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Authors

Miao, Guanhong
Zhang, Ying
Huo, Zhiguang
et al.

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Longitudinal Plasma Lipidome and Risk of Type 2 Diabetes in a Large Sample of American Indians With Normal Fasting Glucose: The Strong Heart Family Study

Guanhong Miao,[1] Ying Zhang,[2] Zhiguang Huo,[3] Wenjie Zeng,[1] Jianhui Zhu,[4] Jason G. Umans,[4,5] Gert Wohlgemuth,[2] Diego Pedrosa,[2] Brian DeFelice,[2] Shelley A. Cole,[6] Amanda M. Fretts,[7] Elisa T. Lee,[8] Barbara V. Howard,[4] Oliver Fiehn,[2] and Jinying Zhao[1]

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EPIDEMIOLOGY/HEALTH SERVICES RESEARCH

OBJECTIVE

Comprehensive assessment of alterations in lipid species preceding type 2 diabetes (T2D) is largely unknown. We aimed to identify plasma molecular lipids associated with risk of T2D in American Indians.

RESEARCH DESIGN AND METHODS

Using untargeted liquid chromatography–mass spectrometry, we repeatedly measured 3,907 fasting plasma samples from 1,958 participants who attended two examinations (~5.5 years apart) and were followed up to 16 years in the Strong Heart Family Study. Mixed-effects logistic regression was used to identify lipids associated with risk of T2D, adjusting for traditional risk factors. Repeated measurement analysis was performed to examine the association between change in lipidome and change in continuous measures of T2D, adjusting for baseline lipids. Multiple testing was controlled by false discovery rate at 0.05.

RESULTS

Higher baseline level of 33 lipid species, including triacylglycerols, diacylglycerols, phosphoethanolamines, and phosphocholines, was significantly associated with increased risk of T2D (odds ratio [OR] per SD increase in log2-transformed baseline lipids 1.50–2.85) at 5-year follow-up. Of these, 21 lipids were also associated with risk of T2D at 16-year follow-up. Aberrant lipid profiles were also observed in prediabetes (OR per SD increase in log2-transformed baseline lipids 1.30–2.19 for risk lipids and 0.70–0.78 for protective lipids). Longitudinal changes in 568 lipids were significantly associated with changes in continuous measures of T2D. Multivariate analysis identified distinct lipidomic signatures differentiating high- from low-risk groups.

CONCLUSIONS

Lipid dysregulation occurs many years preceding T2D, and novel molecular lipids (both baseline level and longitudinal change over time) are significantly associated with risk of T2D beyond traditional risk factors. Our findings shed light on the mechanisms linking dyslipidemia to T2D and may yield novel therapeutic targets for early intervention tailored to American Indians.

[1]Department of Epidemiology, Colleges of Public Health and Health Professions and Medicine, University of Florida, Gainesville, FL
[2]West Coast Metabolomics Center, University of California Davis, Davis, CA
[3]Department of Biostatistics, Colleges of Public Health and Health Professions and Medicine, University of Florida, Gainesville, FL
[4]MedStar Health Research Institute, Hyattsville, MD
[5]Georgetown-Howard Universities Center for Clinical and Translational Science, Washington, DC
[6]Texas Biomedical Research Institute, San Antonio, TX
[7]Department of Epidemiology, University of Washington, Seattle, WA
[8]Department of Biostatistics and Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Corresponding author: Jinying Zhao, jzhao66@ufl.edu

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G.M. and Y.Z. contributed equally to this work.

Dyslipidemia, including high triglycerides and low HDL cholesterol (HDL-c), represents a hallmark of type 2 diabetes (T2D) (1). American Indians are nearly three times more likely to be diagnosed with T2D than non-Hispanic Whites (2). Routine biochemical tests cannot capture all molecular lipids (i.e., lipidome) and thus have limited value in detecting early lipid disturbances implicated in disease. Lipidomics can identify many individual lipids and is well suited for characterizing the perturbed lipids preceding T2D. This is important, because prevention or delay of T2D has proven to be effective via both lifestyle and pharmacological interventions.

Previous studies have reported associations of altered blood lipids with T2D (3–9), obesity (10,11), and insulin resistance (IR) (9,11–13). Altered plasma triacylglycerols (TGs), diacylglycerols (DAGs), sphingomyelins (SMs), phosphoethanolamines (PEs), phosphocholines (PCs), and cholesterol esters (CEs) have been associated with T2D in different populations (3–5,7). However, most existing studies only measured baseline plasma lipids, which did not reflect change in plasma lipidome during T2D development. We are aware of only one lipidomic study that measured 207 known lipids in plasma sample collected at two time points (1 year apart) in a high-risk population, but none was associated with T2D after adjusting for baseline levels (7). Moreover, previous studies included predominately participants of European descent or at-risk individuals (as a result of matching in nested case-control design or inclusion of prediabetes as control). We are aware of two metabolomic studies among normoglycemic individuals with Chinese (4) or European ancestry (14), both of which only measured baseline metabolites and had low coverage of plasma lipidome.

Here we report findings from the first longitudinal lipidome profiling in 3,916 fasting plasma samples from 1,962 normoglycemic American Indians who attended two examinations (~5.5 years apart) and were followed up to 16 years in the Strong Heart Family Study (SHFS). Our goal was to identify novel molecular lipids and lipidomic signatures associated with risk of T2D in American Indians beyond traditional risk factors (TRFs).

RESEARCH DESIGN AND METHODS

Participants

All participants in our study were American Indians in the SHFS (2001–ongoing), a family-based prospective study designed to identify genetic and metabolic factors for cardiovascular disease (CVD) and T2D in American Indians (15). Briefly, 2,780 tribal members (aged 18 or older) in three geographic regions (Arizona, North/South Dakota, Oklahoma) were initially examined in 2001–2003 and reexamined in 2006–2009 (mean 5.5 years apart) using the same protocols. Information on demography, family history, medical records, and lifestyle was collected at each visit. Detailed methods for study design, laboratory protocols (16), and phenotype collection were reported previously (15,16). More information on blood sample collection and sample handling is described in the Supplementary Materials. All participants provided informed consent. The SHFS protocols were approved by the institutional review board of each participating institution and tribe.

Participants in this study met the following criteria: *1*) attended clinical examinations and had available fasting plasma samples at both baseline (2001–2003) and 5-year follow-up (2006–2009), and *2*) were free of overt CVD at baseline. Participants with missing information on fasting glucose or hypoglycemia medications at either time point were excluded.

Definition of Incident T2D and Related Traits

At baseline and 5-year follow-up, diabetes was defined as fasting plasma glucose (FPG) $\geq$7.0 mmol/L or receiving hypoglycemic medications. Impaired fasting glucose (IFG) was defined as FPG of 6.1–6.9 mmol/L and no hypoglycemic medications, and normal fasting glucose (NFG) was defined as FPG $<$6.1 mmol/L. Because SHFS did not collect biospecimens after the 5-year visit, T2D at 16-year follow-up was ascertained based on medical records abstracted by trained research staff. Incident T2D was defined as NFG at baseline (2001–2003) but development of T2D by end of 5-year (2006–2009) or 16-year follow-up (31 December 2018). IR was assessed using HOMA: HOMA-IR = fasting glucose (mg/dL) $\times$ insulin (μU/mL)/405 (17). Pancreatic β-cell function (HOMA-β) was assessed using the following formula: 360 $\times$ fasting insulin (μU/mL)/(fasting glucose (mg/dL) $-$ 63) (17). Insulin sensitivity was estimated by calculating the quantitative insulin sensitivity check index (QUICKI): QUICKI = 1/(log insulin [mU/L] + log baseline glucose [mg/dL]) (18).

Lipidomic Data Acquisition

Fasting plasma was extracted using a modified liquid-liquid extraction method (cold methanol/MTBE/water) (19), then subjected to liquid chromatography–mass spectrometry on both positive and negative ion modes. Raw data were processed using in-house cloud-based software (LC-BinBase) with peak detection and deconvolution algorithms adapted from MS-DIAL (20). Lipid peak intensity results were manually checked against raw data files. Features and peaks with 50% missing values across all samples were removed. Batch effect was corrected by SERRF (21). Details on sample extraction, laboratory protocols, and data preprocessing are described in the Supplementary Materials.

After preprocessing and quality control, we obtained 1,809 lipids (579 known, 1,230 unknown) in 3,916 samples. Coefficient variations of the Bioreclamation and National Institutes of Standards and Technology samples were 9% and 16%, respectively. Relative abundances of duplicated samples were highly correlated ($r > 0.95$).

Statistical Analyses

Prior to statistical analysis, lipidomic data were $\log_2$ transformed, then standardized to zero mean and unit SD. Multimodal distribution was detected by the Hartigans-Dip test (22). Outlier samples were detected by principal component analysis, and those beyond mean ± 5 SD for any of the first three PCs were further removed. Our final analysis included 1,542 lipids (518 known, 1,024 unknown) in 3,907 samples (1,958 at baseline, 1,949 at 5-year follow-up). Of these, 1,945 participants had complete data at both time points. Supplementary Figure 1 describes the procedures for participant selection and statistical analysis.

Prospective Association Analyses

To identify novel molecular lipids associated with risk of T2D, we constructed a

mixed-effects logistic regression model, in which level of baseline lipid was the independent variable and incident status of T2D was the dependent variable, adjusting for TRFs including age, sex, study site, BMI, fasting glucose, IR, HDL-c, and total triglycerides at baseline. The model tested the fixed effect of baseline lipids on risk of T2D and included random effect to account for relatedness among family members. Similar analysis was conducted to identify lipids predictive of risk of prediabetes (IFG). These analyses were performed among 1,161 NFG participants followed through 2006–2009 (mean 5.5-year follow-up).

Of 1,161 NFG participants at baseline, 989 individuals had information on T2D status through 31 December 2018 (mean follow-up 16 years), during which 176 participants developed incident T2D. The same statistical model was used to identify lipids associated with 16-year risk of T2D among these 989 participants. Multivariable adjusted odds ratios (ORs) and 95% CIs were calculated for each lipid. Multiple testing was controlled by false discovery rate (FDR) (23), and an FDR-adjusted *P* value (i.e., *q* value) <0.05 was considered significant.

Repeated Measurement Analyses

To examine the longitudinal association between changes in plasma lipidome and changes in continuous measures of T2D, including fasting glucose, fasting insulin, IR (HOMA-IR), β-cell function (HOMA-β), and insulin sensitivity (QUICKI), we constructed a mixed-effects linear regression model, in which 5-year change in each continuous measure (i.e., difference between 5-year follow-up and baseline, standardized to N [0,1]) was the outcome and change in each lipid was the independent variable, adjusting for age, sex, site, change in BMI, baseline lipid, and trait under study. The model tested the fixed effect of change in lipids on the change in T2D-related traits and included random effect to account for relatedness among family members. We also estimated to what extent the variation in 5-year change of each trait could be explained by change in plasma lipidome using the R package *r2glmm*. In addition, we tested the association of change in lipidome (focusing on known lipids whose 5-year changes were associated with changes in continuous measures of T2D at $q < 0.05$) with risk of T2D at 16-year follow-up, adjusting for TRFs and baseline lipids.

Multivariate Analysis by Partial Least Squares Discriminant Analysis

To identify discriminatory lipids and lipidomic signatures associated with risk of T2D, we conducted partial least squares discriminant analysis (PLS-DA) using the R package *mixOmics* (24). The model included all 1,542 lipids among 1,161 NFG participants at baseline, adjusting for TRFs. Optimal number of components and potential overfitting of the model were assessed with 10-fold cross validation (repeated 100 times). Lipids with variable importance in projection (VIP) scores $\geq$1.5 were considered crucial in identifying participants who developed incident T2D or IFG (cases) from those who remained NFG (controls) over 5 years of follow-up. Discriminant power of the validated model was assessed by area under the receiver-operating curve.

Sensitivity Analyses

To examine whether diet quality (assessed by the Alternate Healthy Eating Index), physical activity (steps per day), or use of lipid-lowering medications (yes/no) affected our results, we additionally adjusted for baseline levels of these covariates in the above-described prospective analyses.

RESULTS

After stringent quality control, we obtained data for 1,542 plasma lipids (both positive and negative ionization) in 3,907 samples from 1,958 participants at both baseline and 5-year follow-up. Distribution of the detected lipid species is shown in Supplementary Fig. 2.

Of the 1,161 NFG participants followed through 2006–2009 (mean follow-up 5.5 years), 205 participants developed incident IFG and 73 developed incident T2D. Of these 1,161 NFG participants, 989 were followed through 31 December 2018 (mean follow-up 16 years), during which 176 participants developed incident T2D. Table 1 presents the baseline characteristics of participants according to diabetic status by end of 5-year and 16-year follow-up. Compared with participants who did not develop incident T2D, those who developed incident T2D had higher levels of BMI, waist circumference, blood pressure, total triglycerides, fasting glucose, fasting insulin, IR, and β-cell function, but lower levels of HDL-c and insulin sensitivity, at baseline. Supplementary Table 1 presents the differences in baseline characteristics of participants between incident T2D or IFG, as compared with those who remained NFG, by end of 5-year follow-up.

Novel Lipid Species Associated With Risk of T2D Over TRFs

After adjusting for TRFs (age, sex, site, BMI, fasting glucose, IR, total triglycerides, and HDL-c) and multiple testing ($q < 0.05$), we found that higher baseline levels of 56 lipids (33 known, 23 unknown) were significantly associated with risk of T2D at 5-year follow-up. The 33 known lipids included 19 glycerolipids (TGs and DAGs) and 14 glycerophospholipids (PCs and PEs) with ORs (per SD increase in log2-transformed baseline lipids) ranging from 1.50 to 2.85 (Supplementary Table 2). Of these, 21 lipids were also associated with risk of T2D at 16-year follow-up (Fig. 1). A full list of all known and unknown lipids associated with 5-year risk of T2D ($q < 0.05$) is shown in Supplementary Table 3.

By contrast, 90 plasma lipids (49 known, 41 unknown) were positively or inversely associated with risk of future IFG at 5-year follow-up ($q < 0.05$) (Supplementary Table 4). Of the 49 known lipids, higher baseline levels of 44 lipids, including glycerolipids (TGs and DAGs), glycerophospholipids (PCs, PEs, PIs, and PGs), SMs, and CEs, were positively (OR per SD increase in log2-transformed baseline lipids ranging from 1.30 to 2.19), while 5 lipids, including LPC(20:4), PC(17:1/22:5), PC (39:6), PC(p-18:1/20:4)/PC(o-18:2/20:4), and SM(d40:3), were inversely (OR per SD increase in log2-transformed baseline lipids ranging from 0.70 to 0.78) associated with risk of future IFG. Twenty-four of 49 lipids were also significantly associated with 5-year risk of T2D (Supplementary Fig. 3). Besides the known lipids mentioned above, our untargeted lipidomics also identified multiple lipids with unknown structures associated with future risk of T2D/IFG. Figure 2 displays the lipidome-wide associations of plasma lipidome with risk of T2D (Fig. 2*A*) and IFG (Fig. 2*B*) during 5-year follow-up.

Table 1—Baseline characteristics of NFG participants according to diabetes status by end of 5-year and 16-year follow-up

Characteristic	5-year follow-up (n = 1,161)			16-year follow-up (n = 989)		
	Cases (n = 73)	Noncases (n = 1,088)	P	Cases (n = 176)	Noncases (n = 813)	P
Age (years)	35.8 ± 11.3	36.1 ± 13.0	0.84	36.7 ± 10.9	35.9 ± 12.9	0.40
Female (%)	44 (60)	693 (64)	0.54	110 (62)	539 (66)	0.347
BMI (kg/m^2)	34.9 ± 7.6	29.6 ± 6.6	1.75×10^{-10}	33.9 ± 7.4	29.2 ± 6.2	4.96×10^{-12}
Waist (cm)	110.4 ± 18.4	97.3 ± 15.9	4.83×10^{-10}	106.8 ± 17.7	96.1 ± 15.0	1.07×10^{-11}
SBP (mmHg)	123.4 ± 14.1	119.4 ± 14.5	0.01	122.6 ± 14.3	118.6 ± 14.3	3.66×10^{-3}
DBP (mmHg)	79.0 ± 9.9	76.0 ± 10.5	7.62×10^{-3}	78.6 ± 10.6	75.7 ± 10.4	1.37×10^{-3}
HDL (mg/dL)	49.1 ± 15.5	53.9 ± 14.9	0.02	48.4 ± 11.6	54.7 ± 15.1	8.01×10^{-9}
LDL (mg/dL)	101.8 ± 29.0	100.3 ± 29.2	0.66	99.4 ± 25.5	101.0 ± 29.4	0.50
Triglycerides (mg/dL)	177.9 ± 124.8	139.0 ± 78.9	8.93×10^{-4}	166.0 ± 118.2	136.7 ± 76.4	3.90×10^{-3}
Total cholesterol (mg/dL)	184.3 ± 35.1	181.8 ± 33.7	0.55	179.8 ± 31.0	182.9 ± 33.8	0.30
Fasting glucose (mg/dL)	91.3 ± 6.5	88.9 ± 6.6	9.36×10^{-3}	90.6 ± 6.4	88.7 ± 6.6	2.58×10^{-3}
Fasting insulin (μU/mL)	21.1 ± 16.0	12.4 ± 10.3	1.34×10^{-7}	17.3 ± 13.0	11.9 ± 10.0	1.56×10^{-5}
HOMA-IR	4.7 ± 3.5	2.8 ± 2.3	8.96×10^{-8}	3.9 ± 3.0	2.6 ± 2.3	1.12×10^{-5}
HOMA-β	295.8 ± 273.5	180.3 ± 155.6	1.39×10^{-7}	237.6 ± 203.3	174.8 ± 153.1	3.87×10^{-4}
QUICKI	0.14 ± 0.01	0.15 ± 0.02	1.40×10^{-8}	0.14 ± 0.01	0.15 ± 0.02	9.05×10^{-10}

Data are presented as mean ± SD or n (%). P values were obtained by generalized estimating equation to account for correlation among family members. Cases are participants who developed incident T2D by end of follow-up. Noncases are participants who did not develop incident T2D by end of follow-up.

Longitudinal Changes in Plasma Lipidome Associated With Changes in T2D Traits

After correction for multiple testing ($q < 0.05$) and adjustments for covariates (age, sex, site, and change in BMI between baseline and follow-up) as well as baseline lipids and the trait under investigation, our repeated measurement analysis identified significant associations between 5-year changes in 568 lipids (230 known, 338 unknown) and changes in one or more T2D-related traits, including fasting glucose, fasting insulin, IR, pancreatic β-cell function, and insulin sensitivity (Supplementary Table 5). The 230 known lipids largely belong to TGs (n = 84), PCs (n = 57), fatty acids (FAs) (n = 23), SMs (n = 15), PEs (n = 13), acylcarnitines (ACs) (n = 13), and DAGs (n = 9). Figure 3 illustrates the longitudinal association patterns between changes in lipidome and changes in T2D-related traits during 5-year follow-up (only lipids from seven main classes are shown). We observed a clear pattern that longitudinal changes in TGs, DAGs, and PEs were positively associated with changes in IR, β-cell function, and fasting insulin and inversely associated with changes in insulin sensitivity. In contrast, longitudinal changes in FAs and ACs were inversely associated with changes in IR, β-cell function, and fasting insulin and positively associated with changes in insulin sensitivity. In addition, changes in SMs were inversely associated with changes in fasting plasma glucose.

To further evaluate the impact of altered plasma lipids on diabetes development, we estimated to what extent the variability in change of each T2D trait could be explained by the change in plasma lipids over 5-year follow-up period. After adjusting for covariates and baseline lipids and the trait under investigation, longitudinal changes in three major classes (TGs, PCs, and FAs) explained up to 8.4% variability in the change of T2D-related traits. These findings indicate a potential large impact of altered plasma lipidome on development of T2D.

To examine the association between change in plasma lipidome and risk of T2D, we focused on the 230 known lipids whose longitudinal changes between baseline and 5-year follow-up were associated with one or more continuous measures of T2D and tested their associations with risk of T2D at 16-year follow-up. We found that 5-year changes in 10 lipids (7 TGs and DAGs, 3 PCs) were significantly associated with 16-year risk of T2D at $q < 0.05$ (Fig. 2*C* and Supplementary Table 6).

Discriminatory Lipidomic Signatures Identified by PLS-DA

Our multivariate analysis revealed a distinct lipidomic signature that was able to separate cases (incident T2D or IFG) from controls (NFG) at 5-year follow-up (Supplementary Fig. 4). The validated PLS-DA model identified four clinical variables (site, baseline BMI, fasting glucose, and IR) and 128 discriminatory lipids with VIP score >1.5 (Supplementary Table 7). Supplementary Figure 5 displays the 67 known lipids with VIP score >1.5. Top discriminatory lipids included TGs, DAGs, PCs, PEs, and SMs.

Results From Sensitivity Analyses

Additional adjustments for diet quality, physical activity, and lipid-lowering drugs did not change the associations between baseline lipids and 5-year risk of T2D (Supplementary Table 8). Of the 21 lipids associated with 16-year risk of T2D, 15 and 12 lipids remained significant after further adjustment for lipid-lowering drugs or physical activity, respectively. Four lipids remained significant after further adjustment for all three covariates

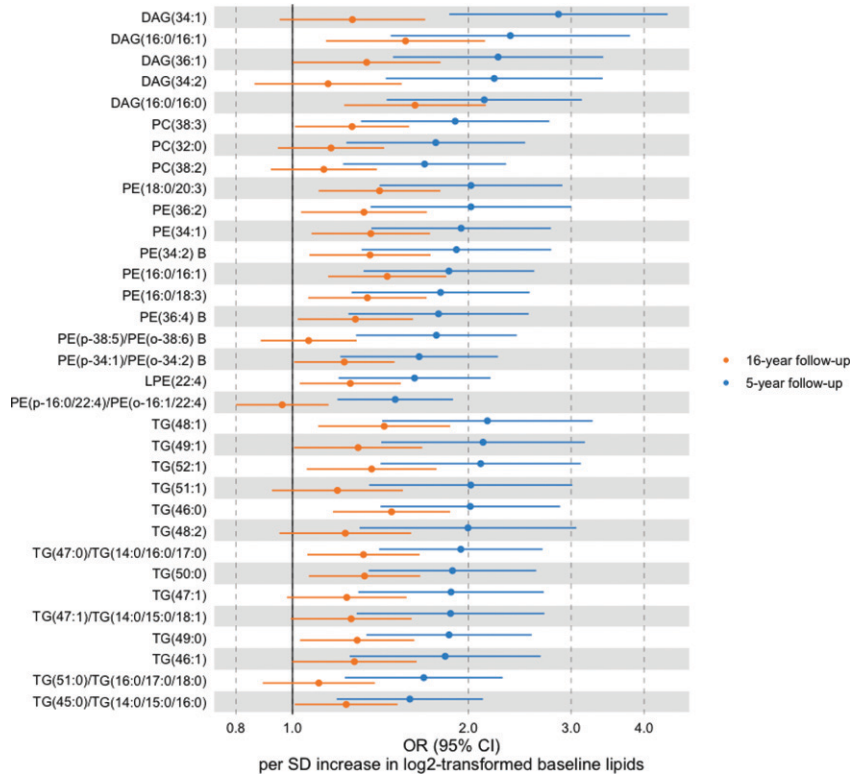

Figure 1—Baseline plasma lipids significantly associated with risk of T2D. Only known lipids are shown. The solid line represents OR = 1. Of the 33 known lipids associated with 5-year risk of T2D ($q < 0.05$), 21 lipids were also associated with 16-year risk of T2D. The mixed-effects logistic regression model adjusted for age, sex, BMI, fasting glucose, IR, HDL-c, and total triglycerides at baseline. Random effect was included in the model to account for relatedness among family members.

(diet quality, physical activity, and lipid-lowering drugs).

CONCLUSIONS

Dyslipidemia may precede overt T2D by many years (25), but a full spectrum of plasma lipid species (i.e., lipidome) predicting the onset and progression of T2D among apparently healthy individuals remains largely unknown. Moreover, few studies have examined the longitudinal association of change in lipidome with T2D development in a large community sample of normoglycemic individuals. Here we report findings from a longitudinal profiling of plasma lipidome in nearly 4,000 fasting plasma samples collected at two time points from 2,000 apparently healthy American Indian men and women. Our study has several novel findings. First, we identified novel molecular lipids associated with risk of future T2D beyond TRFs, including age, sex, BMI, fasting glucose, IR, HDL-c, and total triglycerides. At baseline, individuals who later developed T2D exhibited elevated levels of TGs, DAGs, PCs, and PEs. Altered lipid profiles were also observed in prediabetes (i.e., IFG). These results demonstrate lipid dysregulation occurs years before symptoms appear, and if confirmed, the newly identified molecular lipids may serve as novel biomarkers for risk stratification and early intervention. Second, our repeated measurement analyses identified several lipid species (e.g., TGs, PCs, FAs, and SMs) whose changes over time were associated with changes in T2D traits during 5 years of follow-up. Overall, changes in plasma lipidome explained up to 3.0%, 4.0%, and 8.4% variability in changes of fasting glucose, IR, and insulin sensitivity, respectively. Third, our multivariate analysis identified distinct lipidomic signatures associated with risk of T2D. To our knowledge, the current study represents the first comprehensive assessment of fasting plasma lipidome in relation to risk of T2D in a large community sample of apparently healthy adults among not only American Indians but other racial/ethnic groups as well.

In line with previous studies among European Caucasians (3,7,12,14,26), Mexican Americans (3), and Chinese individuals (4,27,28), we found that glycerolipids (e.g., TGs and DAGs) showed the most significant associations with risk of T2D in American Indians. In our study, higher baseline levels of multiple glycerolipids, including TAG(46:0), TAG(47:0)/TAG(14:0/16:0/17:0), TAG(48:1), TAG(49:1), TAG(49:0), TAG(50:0), TAG(52:1), DAG(36:1), DAG(16:0/16:1), and DAG(16:0/16:0), were consistently associated with risk of future T2D and prediabetes. Many lipids identified in our study, such as TAG(48:1), TAG(48:2), TAG(49:1), TAG(50:0), TAG(51:0)/TAG(16:0/17:0/18:0), TAG(51:1), TAG(52:1), DAG(34:1), DAG(34:2), and DAG(16:0/16:0), were also reported to be associated with diabetes (in the same direction) in European Caucasians (3,12,26), Mexican Americans (3), and Chinese individuals (27,28). The mechanisms through which TGs and DAGs affect T2D are likely related to their roles in membrane fluidity, inflammation, oxidative stress, and insulin signaling, all of which may affect glucose metabolism and IR (29–32).

Besides TGs and DAGs, we found that higher baseline levels of PCs, including PC(32:0), PC(38:2), and PC(38:3), and PEs, including PE(16:0/16:1), PE(18:0/20:3), PE(34:1), and PE(36:2), were also associated with increased risk of T2D in American Indians. Many of these lipids, such as PC(32:0), PC(38:3), PE(34:1), and PE(36:2), were also associated with T2D in other cohorts (3). Moreover, previous studies have reported associations of different species of phospholipids with IR, T2D, and related traits (7,33,34), even though specific lipid molecules were not identical as a result of the use of different analytical platforms and/or sample heterogeneity across studies. PCs and PEs are the major components of cellular membranes, and their roles in regulating glucose metabolism and insulin sensitivity have been reported in both animal models (35–37) and humans (33,38,39). Our results in American Indians, along with findings from other populations, demonstrate that altered plasma lipids precede T2D, and perturbed metabolism in TGs, DAGs, PCs, and PEs is implicated in T2D development.

In agreement with previous studies among European Caucasians and Mexican

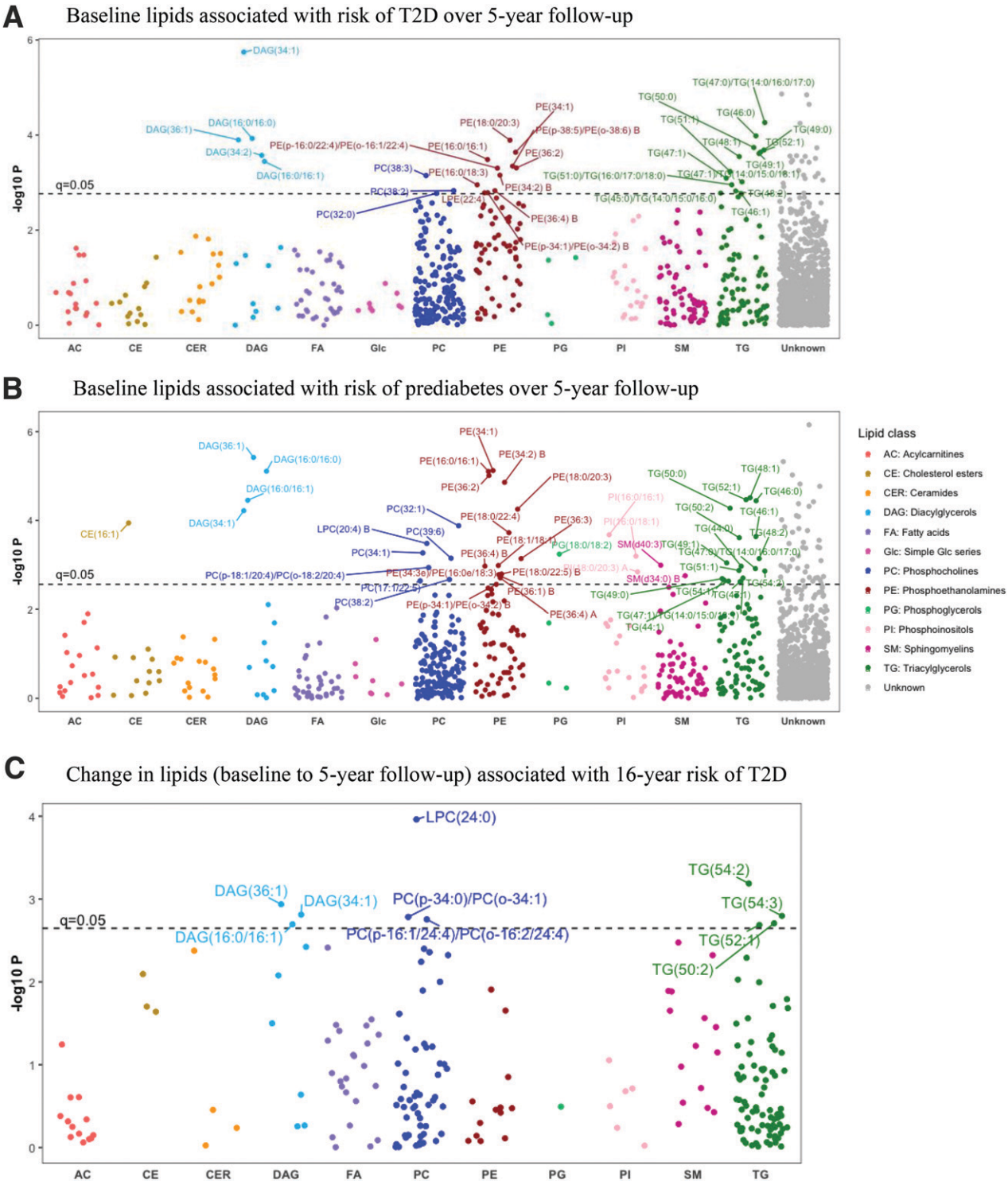

Figure 2—*A* and *B*: Manhattan plots showing the prospective associations of 1,542 baseline plasma lipid species with future risk of T2D (*A*) or prediabetes (*B*) over 5 years of follow-up. The mixed-effects logistic regression model adjusted for age, sex, study site, BMI, fasting glucose, IR, HDL-c, and total triglycerides at baseline. Random effect was included in the model to account for relatedness among family members. *C*: Manhattan plot showing the association between change in plasma lipidome (baseline to 5-year follow-up) and 16-year risk of T2D. *x*-axis indicates lipid class; *y*-axis indicates $-\log_{10} P$. The dashed line represents significance level at $q = 0.05$.

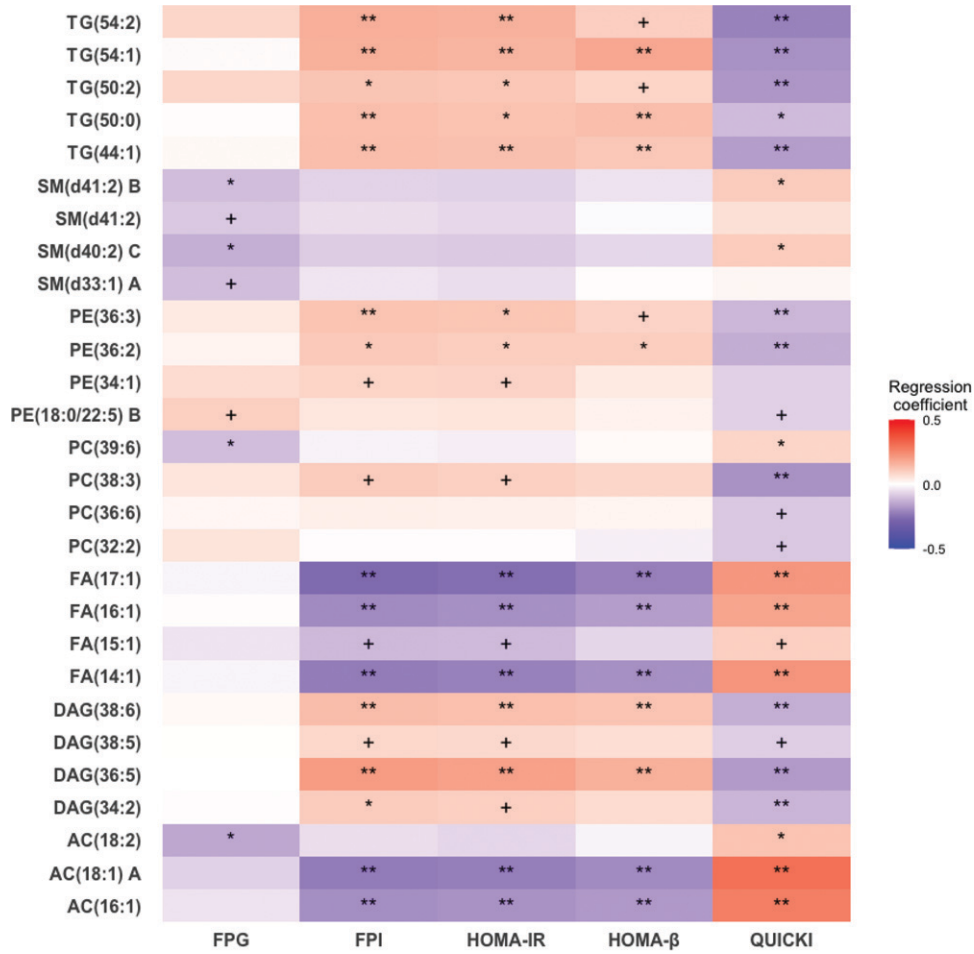

Figure 3—Heatmap illustrating the longitudinal associations between change in plasma lipidome and change in continuous diabetes traits during 5-year follow-up. Each row represents a lipid, and each column represents a phenotype. The heatmap is arranged based on lipid classes, and only top 10 known lipids in each class are shown. Color code is based on regression coefficients obtained from the linear mixed-effect model, in which change in lipid was the independent variable, and change in each of the T2D-related traits was the dependent variable. The model adjusted for age, sex, study site, change in BMI, and baseline levels of the specific lipid and the trait under investigation. Random effect was included in the model to account for relatedness among family members. FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, insulin resistance; HOMA-β, β-cell function; QUICKI, insulin sensitivity. $^{**}P < 0.001$, $^{*}P < 0.01$, $+P < 0.05$.

Americans (3,40), we found that lipid profiles of risk for prediabetes (IFG) were similar to those of T2D in American Indians. Specifically, of the 33 known lipids associated with risk of T2D, 24 lipids (largely TGs, DAGs, and PEs) were also associated with IFG in the same direction, indicating that aberrant lipid metabolism presents in prediabetes and persists during T2D development. Moreover, longitudinal changes (baseline to 5-year follow-up) in four of these 24 lipids, specifically DAG(16:0/16:1), DAG(34:1), DAG(36:1), and TAG(52:1), were also associated with risk of T2D at 16-year follow-up. These consistent findings lend support to an important role of these lipids in diabetes pathogenesis.

In line with previous studies reporting that SMs were either positively or inversely associated with T2D (7,9,12), we found that SM(d40:3) was inversely whereas SM(d34:0) was positively associated with risk of prediabetes. Our repeated measurement analysis showed that 5-year changes in SMs, such as SM(d40:2) and SM(d41:2), were inversely associated with changes in fasting glucose and positively associated with insulin sensitivity. In addition, we found that higher baseline levels of four PCs, including LPC (20:4), PC(39:6), PC(17:1/22:5), and PC(p-18:1/20:4)/PC(o-18:2/20:4), were inversely associated with risk of prediabetes. The observed bidirectional relationship of some SMs and PCs with T2D may be attributable to the different numbers of carbons and/or double bonds in these lipids. For example, previous research reported that lipids with lower numbers of carbons and double bonds were positively, whereas those with higher numbers of carbons and double bonds were inversely, associated with diabetes (12).

Our repeated measurement analysis identified a clear pattern between changes in lipidome and changes in T2D traits. Specifically, 5-year changes in TGs, DAGs, and PEs were positively associated with changes in fasting insulin, IR, and β-cell function and inversely associated with changes in insulin sensitivity. In contrast, 5-year changes in ACs and FAs were inversely associated with changes in fasting insulin, IR, and β-cell function and positively associated with changes in insulin sensitivity. Moreover, we found that of the 230 known lipids whose longitudinal changes between baseline and 5-year follow-up were significantly associated with

changes in continuous T2D traits, 37 lipids were also significantly associated with risk of T2D (both 5-year and 16-year follow-up) or IFG. These consistent results from different analyses support the robustness of our findings. Moreover, these novel and potentially important findings may provide mechanistic insights into the role of molecular lipids in diabetes development.

Our multivariate analysis identified distinct baseline lipidomic signatures able to classify participants into different risk groups. Top discriminatory lipids included TGs, DAGs, PCs, PEs, SMs, and ceramides. Of the 67 known discriminatory lipids, 30 were also identified in prospective and repeated measurement analyses. These results support important roles of these lipids in T2D development among American Indians.

Our study has several strengths. First, the repeatedly measured plasma lipidome in a large number of community-dwelling individuals represents a major strength of our study. To our knowledge, this is by far the largest longitudinal lipidomic study on risk of T2D in any racial/ethnic group. Second, unlike most previous studies that included a mixture of both normoglycemic individuals and individuals with prediabetes (i.e., those with IFG or IGT among whom early metabolic disturbances might have already been present) at baseline, our study focused on normoglycemic individuals who were also free of overt CVD at baseline. This makes our results much easier to interpret. Moreover, because we focused on apparently healthy individuals at baseline, and only a small proportion (1.1%) of participants received lipid-lowering drugs, our results are less likely to be confounded by drug use. Third, compared with previous research, our high-resolution liquid chromatography–mass spectrometry identified a larger number of lipid species spanning five lipid categories, including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, and sterol lipids. The high-coverage plasma lipidome allowed us to identify novel lipid species associated with T2D and provided unprecedented opportunities for future lipidomic research. Fourth, we conducted comprehensive statistical analyses, including prospective association analyses, repeated measurement analyses, and multivariate analyses, to identify novel lipids and lipidomic signatures associated with T2D. Fifth, many lipids were consistently detected by different statistical models and were also reported in previous studies across different populations. Moreover, our analyses adjusted for a comprehensive list of clinical covariates, including age, sex, BMI, fasting glucose, IR, total triglycerides, HDL-c, diet, physical activity, and use of lipid-lowering medications, signifying the robustness of our findings.

Our study has several limitations. First, although we detected more than 1,500 lipids covering a wide range of molecular lipid species, we were unable to match many lipids to the current databases. We were also unable to distinguish and identify isomeric lipids. These unknown compounds and isomers need to be characterized using de novo identification or additional experiments if considered of interest. Second, because of our focus on normoglycemic participants who were also free of overt CVD at baseline, the number of incident T2D cases during the 5-year follow-up period was relatively small, and therefore, our power in detecting predictive lipids was limited. However, there was a large number of incident T2D cases during 16-year follow-up. Third, T2D ascertainment was based on one single blood test at baseline and 5-year follow-up, and the diagnosis of T2D at 16-year follow-up was based on medical records (because of lack of blood samples at 16-year follow-up). Fourth, although our analyses adjusted for many known risk factors, we cannot exclude the possibility of potential confounding by unknown or unmeasured factors (e.g., changes in lifestyle factors, insulin secretion, and resistance over time). Fifth, although our analysis included a large number of American Indians, we did not have an external validation because of the lack of comparable cohorts comprising normoglycemic individuals who developed T2D for whom longitudinal lipidomic data were also available. However, many lipids were consistently identified in different models in our own study and were also previously associated with diabetes (in the same direction) in different populations. This increases the confidence in and signifies the robustness of some of our findings. Finally, the observational nature of our study precludes any causal inference regarding the role of altered lipids in diabetes etiology.

In summary, we identified novel lipid species (both baseline level and longitudinal change over time) and lipidomic signatures associated with risk of T2D beyond conventional risk factors. The newly identified lipids were altered years before the onset of T2D or prediabetes and could help identify high-risk individuals who may benefit from early intervention. Targeting metabolic pathways involving these newly identified lipids would help develop precision strategies tailored to American Indians.

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References

1. Hermans MP, Valensi P. Elevated triglycerides and low high-density lipoprotein cholesterol level

as marker of very high risk in type 2 diabetes. Curr Opin Endocrinol Diabetes Obes 2018;25:118–129
2. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2020. Atlanta, GA, Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services, 2020
3. Meikle PJ, Wong G, Barlow CK, et al. Plasma lipid profiling shows similar associations with prediabetes and type 2 diabetes. PLoS One 2013;8:e74341
4. Lu J, He J, Li M, et al.; 4C Study Group. Predictive value of fasting glucose, postload glucose, and hemoglobin A1c on risk of diabetes and complications in Chinese adults. Diabetes Care 2019;42:1539–1548
5. Suvitaival T, Bondia-Pons I, Yetukuri L, et al. Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men. Metabolism 2018;78:1–12
6. Cummings DE, Overduin J, Foster-Schubert KE. Gastric bypass for obesity: mechanisms of weight loss and diabetes resolution. J Clin Endocrinol Metab 2004;89:2608–2615
7. Razquin C, Toledo E, Clish CB, et al. Plasma lipidomic profiling and risk of type 2 diabetes in the PREDIMED trial. Diabetes Care 2018;41:2617–2624
8. Wong G, Barlow CK, Weir JM, et al. Inclusion of plasma lipid species improves classification of individuals at risk of type 2 diabetes. PLoS One 2013;8:e76577
9. Chew WS, Torta F, Ji S, et al. Large-scale lipidomics identifies associations between plasma sphingolipids and T2DM incidence. JCI Insight 2019;5:e126925
10. Mousa A, Naderpoor N, Mellett N, et al. Lipidomic profiling reveals early-stage metabolic dysfunction in overweight or obese humans. Biochim Biophys Acta Mol Cell Biol Lipids 2019;1864:335–343
11. Yin X, Willinger CM, Keefe J, et al. Lipidomic profiling identifies signatures of metabolic risk. EBioMedicine 2020;51:102520
12. Rhee EP, Cheng S, Larson MG, et al. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. J Clin Invest 2011;121:1402–1411
13. Kopprasch S, Dheban S, Schuhmann K, et al. Detection of independent associations of plasma lipidomic parameters with insulin sensitivity indices using data mining methodology. PLoS One 2016;11:e0164173
14. Merino J, Leong A, Liu C-T, et al. Metabolomics insights into early type 2 diabetes pathogenesis and detection in individuals with normal fasting glucose. Diabetologia 2018;61:1315–1324
15. North KE, Howard BV, Welty TK, et al. Genetic and environmental contributions to cardiovascular disease risk in American Indians: the strong heart family study. Am J Epidemiol 2003;157:303–314
16. Lee ET, Welty TK, Fabsitz R, et al. The Strong Heart Study. A study of cardiovascular disease in American Indians: design and methods. Am J Epidemiol 1990;132:1141–1155
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419
18. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402–2410
19. Cajka T, Smilowitz JT, Fiehn O. Validating quantitative untargeted lipidomics across nine liquid chromatography-high-resolution mass spectrometry platforms. Anal Chem 2017;89:12360–12368
20. Tsugawa H, Cajka T, Kind T, et al. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. Nat Methods 2015;12:523–526
21. Fan S, Kind T, Cajka T, et al. Systematic error removal using random forest for normalizing large-scale untargeted lipidomics data. Anal Chem 2019;91:3590–3596
22. Hartigan JA, Hartigan PM. The dip test of unimodality. The Annals of Statistics 1985;13:70–84
23. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 1995;57:289–300
24. Rohart F, Gautier B, Singh A, Lê Cao K-A. mixOmics: an R package for 'omics feature selection and multiple data integration. PLOS Comput Biol 2017;13:e1005752
25. Schofield JD, Liu Y, Rao-Balakrishna P, Malik RA, Soran H. Diabetes dyslipidemia. Diabetes Ther 2016;7:203–219
26. Fernandez C, Surma MA, Klose C, et al. Plasma lipidome and prediction of type 2 diabetes in the population-based Malmö diet and cancer cohort. Diabetes Care 2020;43:366–373
27. Lu J, Lam SM, Wan Q, et al. High-coverage targeted lipidomics reveals novel serum lipid predictors and lipid pathway dysregulation antecedent to type 2 diabetes onset in normoglycemic Chinese adults. Diabetes Care 2019;42:2117–2126
28. Lu L, Koulman A, Petry CJ, et al. An unbiased lipidomics approach identifies early second trimester lipids predictive of maternal glycemic traits and gestational diabetes mellitus. Diabetes Care 2016;39:2232–2239
29. Erion DM, Shulman GI. Diacylglycerol-mediated insulin resistance. Nat Med 2010;16:400–402
30. Kraegen EW, Cooney GJ, Ye JM, Thompson AL, Furler SM. The role of lipids in the pathogenesis of muscle insulin resistance and beta cell failure in type II diabetes and obesity. Exp Clin Endocrinol Diabetes 2001;109(Suppl. 2):S189–S201
31. Castoldi A, Monteiro LB, van Teijlingen Bakker N, et al. Triacylglycerol synthesis enhances macrophage inflammatory function. Nat Commun 2020;11:4107
32. Glass CK, Olefsky JM. Inflammation and lipid signaling in the etiology of insulin resistance. Cell Metab 2012;15:635–645
33. Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. Nat Rev Endocrinol 2017;13:79–91
34. Imamura F, Fretts A, Marklund M, et al.; InterAct Consortium; Fatty Acids and Outcomes Research Consortium (FORCE). Fatty acid biomarkers of dairy fat consumption and incidence of type 2 diabetes: a pooled analysis of prospective cohort studies. PLoS Med 2018;15:e1002670
35. Funai K, Lodhi IJ, Spears LD, et al. Skeletal muscle phospholipid metabolism regulates insulin sensitivity and contractile function. Diabetes 2016;65:358–370
36. Funai K, Song H, Yin L, et al. Muscle lipogenesis balances insulin sensitivity and strength through calcium signaling. J Clin Invest 2013;123:1229–1240
37. Selathurai A, Kowalski GM, Burch ML, et al. The CDP-ethanolamine pathway regulates skeletal muscle diacylglycerol content and mitochondrial biogenesis without altering insulin sensitivity. Cell Metab 2015;21:718–730
38. Newsom SA, Brozinick JT, Kiseljak-Vassiliades K, et al. Skeletal muscle phosphatidylcholine and phosphatidylethanolamine are related to insulin sensitivity and respond to acute exercise in humans. J Appl Physiol (1985) 2016;120:1355–1363
39. Pilon M. Revisiting the membrane-centric view of diabetes. Lipids Health Dis 2016;15:167
40. Mamtani M, Kulkarni H, Wong G, et al. Lipidomic risk score independently and cost-effectively predicts risk of future type 2 diabetes: results from diverse cohorts. Lipids Health Dis 2016;15:67