in PEs, MGs, and PEOs (all $r < 0.34$) (Supplementary Fig. 4*B*). Correlations of individual PUFAs across lipid classes were strongest between DGs and TGs. For example, DG(18:2) was positively correlated with TG(18:2) (r = 0.87), as well as DG(20:4) with TG(20:4) (r = 0.83). However, these lipids were not significantly correlated with the same FAs in the phospholipid classes.

Longitudinal Associations With Type 2 Diabetes Risk

The associations of PUFAs with incident diabetes differed according to the lipid class (Fig. 2 and Supplementary Table 2). Higher 18:2 abundance was inversely associated with risk in several lipid classes, but adjustment for OCFAs and DNLFAs attenuated associations for total plasma, total phospholipids, PCs, DGs, and TGs. After this adjustment, inverse associations became stronger for PE(18:2) (HR 0.73; 95% CI 0.51–1.05), PI(18:2) (HR 0.80; 0.63–1.02), and LPC(18:2) (HR 0.53; 0.23–1.26) and became apparent for MG(18:2) (HR 0.59; 0.38–0.92). Higher 20:3 abundance was positively associated with risk in most classes, particularly PC(20:3), PE(20:3), PEP(20:3), PI(20:3), FFA(20:3), and CE(20:3). The concentration of 20:4 in total plasma, total phospholipids, and most lipid classes appeared not to be associated with diabetes risk. These associations were not different by sex (all $P_{\text{interaction}} > 0.1$). After performing a multiple testing correction in the fully adjusted model, 20:3 in total plasma, total phospholipids, PC(20:3), PE(20:3), PEP(20:3), PI(20:3), FFA(20:3), and CE(20:3) reached statistical significance (FDR-adjusted $P < 0.05$) (Supplementary Table 3).

Spline regressions suggested that some lipids were nonlinearly associated (Supplementary Figs. 5–7). However, most association-based quintile comparisons revealed the same direction of association as the linear models (Supplementary Fig. 8).

Estimated Desaturase Activity, *FADS* Genotypes, and Type 2 Diabetes Risk

Estimated D5D activity was highest in PEs, PEPs, and PEOs, followed by CEs and other phospholipid classes (PIs, LPEs, and PCs), whereas it was lowest in MGs (Supplementary Fig. 10). When correlating estimated D5D activity among different classes, highest correlations were among PCs, LPCs, and CEs (r = 0.79–0.85), while the correlations of estimated D5D activity in MGs with those in all the other classes were low ($r \leq 0.09$) (Supplementary Fig. 11).

After adjusting for OCFAs and DNLFAs, higher estimated D5D activity was significantly associated with reduced diabetes risk when determined in phospholipids (PCs, PEs, PEPs, and PIs), CEs, and FFAs but not in MGs, DGs, and TGs (Fig. 3). The inverse association appeared strongest in PEs (HR 0.75; 95% CI 0.59–0.95) and CEs

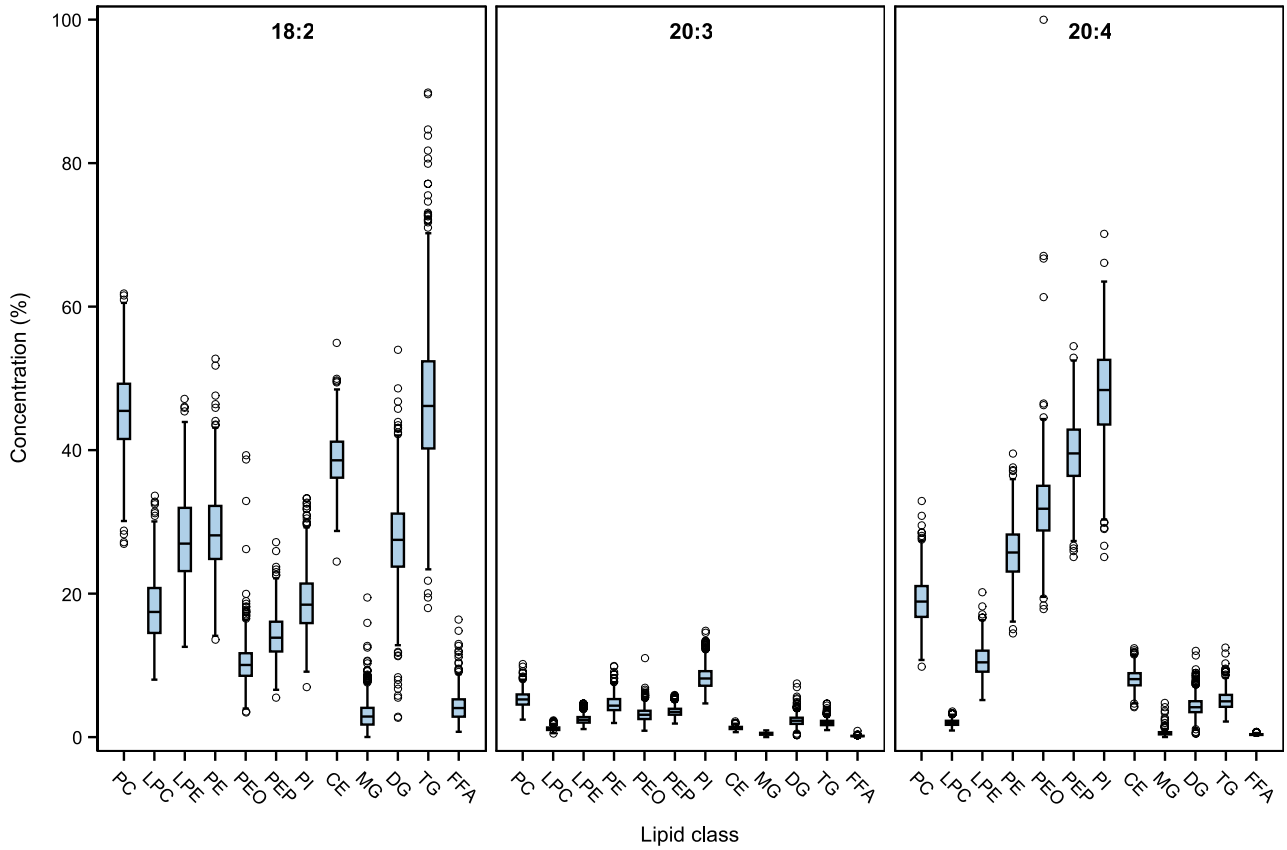

Figure 1—Fatty acid abundance (%) in each lipid class (subcohort *n* = 1,084).

(HR 0.80; 0.68–0.93) (Supplementary Table 2).

Some associations were nonlinear in spline regressions (Supplementary Fig. 9). The association appeared to be U-shaped in phospholipids (PEs, PEPs, PEOs, PIs, and LPCs) and J-shaped in FFAs (Supplementary Fig. 8). Genetic analysis revealed that the *FADS1* SNP rs174546 explained variance in the estimated D5D activity, depending on lipid class: 10–18% in phospholipid classes (PCs, PIs, LPCs, PEPs, PEs), 15% in CEs, 9% in FFAs, 5–7% in LPEs and PEOs, and ≤3% in glycerolipids (TGs, DGs, MGs) (Supplementary Table 4).

CONCLUSIONS

In this study, we evaluated associations of n-6 PUFAs in the lipidome, including 10 lipid classes and 2 subclasses, with type 2 diabetes risk. The FA 18:2 was highly abundant in phospholipid classes (particularly PCs), CEs, and TGs; 20:3 represented a small fraction of FAs in most lipid classes, whereas 20:4 accounted for a large proportion of circulating PIs and PEs. After adjusting for other FAs, inverse associations of 18:2 in most classes did not reach statistical significance; only MG(18:2) was significantly inversely associated with diabetes risk. The higher concentrations of 20:3 in phospholipids classes, FFAs, and CEs remained statistically significantly associated with diabetes incidence, even after correction for multiple testing. The FA 20:4 was unrelated to risk in most lipid classes. Explained variance of estimated D5D activity by genomic variation in the *FADS* locus was highest in PCs, PIs, LPCs, PEPs, PEs, and CEs, in which the estimated D5D activity was inversely associated with type 2 diabetes.

The heterogeneous integration of n-6 PUFAs into lipids across different lipid classes is influenced by diet, endogenous synthesis, preferential oxidation of certain FAs, and the adipose tissue's metabolic regulation of uptake and release. In phospholipids, the different FA compositions and, therefore, varying membrane properties are regulated by the specificity/selectivity of the enzymes in the phospholipid biosynthesis or remodeling pathways (22). Our results align with previous evidence showing that PIs had the highest enrichment of 20:4n-6 of all phospholipids (23), attributed to the acyl chain remodeling by acyltransferases that selectively incorporate 20:4n-6 into PIs (24). In contrast, 20:4n-6 is a poor substrate for TG synthesis (23), reflected in the low concentrations of TG(20:4) in our data. Another example is the lecithin-cholesterol acyltransferase, a catalyzer of the synthesis of CEs by transferring one FA from PC to CE, with specificity for 18:2n-6 (25), which could explain our observation of a high concentration of 18:2 in CEs (39%) and the positive correlation between CE(18:2) and PC(18:2). The lecithin-cholesterol acyltransferase specificity is lower for 20:4n-6, indicating a lower proportion of 20:4 in CEs (8%). The diverse distribution of PUFAs across the lipid classes supports the differential role and metabolic control of PUFAs in the different lipid compartments.

Associations of n-6 PUFAs with type 2 diabetes risk varied in direction, strength, and precision across different lipid classes. The 18:2n-6 levels in total phospholipids and total serum have been related to reduced risk in prospective cohort studies (5,6). Our findings indicate that the inverse association of phospholipid 18:2 and diabetes is strongest in LPCs,

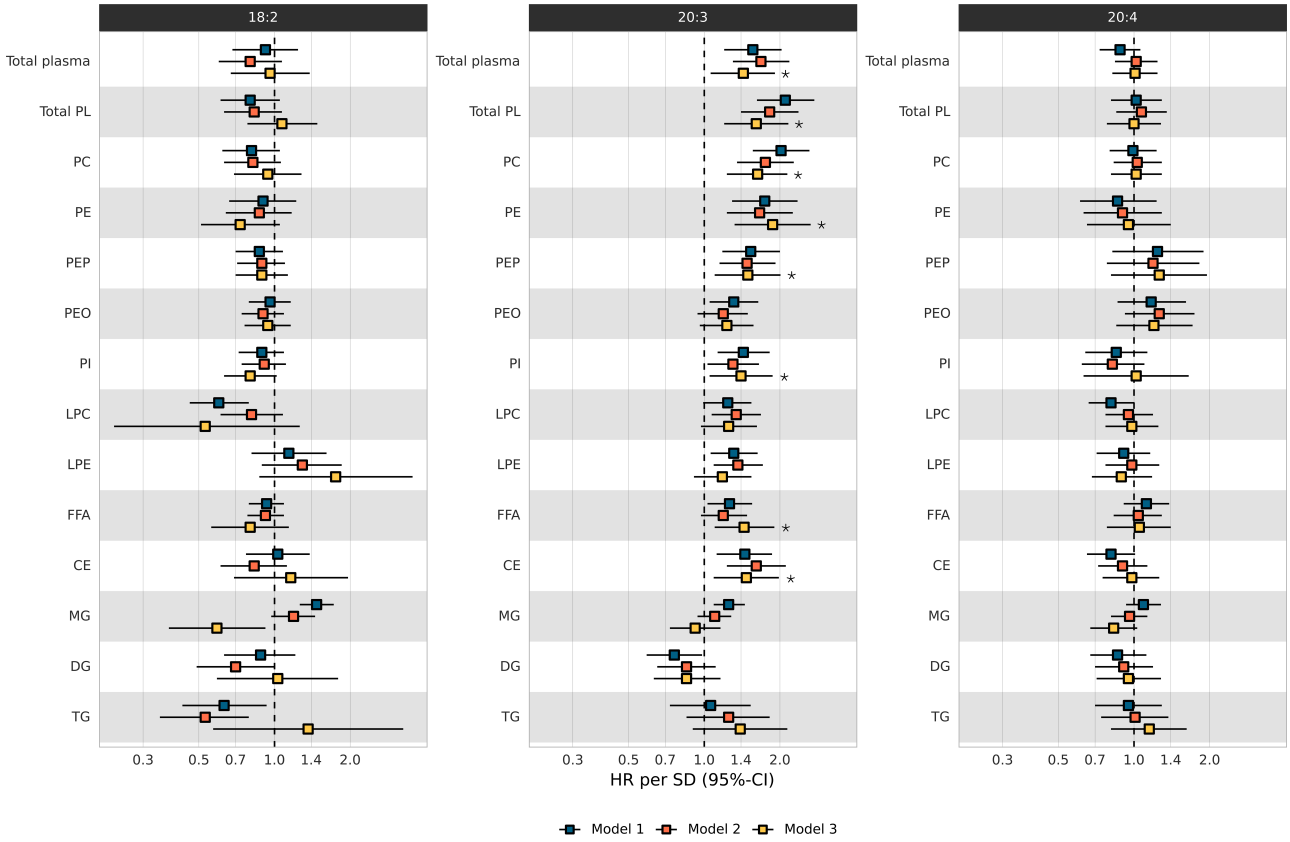

Figure 2—Associations of PUFAs with type 2 diabetes (subcohort *n* = 1,084; cases = 536). HRs and 95% CIs per 1-SD higher plasma concentration derived from the following models: model 1, adjusted for age (as underlying time variable), sex, waist circumference, height, leisure time physical activity (h/week), highest achieved education level (in or no training, skilled worker, technical school, or university degree), smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/day), fasting status at blood draw, antihypertensive medication, lipid-lowering medication, acetylsalicylic acid medication, and respective lipid class sum; model 2, model 1 with further adjustment for classical blood lipids (total cholesterol, HDL cholesterol, and standard clinical TGs except in the TG class), and model 3, model 2 with further adjustment for OCFAs (15:0 and 17:0) and DNLFAs (16:0, 16:1, 18:0, and 18:1) pathways in the respective lipid class. *FDR-adjusted $P < 0.05$ in model 3.

although the association did not withstand multiple testing correction. Other prospective cohort studies have linked higher concentrations of LPC(18:2) with lower diabetes risk (26–29). Of note, LPC(18:2) has also been associated with lower coronary heart disease risk (30). Although the mechanisms underlying the protective effects are not well understood, LPC(18:2) was inversely associated with C-reactive protein and plasminogen activator inhibitor 1 in humans (30). Other LPC metabolites have been reported to stimulate glucose uptake in adipocytes, improving glycemia in mice (31).

TG(18:2) and DG(18:2) were inversely associated and MG(18:2) positively associated with diabetes before adjusting for OCFAs and DNLFAs. Accounting for these FAs nullified the associations of TG(18:2) and DG(18:2) and reversed the association of MG(18:2) (statistically significant only before adjusting for multiple testing). These changes appeared to be largely driven by the adjustment for TG(16:0) and DG(16:0) in the associations of TG(18:2) and DG(18:2) and adjustment for MG(18:1) for MG(18:2) associations (data not shown). Stable isotope studies indicated that FAs in TG reflect dietary FA intake (32), and n-6 PUFA–rich diets increased the concentrations of TG(18:2) while decreasing TG(16:0) and DG(16:0) in randomized trials (13,33). In our study, TG(16:0) and DG(16:0) were negatively correlated with TG(18:2) and DG(18:2), respectively, when adjusting for total TGs and DGs (data not shown). It is therefore conceivable that the beneficial effects of 18:2n-6 intake (2,3) are mirrored in a replacement of 16:0 by 18:2 in TGs and DGs. In contrast, MG(18:2) was not found to be significantly influenced by diet and was positively correlated with MG(18:1) (13). Previous prospective studies indicating that MG(18:2) was positively associated with diabetes incidence (34) may have been confounded by MG(18:1), which we previously reported to be associated with an increased risk (13).

In our study, higher concentrations of 20:3 were related to increased diabetes risk in most lipid classes, consistent with prospective studies using total plasma phospholipids (5,7), total serum (9), and CEs (7,8). We did not find significant associations of 20:3 in TGs, similar to one previous study (7). In contrast to 18:2n-6, dietary intake of 20:3n-6 is negligible. The fact that plasma 20:3n-6 was not increased after diets supplemented with 18:2n-6 (35) suggests that endogenous regulation has more impact on plasma 20:3n-6 concentrations than dietary intake of the substrate for its endogenous production. In previous analyses, we detected a minor influence of dietary PUFA content on 20:3-containing plasma

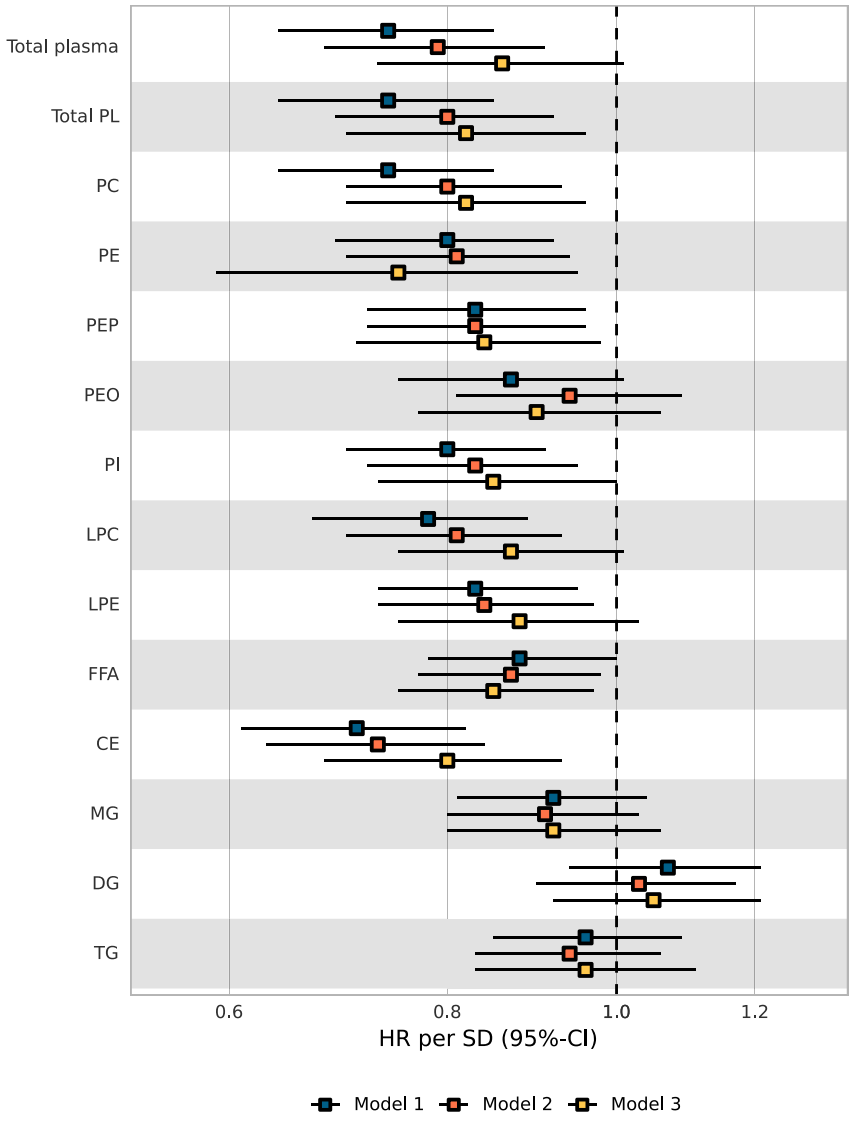

Figure 3—Associations of estimated D5D activities with type 2 diabetes (subcohort *n* = 1,084; cases = 536). HRs and 95% CIs per 1-SD higher plasma concentration derived from the following models: model 1, adjusted for age (as underlying time variable), sex, waist circumference, height, leisure time physical activity (h/week), highest achieved education level (in or no training, skilled worker, technical school, or university degree), smoking status (never, past, current, <20 cigarettes/day, or current >20 cigarettes/day), alcohol intake (0, 0.1–5.0, 5.1–10.0, 10.1–20.0, 20.1–40.0, or >40.0 g/day), fasting status at blood draw, antihypertensive medication, lipid-lowering medication, acetylsalicylic acid medication, and respective lipid class sum; model 2, model 1 with further adjustment for classical blood lipids (total cholesterol, HDL cholesterol, standard clinical TGs except in the TG class); and model 3, model 2 with further adjustment for OCFAs (15:0 and 17:0) and DNLFAs (16:0, 16:1, 18:0, and 18:1) pathways in the respective lipid class.

lipids (13). Lower concentrations of 20:3n-6 may result from a reduced enzymatic conversion of its precursors (18:2n-6 and 18:3n-6) or increased desaturation of 20:3n-6 to form 20:4n-6 (4).

Our finding that 20:4 enrichment in most phospholipid classes and CEs was not associated with diabetes risk is consistent with other prospective cohort studies that measured 20:4n-6 in total phospholipids and CEs (5,6). Endogenous production of 20:4n-6 (and therefore the decrease in the concentration of 20:3n-6) is regulated by the enzyme D5D, whose activity is commonly estimated with FA ratios in different lipid pools in epidemiological studies. The high intercorrelations between the estimated D5D activities in CEs and phospholipid classes in the current study confirm results from a previous study (36). In another study, the correlation between D5D in serum total phospholipids and serum FFAs was moderate (r = 0.31) (37), similar to our observed correlations between plasma phospholipid classes and plasma FFAs (r = 0.23–0.46). The estimated D5D activities in CEs and adipose tissue were reported to be only moderately correlated (r = 0.36) (15). Assuming that plasma FFAs are markers of adipose tissue FA composition, our results are comparable (correlation of CEs and FFAs: r = 0.40).

Previous prospective cohort studies observed inverse associations of the estimated D5D activity with type 2 diabetes risk for total phospholipids (5,7). Our study extends these findings by detailing PCs, PEs, PEPs, and PIs as the phospholipid classes with stronger inverse associations. Estimated D5D activity in CEs was also inversely associated in our study as in earlier research (7). We also found that the estimated D5D activities in phospholipid classes and CEs were more strongly influenced by genomic variance than the activity estimated in other lipid classes. *FADS1* variants have previously been associated with the estimated D5D activities in plasma phospholipids (11), individual phospholipids species (PCs, PEs, and PIs) (14), and CEs (15). The consistent associations of estimated D5D activities in plasma phospholipids and CEs with diabetes risk and with *FADS* variants support that FA composition in these lipid classes reflects the pathogenic involvement of PUFA metabolism in diabetes etiology. In our study, the *FADS1* variant was not related to D5D activity in glycerolipids (MGs, DGs, and TGs). Thus, FAs in glycerolipids may not reflect desaturase activity.

A major strength of the current study is the comprehensive lipidomics data, with PUFA concentrations likely reflecting 18:2n-6, 20:3n-6, and 20:4n-6 determined across 12 lipid (sub)classes in the context of a large prospective cohort study on incident diabetes. However, our study has some limitations. As we focused on relative concentrations of specific PUFA-containing lipids, higher concentrations of one particular lipid may actually be reflecting lower concentrations of other lipids within the class. Still, in our third model, we adjusted for FAs commonly associated with type 2 diabetes. Our lipidomics data did not provide information about the

conformation (n-3 vs. n-6) of the lipids. However, the high correlations with n-6 PUFAs measured in total phospholipids by GC in the same study population support that our exposures reflect n-6 PUFAs. For lipids with more than one FA (PCs, PEs, DGs, and TGs), we only considered one FA; thus, our modeling approach is not sensitive to a potential interaction between the FAs bound in the same lipid metabolite. Testing multiple classes increased the risk of false-positive associations; therefore, we accounted for multiple testing. Finally, additional studies are needed to replicate and judge the generalizability of our findings.

In conclusion, our results indicate a complex variability in n-6 PUFA incorporation into the different plasma lipid classes. The n-6 PUFAs were associated differently with type 2 diabetes incidence, depending on the specific FA and the lipid class. Phospholipid 18:2 was mostly related to lower diabetes risk, most strongly in LPCs and MGs, after accounting for OCFAs and DNLFAs. While 20:3 was linked to higher risk in most lipid classes, 20:4 was rather neutral. Evaluation of estimated D5D activities and *FADS* gene variants further supports that the plasma lipidome is an important reflection of diabetes-related PUFA metabolism.

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