Underlying dyslipidemia postpartum in women with a recent GDM pregnancy who develop Type 2 diabetes

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Abbreviations

TAG Triacylglycerol, MAG Monoacylglycerol, DAG Diacylglycerol, FFA Free fatty acid, CE Cholesteryl ester, PC Phosphatidylcholine, LPC Lysophosphatidylcholine, PE Phosphatidylethanolamine, LPE Lysophosphatidylethanolamine, PI Phosphatidylinositol, DCER Dihydroceramide, CER Ceramide, SM Sphingomyelin, HCER Hexosylceramide, LCER Lactosylceramide, SFA saturated fatty acid, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids

Abstract

Approximately 35% of women with Gestational Diabetes (GDM) progress to Type2 Diabetes (T2D) within 10 years. However, links between GDM and T2D are not well understood. We used a well-characterised GDM prospective cohort of 1,035 women following up to 8 years postpartum. Lipidomics profiling covering >1000 lipids, was performed on fasting plasma samples from participants 6-9week postpartum (171 incident T2D vs. 179 controls). We discovered 311 lipids positively and 70 lipids negatively associated with T2D risk. The upregulation of glycerolipid metabolism involving triacylglycerol and diacylglycerol biosynthesis suggested activated lipid storage before diabetes onset. In contrast, decreased sphingomyelines, hexosylceramide and lactosylceramide indicated impaired sphingolipid metabolism. Additionally, a lipid signature was identified to effectively predict future diabetes risk. These findings demonstrate an underlying dyslipidemia during the early postpartum in those GDM women who progress to T2D and suggest endogenous lipogenesis may be a driving force for future diabetes onset.

Keywords

Gestational diabetes mellitus, Prospective study, Pathophysiology, Lipidomics, Triacylglycerol, Sphingolipid metabolism.

Introduction

Gestational diabetes mellitus (GDM) develops during pregnancy, affecting 1%-14% of all pregnancies depending on diagnostic criteria and the population characteristics [1,2]. The

majority of women with a history of GDM were not known to have overt diabetes before pregnancy and return to non-diabetes post-delivery. However women with a history of GDM are ~7 times more likely to develop type 2 diabetes (T2D) during the child bearing years compared to women who had no previous GDM [1,3,4]. In fact, it is estimated that 35%-50% of women with GDM may progress to T2D within 10 years after delivery [3,5]. Within 15 to 25 years, the lifetime maternal risk for overt diabetes is estimated to reach >50% [6,7]. Therefore, it is critical to uncover the underlying metabolic changes and understand the distinctive pathophysiology in T2D progression/development following GDM.

In the past decade, omics-based approaches have been used to discover novel metabolic fluctuations in humans, providing insight into pathophysiology of disease and identifying biomarkers of future disease including diabetes [8-10]. In particular, lipidomics has emerged as a more specialized omics platform that enables the measurement of a wide spectrum of lipid species. This approach has greatly expanded our understanding of the complexity of lipid dysregulation in metabolic diseases. Recently, an increasing number of lipidomics studies have aimed to link lipid dysregulation to diabetes pathology [11-20]. In the Framingham Heart Study cohort, more than 100 lipid analytes were measured and a group of triacylglycerols (low total carbon number and carbon double bonds) were found to be associated with increased risk of T2D[14]. In the PREDIMED trial, 207 plasma lipids were measured in which lysophosphatidylcholines (LPCs), phosphatidylcholine-plasmalogens (PC-PLs), sphingomyelins (SMs), and cholesteryl esters (CEs) were found to be inversely associated with T2D risk while triacylglycerols (TAGs), diacylglycerol (DAGs) and phosphatidylethanolamine (PEs) were positively associated with T2D risk [20]. A total of 277 plasma lipids were analyzed using a

lipidomics approach in Finnish males in which 5 lipids were selected to predict progression to Type 2 diabetes (T2D) [19]. In this cohort, higher levels of specific TAGs and diacyl-phospholipids and lower levels of alkylacyl-phosphatidylcholines were also observed in those who progressed to T2D[19]. In a very recent lipidomics study of a Chinese cohort, 250 lipids were tested and 38 significantly associated with T2D risk, including TAGs, LPCs, PCs, polyunsaturated fatty acid (PUFA)–plasmalogen phosphatidylethanolamines (PUFA-PEps), and CEs [15]. A lipid panel including 6 lipids significantly improved T2D prediction compared to that achieved by conventional risk factors [15]. In all of these studies, the positive association of TAG/DAG and T2D risk was consistently reported. However, a convergence on other specific lipids were not evident. This could be due to the differences in study design, cohort background and methodology including, importantly, limitations in coverage - expressed lipids in each study were not consistent.

Lipidomics has also been performed in GDM cohorts, including the measurement of 181 lipids in serum samples obtained from GDM women in their early second trimester. Four lipid biomarkers (TG(51:1), TG(48:1), PC(32:1), and PCae(40:4)) were identified for GDM prediction with moderate accuracy 71% [18]. Another lipidomic study measuring ~300 lipid species in blood samples from 104 women with recent GDM at 12-week post-delivery, of whom 21 cases later developed T2D, showed 84% accuracy in T2D prediction based on three lipids [i.e., PE(P-36:2), PS38:4, CE20:4] in combination with six other risk factors (i.e., age, BMI, prenatal fasting glucose, postpartum fasting glucose, total triglycerides, and total cholesterol) which were not matched for the analysis [21]. Our team identified 7 lipids from early postpartum blood samples to predict later incident T2D with an AUC of 0.92 in a very small subset of women with recent

GDM in our large prospective cohort (55 matched pairs of incident cases controls) [9]. To date however, no consensus has been achieved in terms of lipidomic dysregulation in GDM progression to T2D, likely due to limitation in the coverage of lipidome, cohort size, clinical data including diagnosis and follow-up years. Lipidomic changes within a large prospective cohort of women with GDM followed from the early postpartum period have not been evaluated. A comprehensive evaluation of lipidomic changes in relation to progression to T2D could elucidate the pathogenesis of transition from GDM to T2D, and thereby improve our understanding of the clinical targets for therapeutic interventions.

In the present study, lipidomics of 1008 lipid species from 15 lipid classes and 296 fatty acids was measured in a well-characterised prospective cohort of 1,010 women with recent GDM pregnancy and no diabetes, followed from 6-9 weeks post-delivery (baseline), retested with OGTTs for 2 years and followed via clinical laboratory testing and diagnoses up to 8 years later. Our aims were to systematically investigate lipidomic dysregulation in the transition from no diabetes to incident T2D following a GDM pregnancy and uncover lipid markers that may facilitate the early prediction of T2D incidence with clinical risk factors.

Results

Clinical characterization of the participants at baseline

The SWIFT cohort enrolled a total of 1,035 women diagnosed with GDM. Of these, 1010 did not have T2D at 6–9 weeks postpartum (baseline) and 989 had follow up testing for glucose tolerance up to 8 years post-baseline. Fasting blood samples were collected at baseline. During the follow-up period, 197 women had developed incident T2D and 791 did not (Figure 1). The

total years of follow-up were similar between incident T2D and control groups. All research participants underwent 2-h 75-g OGTTs and other assessments at baseline and thereafter annually for 2 years and subsequent medical diagnoses of diabetes was retrieved from electronic medical records for 8 years post-baseline. In our current study, 171 women with incident T2D cases had available plasma samples at baseline, and 179 controls who did not develop T2D in 8 years' follow-up (350 participants in total) were profiled for lipidomics. A total of 1008 lipid species from 15 lipid classes as well as 296 fatty acids were assessed in the plasma samples of all participants (Figure 1). Socio-demographic and clinical parameters of the 350 participants at baseline are summarized in Table 1. There was no significant difference in age, race, parity, pre-pregnancy BMI, family history of diabetes, postpartum BMI, total cholesterol, LDL-C, HOMA-B, smoker, dietary glycemic index, dietary intake and physical activity score. Compared to the control group, a higher percentage of participants who developed T2D later on had been treated with insulin or oral medications during pregnancy ($p<0.001$). Prenatal 3-hr 100g OGTT (sum of the 4 z-scores for glucose values; fasting, 1 hour, 2 hour and 3 hours post-load, $p<0.001$) for the incident T2D case group were higher than the control group. At 6-9 weeks postpartum, compared to controls, women in the incident T2D group had higher mean FPG ($p<0.001$), 2hPG ($p<0.001$), fasting insulin ($p=0.001$), 2h insulin ($p<0.001$), fasting TAG ($p=0.003$), median HOMA-IR ($p<0.001$) and hypertension ($p=0.04$), but lower mean fasting HDL-C ($p=0.017$).

Lipids associated with future T2D risk

Lipid biosynthesis and metabolism have been implicated in the development and progression of T2D. However, in previous studies, it has been an understudied component of metabolomics

profiling in the GDM transition to T2D. Thus, we have launched a broad spectrum lipidomics analysis, screening lipid metabolites and providing a comprehensive linkage of lipid metabolism to T2D. With a total of 1008 lipid species, we excluded lipids with >5% missing values among subjects, allowing only robust lipids (816 species) to be included in further analysis. Supervised PCA indicated partial separability of lipid profiles between case and control groups (Figure 2–figure supplement 1). By applying multiple logistic regression analysis, we assessed the association of lipids with future diabetes risk after adjusting for age, race and BMI. Of the 816 lipid species, 311 were positively and 70 were negatively associated with T2D risk (Figure 2A, Figure 2–figure supplement 2, FDR<0.05). Of the 311 lipids positively associated with risk, 293 were from TAG class while 17 from DAG class and 1 from PE class (Figure 2A-B). Of the 70 lipids negatively associated with T2D, 31 were from SM class, 27 from PC class, 7 from CE class, 4 from FFA class and 1 from TAG class (Figure 2A-B).

Most notably, 57.2% of all TAG species measured (293 out of 512 TAG) were significantly positively associated with T2D risk (Figure 2B). Plasma TAG, a transporter of dietary fats, increased, suggesting an overload of lipids in circulation before T2D onset. Additionally, 17 out of 54 DAGs, intermediates of TAG synthesis, were upregulated, further suggesting TAG biosynthesis was abnormally active (Figure 2B). In contrast, 40% (22 out of 55) measured PC and 25% (3 out of 12) measured LPC were negatively associated with T2D risk (Figure 2B). Similarly, 62% measured sphingolipids (31 out of 50) were inversely associated with T2D risk, particularly in classes of HCER (6 out of 9), LCER (9 out of 10) and sphingomyelins (10 out of 12) (Figure 2B). These findings suggested an inverse association of phospholipids and sphingolipids and increased risk of T2D.

More strictly, by using a cut-off of FDR<0.001, we demonstrated 107 lipids were significantly associated with T2D progression (Figure 3A). In this panel, 97 TAGs spanning carbon atom numbers from 42 to 56 with double bonds from 0-8, along with one saturated DAG(16:0/16:0) were consistently associated with increased diabetes risk. One monounsaturated PC(17:0/18:1) and 3 polyunsaturated PC(17:0/18:2), PC(18:1/20:4), PC(18:2/16:1) were inversely associated with future diabetes risk. Similarly, SM (18:1), SM(20:1), SM(24:1), HCER(24:1), and LCER(16:0) from the sphingolipid class were negatively associated with diabetes risk. Correlations between the 107 incident T2D associated lipids and conventional clinical parameters (BMI, FPG, 2hPG, fasting insulin HOMA-IR and HOMA-B) were assessed (Figure 3B). TAGs and DAG demonstrated a weak to moderate positive correlation with fasting insulin and HOMA-IR while sphingolipids and phospholipids were shown to have a weak negative correlation (Figure 3B). In contrast, those 107 lipids showed little correlation with 2hPG, age and BMI (Figure 3B).

Association between diabetes risk and lipid biochemical configuration

Lipidomics profiling provided a comprehensive coverage of plasma lipids for us to gain insight into the associations of lipid species biochemical structure (i.e. chain length, numbers of carbon atoms, double bonds) with diabetes risk. Among all the TAGs detected (carbon atoms from 36-60), those significantly associated with diabetes risk contained between 40-56 carbon atoms and 0-8 double bonds. Within those TAGs containing 40-56 carbon atoms, T2D risk increased in step with the number of carbon atoms (except carbon atom 55). TAGs most significantly associated with T2D risk were clustered in the range of carbon atoms 50-54 and double bond 0-4, particularly with even carbon atoms 52 and 54 (Figure 4A). DAGs with an even number of carbon atoms 30, 32, 34, 36 but not odd numbers were associated with diabetes risk more

prominently. There was no clear pattern of association with incident T2D by numbers of carbon atoms or double bonds in other lipid classes (Figure 4A). From the perspective of specific fatty acid chains in lipids, a relationship between diabetes risk and fatty acid composition was revealed. For total fatty acids, three SFAs (FA12:0, FA14:0 and FA16:0) as well as a PUFA (FA18:3) were positively associated with T2D risk and two very long chain MUFAs (FA24:1, FA26:1) were negatively associated with T2D risk (Figure 4B). Considering lipid classes, positively associated fatty acids were mainly from DAGs and TAGs including long chain SFAs (C12-C20), MUFA (C14 and C16) and PUFA (C20 and C22) (Figure 4B). In contrast, in PC and LPC classes, odd chain fatty acids (C15 and 17) were negatively associated with T2D risk. Interestingly, in the sphingolipid class, only even chain saturated and MUFAs were negatively associated with T2D risk (Figure 4B).

Metabolic pathways associated with future diabetes

To identify metabolic pathways associated with future diabetes, 381 lipids with significant association with diabetes risk (FDR<0.05) (Figure 2–figure supplement 2) were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Glycerolipid metabolism, which involves TAG and DAG biosynthesis, was significantly up-regulated (p=0.01). In contrast, sphingolipid (p=2.11E-05), linoleic acid (p=0.016) and alpha linoleic acid (p=0.041) metabolism were found to be significantly down-regulated (Figure 5A). Specifically, in the glycerolipid biosynthesis pathway, the TAG class was increased with strong significance (p=0.003), suggesting an induced process of lipid storage (Figure 5B). While as a whole the phospholipid metabolism pathway was not significantly altered, the PC class of lipids was significantly reduced (p=0.015) along with a modest decrease in the downstream LPC class (p<0.2),

198 suggesting the potential inhibition of pathway from DAG to PC class. In sphingolipids
199 metabolism, the central metabolite ceramide, which is a precursor for complex sphingolipids,
200 was marginally down-regulated ($p<0.2$). However, classes of SM ($p=0.002$), HCER ($p=0.006$) and
201 LCER ($p=0.0005$), which are downstream of sphingolipid metabolism were highly reduced,
202 suggesting the inhibition in the process of deriving of complex sphingolipids from ceramide
203 (Figure 5B).

204 **Selective lipids can predict future diabetes and complement clinical diagnostics**

205 The 107 lipids are the most significantly associated with future diabetes (odds ratio FDR cut-off
206 <0.001) (Figure 3A). It is intuitive that some may actually have predictive properties, and this
207 was tested. By using stepwise logistic regression modelling, we identified a panel of 11 lipids
208 (10 TAGs and 1 PC) with excellent ability to predict future diabetes in the cohort examined
209 (Figure 6A). With these lipids alone, we achieved the prediction ability as AUC of 0.739 (Figure
210 6B). The classical clinic predictive parameter FPG showed the prediction power of AUC 0.703
211 which was improved to AUC 0.795 by adding lipids (Figure 6B). The clinic predictive parameter
212 2hPG showed the prediction power of AUC 0.704 which was improved to AUC 0.809 by adding
213 lipids (Figure 6B). The combination of two clinical parameters 2hPG and FPG can achieve an
214 AUC 0.775. Importantly, combining the 11 lipid panel outcomes with FPG and 2hPG, the
215 discriminative power was significantly improved to AUC 0.842 (Figure 6B). This demonstrates
216 that the circulating levels of specific lipids can in part be used to assess future diabetes risk and
217 when applied, can improve diabetes prediction, especially when combined with routine clinical
218 parameters (2hPG and FPG) during the early postpartum period.
219

Discussion

In the present study, lipidomic profiling was used to assess the lipid changes at early post-partum (6 to 9 weeks) in a well-characterized, racially and ethnically diverse prospective cohort of postpartum women with recent GDM. A lipid signature associated with future diabetes risk was uncovered which contributes new knowledge into understanding the aetiology of diabetes in women associated with GDM. Importantly, our data indicate that women with recent GDM who later develop new onset T2D have clear differences in their lipidome compared to controls after delivery. This clearly shows they already exhibit lipid dysregulation in the early post-partum period.

Among the 311 lipids positively associated with progression to T2D, we found 293 belonging to TAG classes. This is equivalent to an impressive 57.2% of all measured TAG (293 out of 512) (Figure 2B). In addition, among the lipids associated with the most significant T2D risk, 91% of them were TAGs (97 out of 107) (Figure 3A). This finding fits our clinical measurements showing elevated TAG in T2D incident cases (Table 1) and is consistent with other studies [9,11,14-17,19,20,22]. TAGs, belonging to neutral lipids, are the energy storage in adipocytes and are an efficient energy source for muscle. In plasma, TAGs enable the bidirectional flow of fat from adipose tissue storage and blood glucose from the liver. Therefore, it is not surprising that TAGs outweigh other lipids as the dominant lipid species in terms of reflecting the changes of lipid metabolism in the body. The source of TAGs could be from food intake or endogenous TAG biosynthesis, such as lipogenesis. Our KEGG analysis demonstrated that the glycerolipid metabolism pathway was upregulated, suggesting the accumulation of TAGs could be attributed to the up-regulation of TAG biosynthesis (Figure 5). It was reported high sugar could

stimulate de novo lipogenesis in liver thereby increasing serum TAG level [23]. This process could be activated directly through transcriptional factor carbohydrate responsive element binding protein (ChREBP) to promote expression of lipogenic enzymes. Alternatively, lipogenesis could also be regulated by insulin through sterol regulatory element binding protein-1 (SREBP1). The elevated level of plasma hexose and insulin in those incident T2D cases at baseline could be associated with the enhanced endogenous lipogenesis.

In contrast, classes of glycerophospholipids (PC and LPC classes) are inversely associated with T2D risk (Figure 2B). Glycerophospholipids (through DAG) and TAGs share the same precursor glycerol-3-phosphate. Therefore, the downward trend in glycerophospholipids could be linked to the up-regulation of TAG biosynthesis. In addition to the phospholipids, an impressive 62% of measured sphingolipids (31 out of 50 tested) were inversely associated with T2D risk (Figure 2B). Particularly SM(18:1), SM(20:1), SM(24:1), HCER(24:1), and LCER(16:0) were among the lipids with the most significant risk associated with diabetes (Figure 3A). KEGG analysis revealed that sphingolipid metabolism was most significantly down-regulated (p=2.11E-05), further supporting the inverse association between sphingolipids and diabetes risk. So far, the relationship between sphingolipids and T2D risk has not been unequivocally ascertained. Several cross-sectional clinical studies have shown that CERs (upstream node of the sphingolipids pathway) are elevated in obese subjects with T2D [11,24-26]. We and others, however, have previously shown a negative association of SMs (downstream node of the whole pathway) with diabetes risk [9,10,20,27,28]. Further biological testing in humans and models of diabetes risk are required to validate the association between sphingolipids and diabetes onset.

263 Glycerophospholipids (through DAG) and TAGs share the same precursor glycerol-3-phosphate
264 (G3P). The higher G3P induced by higher plasma glucose levels could shift the acyl-CoA to
265 lipogenesis from sphingolipids and phospholipids pathways. Therefore, in those incident T2D
266 cases, the downward trend in glycerophospholipids and sphingolipids could be associated with
267 the up-regulation of TAG biosynthesis. In normal physiological conditions, de novo lipogenesis
268 mainly occurs in the liver and adipose tissue and is a minor contributor to serum TAG
269 homeostasis. However, an up-regulated lipogenesis could break the balance causing lipidemia.
270 In addition, down-regulation of glycerophospholipids and sphingolipids biosynthesis impairs the
271 integrity of cell membrane structure, which might contribute to insulin resistance. Although
272 higher glucose level could correlate with higher TAG, TAG is not simply an indirect measure of
273 glucose. Instead, increased TAG along with decreased phospholipids and sphingolipids could be
274 a cue of up-regulated endogenous de novo lipogenesis, a driving force of T2D.
275 Investigating the composition of the fatty acids in the lipids showed long chain SFA myristic acid
276 (C14:0) and palmitic acid (C16:0) were positively associated with T2D risk. Previously, palmitic
277 acids were reported to cause pancreatic beta cell dysfunction and were shown to be associated
278 with diabetes [29,30]. A previous study on a large prospective cohort EPIC-InterAct case
279 suggested that even-chain SFA in phospholipids were positively associated with diabetes risk
280 while odd-chain SFA had a negative association [31]. Similarly, we detected odd-chain SFA from
281 phospholipids were negatively associated with T2D risk. However, the association between
282 even-chain SFAs and T2D risk was more complicated depending on the lipid classes from which
283 they were derived. Even-chain SFAs from glycerol lipids (TAGs and DAGs) were positively
284 associated with T2D risk while those from sphingolipids had a negative association. No

285 significant association to T2D risk was detected in even-chain SFAs from phospholipids (Figure
286 4B). Odd-chain SFAs (C15:0 and C17:0) are mainly exogenously derived from dairy fat intake
287 [32-34]. In contrast, even-chain SFAs are from an endogenous source, such as increased
288 lipolysis from adipose tissue or de-novo lipogenesis from excess carbohydrates [34-38].
289 In addition to the carbon numbers of fatty acids, we also showed the association between the
290 degree of fatty acid unsaturation (number of double bonds) with diabetes. MUFAs, particularly
291 those from sphingolipids, were negatively associated with T2D risk; however, PUFAs from TAGs
292 were positively associated. These findings suggest that fatty acids from different sources and
293 lipid classes have opposite influences on diabetes risk. This would provide novel insight into the
294 role of lipid metabolism in diabetes onset and further develop guidelines for a healthy diet to
295 prevent diabetes.
296 In addition to investigating the pathology of diabetes onset, we also developed an 11-lipid
297 panel to predict future diabetes. Traditional clinical parameters such as FPG and 2hPG can
298 achieve a prediction power AUC of 0.775. When we combined this lipid panel with FPG and
299 2hPG, we improved the prediction power from 0.775 to 0.842. Among those 11 lipids, 10
300 belong to TAG and 1 is PC, suggesting specific metabolites of the TAG and PC classes play a
301 critical role in early prediction for detecting in the early postpartum period of GDM women who
302 have the highest risk of transitioning to T2D. Diabetes is a metabolic disorder involving
303 dysmetabolism of carbohydrate, lipids and amino acids. Therefore, it is not surprising that a
304 combination of biomarkers from both the carbohydrate and lipid metabolism could improve
305 the predictive power compared to using those from the carbohydrate metabolism pathway
306 alone. Based on our data, we would envision that adding a specific lipidomic signature to

existing clinical parameters for testing, perhaps including other metabolites (ie. biogenic amines and amino acids) will provide a more accurate assessment of future T2D risk. Nonetheless, our study provides an important clinical application for early prediction of diabetes when most GDM women return to normoglycemia after delivery. The early prediction will contribute to early intervention and prevention of diabetes.

Materials and Methods

SWIFT cohort

The Study of Women, Infant Feeding, and Type 2 Diabetes Mellitus After GDM Pregnancy (SWIFT) is a prospective cohort that conducted in-person research exams among 1,035 women with GDM diagnosed based on the 3-h 100-g OGTT via Carpenter and Coustan's criteria, and no prior history of diabetes or other serious health conditions (age 20–45 years, diverse ethnicities) within the Kaiser Permanente Northern California Healthcare System (KPNC) [39]. Details of the cohort recruitment, selection criteria, methodologies have been described previously [40]. Of 1,035 women with GDM who consented to participate in the 3 in-person research exams for the SWIFT Study, 1,010 participants did not have T2D at baseline (6–9 weeks postpartum) based on 2-h 75g oral glucose tolerance tests (OGTTs). All research participants underwent annual research 2-h 75-g OGTTs and other assessments at baseline throughout 2 years of follow-up, and subsequently for medical diagnoses of diabetes confirmed by laboratory testing from electronic medical records up to 8 years post-baseline. Research methodology included monthly quantitative assessment of lactation intensity and duration, socio-demographics, medical conditions, medication use, reproductive history, depression, subsequent births,

lifestyle behaviors, body composition and anthropometry [40]. Fasting and 2-h postload plasma samples from 75g OGTTs (baseline, 1 year, and 2 years post-baseline) were analyzed within several weeks for glucose and insulin levels, and fasting stored samples from the SWIFT Biobank (-80°C) were used to measure a lipid panel, free fatty acids and adipokines, as previously described[41,42]. Follow-up assessments to determine new onset T2D status were based on research 2-hour 75 g OGTTs and KPNC electronic medical records data based on mediation, ICD codes and laboratory tests for glucose tolerance[43]. T2D diagnosis was based on the American Diabetes Association (ADA) criteria[44]. The study design and all procedures were approved by the Kaiser Permanente Northern California Institutional Review Board (protocol numbers #CN-04EGund-03-H and #1279812-10) and Office of Research Ethics at University of Toronto (protocol number #38188). All participants gave written informed consent before taking part in the research exams.

Lipidomics assay

Baseline fasting plasma from 350 samples from a subset of the cohort (171 incident T2D vs 179 non-T2D controls) were sent to Metabolon, Inc. (Morrisville, NC) and measured by GC-MS and LC-MS. Lipids were extracted from the bio-fluid in the presence of deuterated internal standards using an automated BUME extraction according to the method of Lofgren et al [45]. The extracts were dried under nitrogen and reconstituted in ammonium acetate dichloromethane: methanol. The extracts were transferred to vials for infusion-MS analysis, performed on a Shimadzu LC with nano PEEK tubing and the Sciex SelexIon-5500 QTRAP. The samples were analyzed via both positive and negative mode electrospray. The 5500 QTRAP was operated in MRM mode with a total of more than 1,100 MRMs. 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. Lipid class concentrations were calculated from the sum of all molecular species within a class, and fatty acid compositions were determined by calculating the proportion of each class comprised by individual fatty acids. In this study, a total of 1008 lipid species from 15 classes and 296 fatty acid were measured. In particular, in the natural lipid group, 26 cholesterol esters (CE), 26 monoacylglycerol (MAG), 59 diacylglycerol (DAG), 493 triacylglycerol (TAG), and 26 free fatty acids (FFA) were detected. In phospholipid group, 140 phosphatidylcholine (PC), 216 phosphatidylethanolamine (PE), 28 phosphatidylinositol (PI), 26 lysophosphatidylcholine (LPC), and 26 lysophosphatidylethanolamine (LPE) were measured. In sphingolipid group, levels of 13 dihydroceramide (DCER), 12 ceramide (CER), 12 hexosylceramide (HCER), 12 lactosylceramide (LCER), and 12 species of sphingomyelin (SM) were tested.

Data Analyses

Data processing was performed for further statistical analysis. Lipids with >5% missing values were removed from the data allowing only the most robust lipids for the following statistical analysis. After this filtering step, 1008 species were reduced to 816 for further analysis. Remaining missing values were imputed as 1/2 minimum value for each specific lipid. Sample normalization was performed by normalizing each value within the sample to the total value of the sample to adjust differences among the samples. Log-transformation was performed. Odds ratios (ORs) of each lipid for T2D incidence were calculated by applying logistic regression models adjusting effects from race/ethnicity, age and BMI. FDR was calculated by correcting p-

value by Benjamini-Hochberg method for multiple comparison. A cut-off of FDR<0.05 was used for significance. Lipids with FDR of odds ratio <0.001 were subjected for lipid predictor selection. By applying a conditional logistic regression model with stepwise method (including forward and backwards), 11 lipids were selected for prediction models. Classification models were built with logistic regression and 10-fold cross validation was performed to evaluate the prediction performance. Prediction performance were presented as receiver operating characteristic (ROC) curves. Because association of lipids with diabetes risk can differ based on acyl chain length and unsaturation degree, lipids were grouped and further analyzed based on carbon atom and double bond numbers. All the analysis above was performed in open-source, statistical software, R v3.2.4. Pathway analysis was performed using positive- or negative- associated lipids in the web tool MetaboAnalyst 4.0 [46].

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508

Table 1. Prenatal and Study Baseline (6-9 weeks postpartum) Characteristics of Women with Gestational Diabetes Mellitus in Prospective Cohort Follow Up Study of Incidence of Diabetes (n = 350)

Prenatal characteristics	**Case** Diabetes at follow up (N=171)	**Control** No Diabetes at follow up (N=179)	p-value
Age (years), Mean (SD)	33.3 (5.2)	33.0 (4.5)	0.63
Race/ethnicity, n (%)			0.72
White	31 (18.1)	27 (15.1)	
Asian	51 (29.8)	55 (30.7)	
Black	21 (12.3)	16 (8.9)	
Hispanic	66 (38.6)	79 (44.1)	
Other	2 (1.2)	2 (1.1)	
Parity, n (%)			0.80
Primiparous (1 birth)	56 (32.7)	54 (30.2)	
Biparous (2 births)	62 (36.3)	64 (35.8)	
Multiparous (>2 births)	53 (31.0)	61 (34.1)	
GDM treatment, n (%)			<0.001
Diet only	74 (43.3)	128 (71.5)	
Oral medications	79 (46.2)	47 (26.3)	
Insulin	18 (10.5)	4 (2.3)	
Pre-pregnancy BMI (kg/m^2), Mean (SD)	33.6 (8.2)	32.3 (6.9)	0.10
Sum of Prenatal 3-hr 100 g OGTT glucose z-scores, Mean (SD)	1.4 (3.1)	-0.2 (2.5)	<0.001
Family history of diabetes, n (%)	101 (59.1)	89 (52.0)	0.08

Baseline characteristics at 6-9 weeks Postpartum			
BMI (kg/m^2), Mean (SD)	33.5 (7.4)	32.4 (6.3)	0.18
Fasting plasma glucose (FPG), mg/dl, Mean (SD)	101.5 (10.4)	94.3 (7.7)	<0.001
2-hr Post-load plasma glucose (75 g OGTT), mg/dl, Mean (SD)	131.0 (29.5)	109.8 (27.4)	<0.001
Fasting insulin, µU/ml, Median (IQR)	26.5 (20.7)	22.1 (17.4)	0.001
2-hr insulin, µU/ml, Median (IQR)	111.5 (85.7)	83.3 (73.6)	<0.001
Fasting plasma Triglycerides, mg/dl, Median (IQR)	119.0 (103.0)	94.0 (72.0)	0.003
Fasting plasma HDL-C, mg/dl, Mean (SD)	49.0 (16.0)	52.0 (19.0)	0.017
Fasting plasma Total Cholesterol, Mean (SD)	199.4 (34.5)	203.5 (35.5)	0.27
Fasting plasma LDL-C, Mean (SD)	121.0 (31.1)	126.4 (31.2)	0.10
HOMA-IR, Median (IQR)	6.8 (5.6)	5.0 (4.3)	<0.001
HOMA-B, Median (IQR)	268.1 (192.1)	256.0 (176.2)	0.61
Hypertension, n (%)	**14 (8.2)**	**5 (2.8)**	**0.04**
Smoker, n (%)	5 (2.9)	4 (2.2)	0.68
Dietary glycemic index, Mean (SD)	242.5 (106.7)	246.5 (112.5)	0.73
Dietary Intake, Percentage of Kcal as animal fat, Mean SD	27.0 (7.7)	25.6 (8.6)	0.10
Physical activity score, met-hrs per week, Mean (SD)	50.7 (23.4)	47.4 (20.6)	0.16

Data are presented as the mean (SD) unless otherwise noted.
Variables obtained from the SWIFT Study that administered the research 2-hr 75 g OGTTs and other assessments at in-person research visits (baseline).
Participants did not have diabetes at study baseline and underwent annual 2-hr 75 g OGTTs at baseline and annually for two years, and thereafter evaluated for diabetes onset from electronic medical records.
P-values are for incident diabetes case versus no diabetes controls at follow-up.
Statistically significant differences between group characteristics are shown in boldface type.

509

510 **Figure Legends**

511

512 **Figure 1.** SWIFT cohort and study design. SWIFT prospective cohort. 1,035 women diagnosed
513 with GDM in 2008-2011 were enrolled at 6–9 weeks postpartum (baseline). 1,010 of the 1,035
514 participants were confirmed via 2h 75 g OGTT without diabetes at baseline. Up to 8 years' post-
515 baseline, a total of 197 (19.5%) women developed T2D. At baseline, samples of 171 available

516 cases with 179 controls were measured using lipidomics. A total of 1008 lipid species from 15
517 lipid classes and 296 fatty acids were assessed in the plasma samples of all participants.
518
519 **Figure 2.** Overview of T2D associated lipids. (A) Volcano plot showed -log10(FDR) against
520 log2(odds ratio) of 816 lipid species in the association with T2D risk. Grey circles were denoted
521 as no significant association with T2D risk. Of those that are significantly associated, red circles
522 denote as neutral lipids, orange as phospholipids, blue as sphingolipids. (B) Number of T2D
523 positive-, negative- and non- associated lipids in each lipid class were shown. Orange, green and
524 blue bars denote positive, negative and non- associated lipids respectively. Significance was
525 indicated by FDR<0.05.
526 **Figure supplement 1.** Supervised PCA indicated partial separability of lipid profiles between
527 case and control groups.
528 **Figure supplement 2.** Odds ratio and 95% CI of 311 lipids associated with T2D risk (FDR<0.05).
529 **Source data 1.** Odds ratio, 95%CI and FDR values of all lipids. Lipids with FDR<0.05 were
530 highlighted.
531
532 **Figure 3.** Lipids strongly associated with risk of incident T2D. (A) Odds ratio and 95% CI of 107
533 lipids strongly associated with T2D risk (FDR<0.001) were indicated. The multivariate logistic
534 regression model was adjusted for race, age and BMI. (B) Correlation between 107 T2D-risk
535 associated lipids and conventional clinical parameters was indicated by correlation coefficient
536 (r). Orange color indicates positive correlation while blue denotes negative correlation.

Source data 1. Odds ratio, 95%CI and FDR values of all lipids. Lipids with FDR<0.001 were highlighted.

Source data 2. Correlation r values of T2D risk associated lipids with clinical parameters.

Figure 4. Association between diabetes risk and lipid structure. (A) Relationship between diabetes risk and total number of carbon atoms and double bonds in lipid species. Odds ratios were represented with dots, color denoting odds ratio value, dot size denoting significance by FDR value. (B) Relationship between diabetes risk and fatty acid composition in lipids. Red and blue color denotes log2(odds ratio) with significance (FDR<0.05), white denotes values with no significance, grey denotes fatty acids not detected.

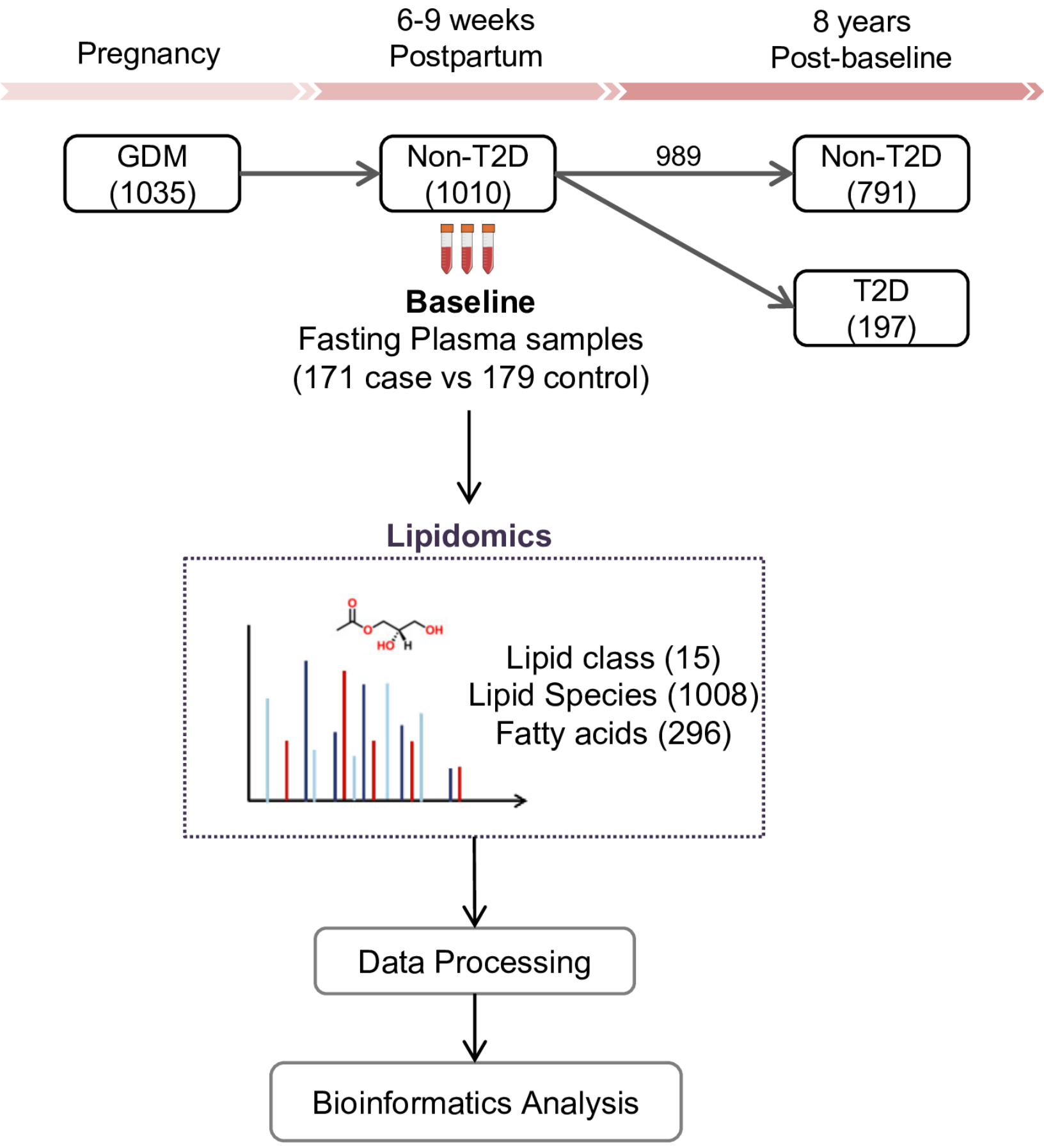
Source data 1. Odds ratio values, FDR values, numbers of carbon atoms and double bonds in all lipid species.

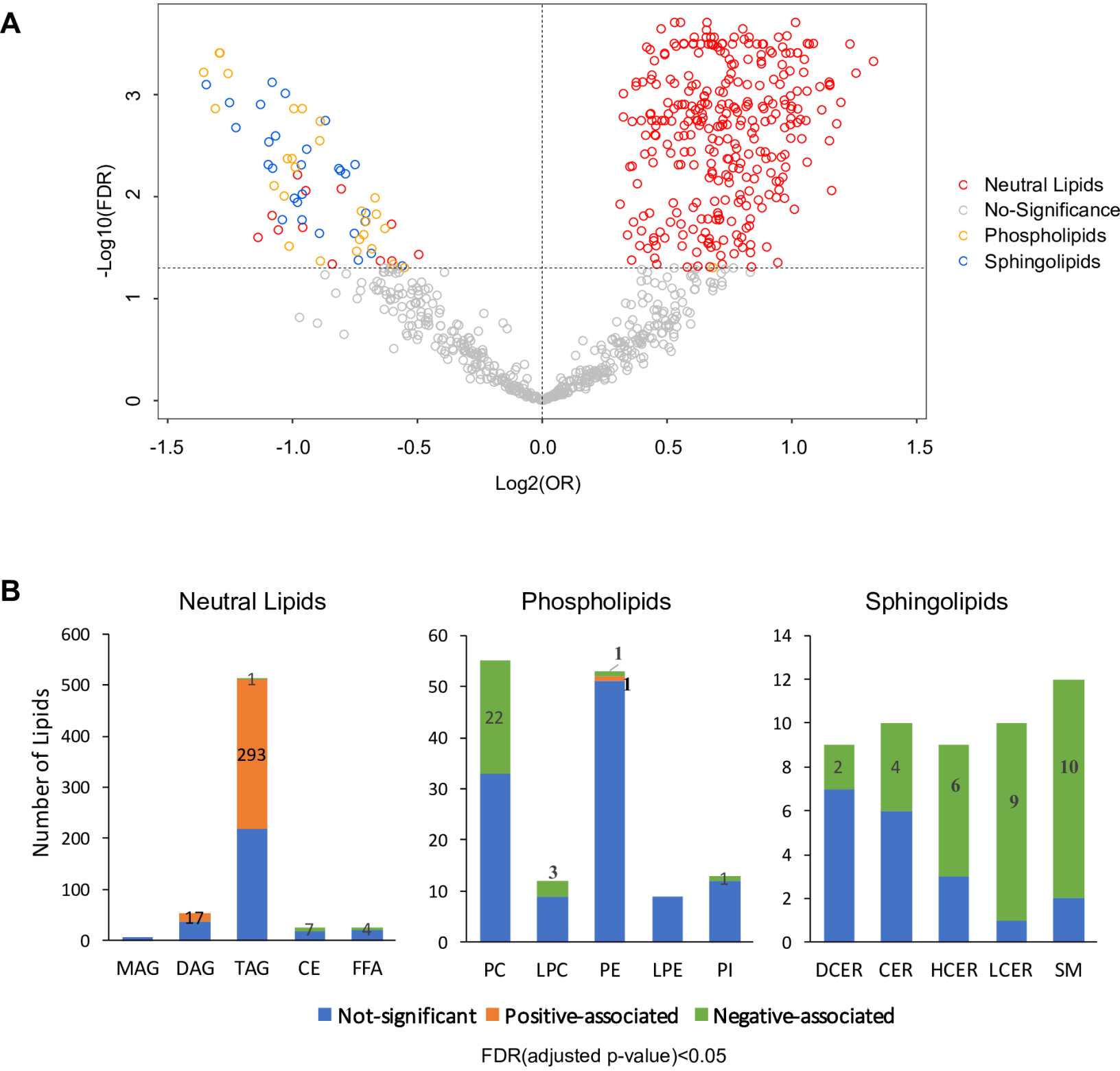
Source data 2. Relationship between diabetes risk and fatty acid composition in lipids.

Figure 5. Pathways associated with future T2D at baseline. (A) Significantly regulated biological pathways associated with future diabetes onset analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG). Blue denotes the down-regulated pathways and red denotes the up-regulated pathway. (B) The altered lipid classes in an integrated lipid metabolism pathway. Red denotes positive association whereas blue denotes negative association with significance of p-value indicated.

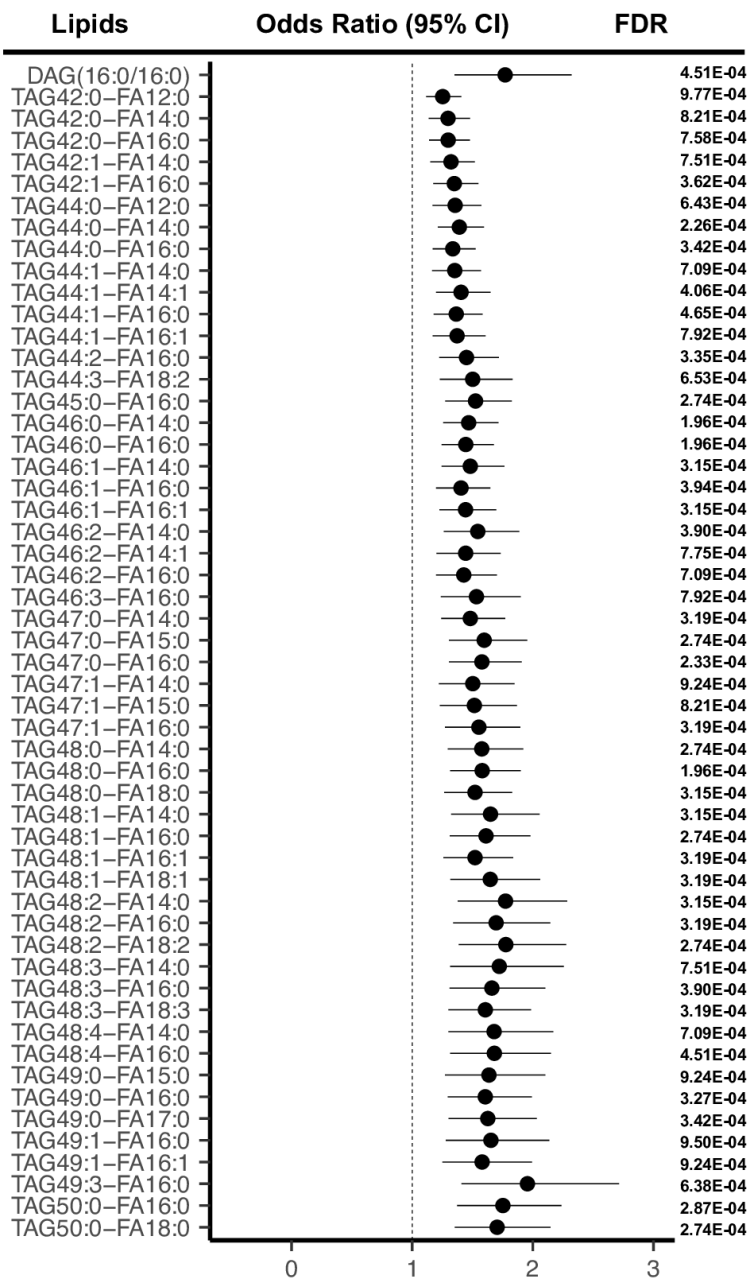
Figure 6. Selected lipid signature predicting future T2D. (A) Top 11 lipids with the best predictive performance were selected for building a model to predict future T2D. Their odds ratio and 95% CI of T2D association were shown. (B) Predictive performance of logistic regression model was demonstrated as ROC curve. The area under the curve and 95% CI in each model were shown.

Source data 1. Predictive performance of logistic regression model.


Pregnancy
6-9 weeks
Postpartum
8 years
Post-baseline
GDM
(1035)
Non-T2D
(1010)
989
Non-T2D
(791)
T2D
(197)
Baseline
Fasting Plasma samples
(171 case vs 179 control)
Lipidomics
Lipid class (15)
Lipid Species (1008)
Fatty acids (296)
Data Processing
Bioinformatics Analysis


A
-Log10(FDR)
Log2(OR)
Neutral Lipids
No-Significance
Phospholipids
Sphingolipids
B
Neutral Lipids
Phospholipids
Sphingolipids
Number of Lipids
MAG
DAG
TAG
CE
FFA
PC
LPC
PE
LPE
PI
DCER
CER
HCER
LCER
SM
Not-significant
Positive-associated
Negative-associated
FDR(adjusted p-value)<0.05

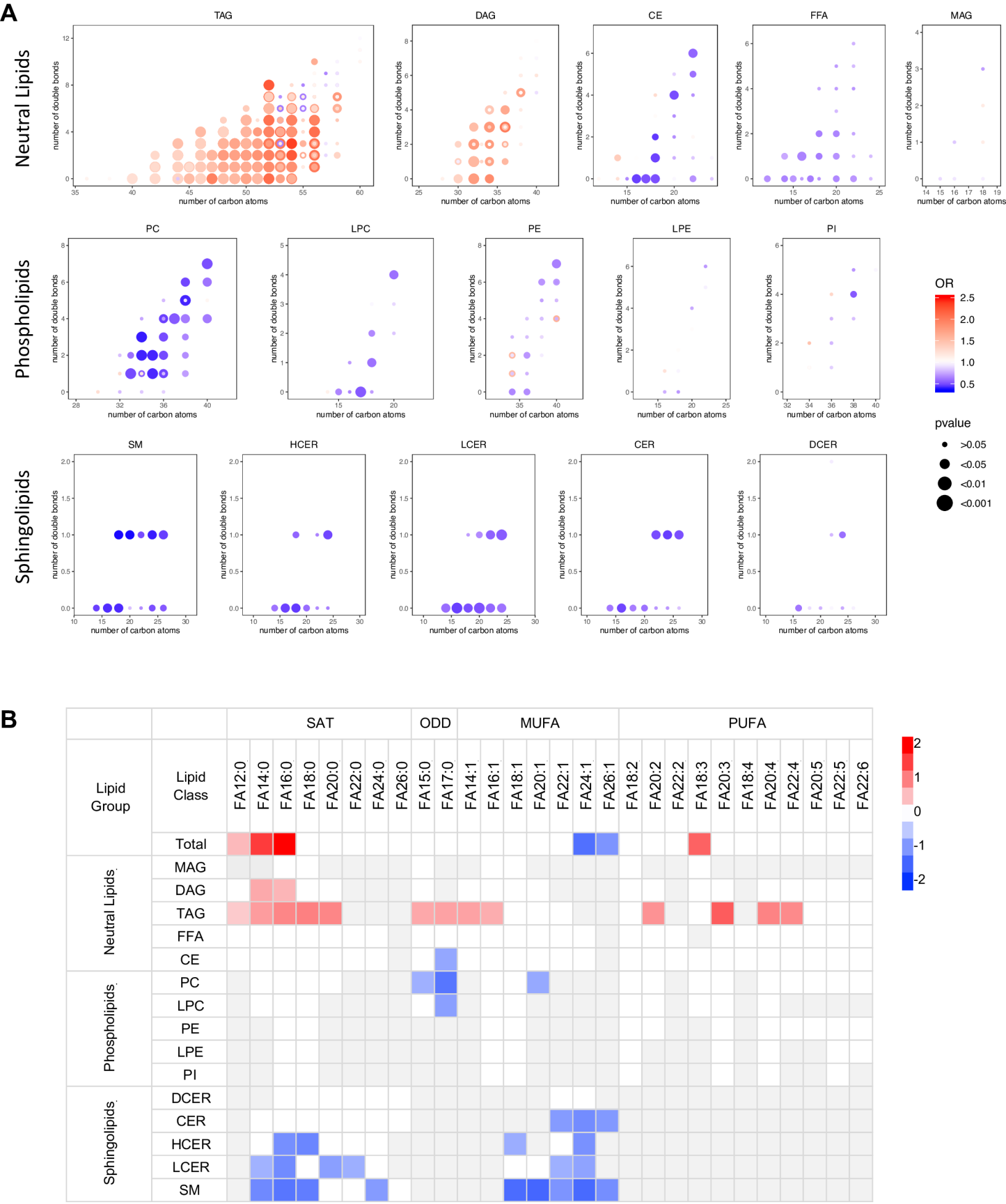
A

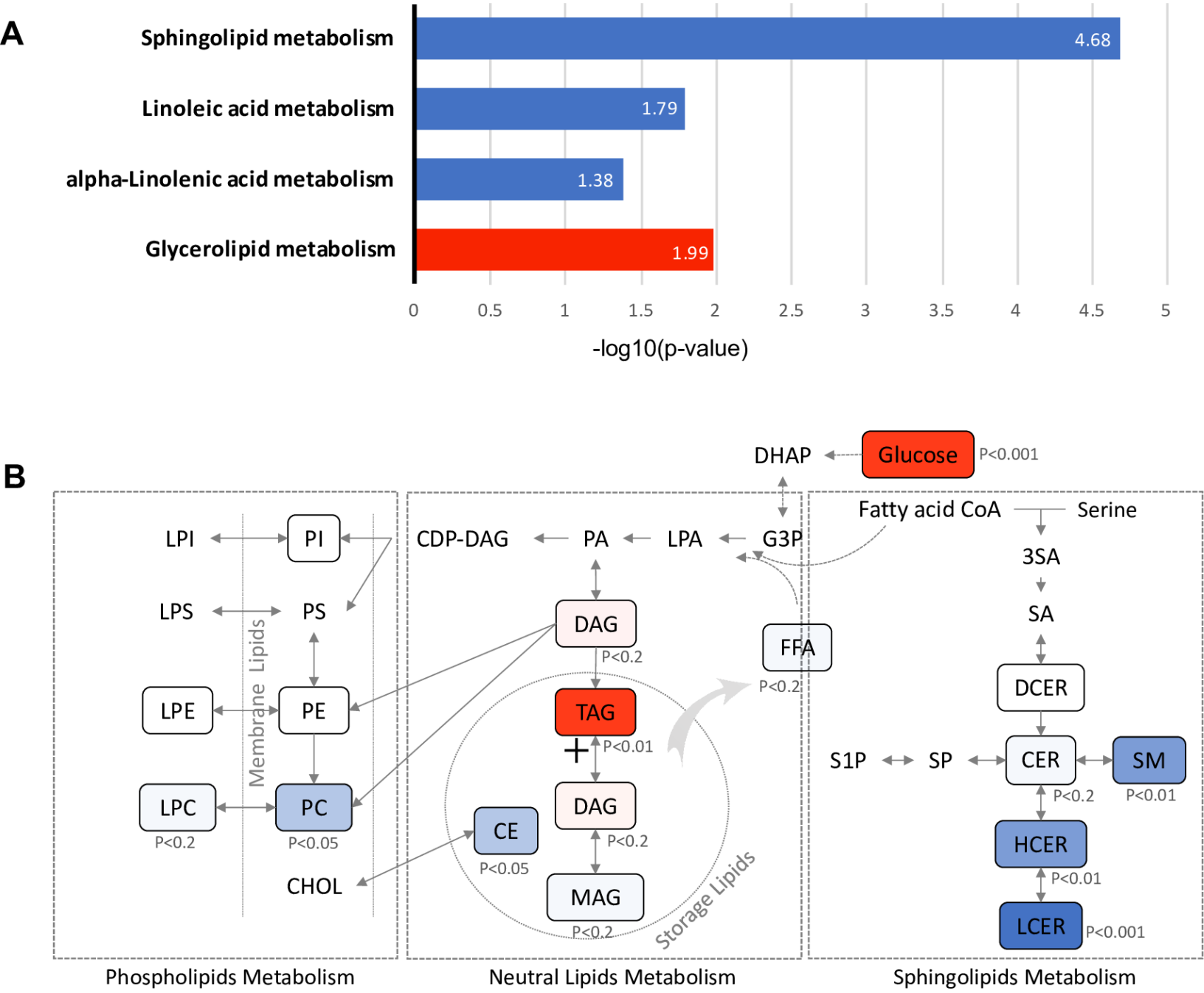


B

Coefficient r	FPG	2hPG	Fasting Insulin	HOMA-IR	HOMA-B	mom_age	pre_bmi
DAG & TAG	0.28 to 0.41	-0.03 to 0.20	0.36 to 0.59	0.37 to 0.61	0.20 to 0.40	-0.09 to 0.07	-0.05 to 0.21
Phospholipids & Sphigolipids	-0.34 to -0.23	-0.21 to -0.12	-0.43 to -0.33	-0.45 to -0.34	-0.28 to -0.16	-0.06 to 0.11	-0.13 to 0.12

	Positive	Negative
Strong	0.8 to 1	-1 to -0.8
Moderate	0.5 to 0.8	-0.8 to -0.5
Weak	0.3 to 0.5	-0.5 to -0.3
No Correlation	0 to 0.3	-0.3 to 0

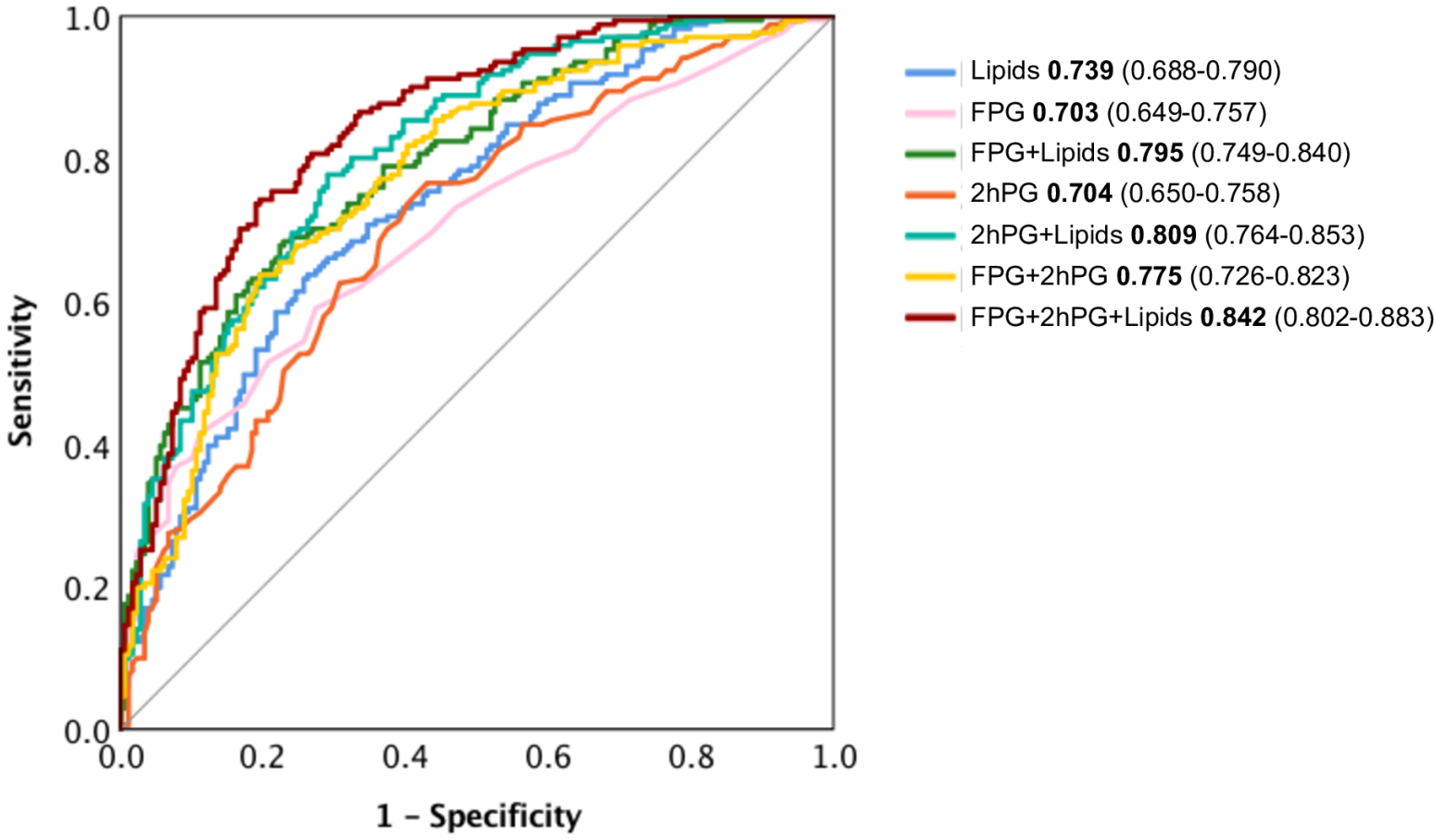

A
Neutral Lipids
Phospholipids
Sphingolipids
TAG
DAG
CE
FFA
MAG
PC
LPC
PE
LPE
PI
SM
HCER
LCER
CER
DCER
number of double bonds
number of carbon atoms
OR
2.5
2.0
1.5
1.0
0.5
pvalue
>0.05
<0.05
<0.01
<0.001
B
Lipid Group
Lipid Class
SAT
ODD
MUFA
PUFA
FA12:0
FA14:0
FA16:0
FA18:0
FA20:0
FA22:0
FA24:0
FA26:0
FA15:0
FA17:0
FA14:1
FA16:1
FA18:1
FA20:1
FA22:1
FA24:1
FA26:1
FA18:2
FA20:2
FA22:2
FA18:3
FA20:3
FA18:4
FA20:4
FA22:4
FA20:5
FA22:5
FA22:6
Total
MAG
DAG
TAG
FFA
CE
PC
LPC
PE
LPE
PI
DCER
CER
HCER
LCER
SM
Neutral Lipids
Phospholipids
Sphingolipids
2
1
0
-1
-2

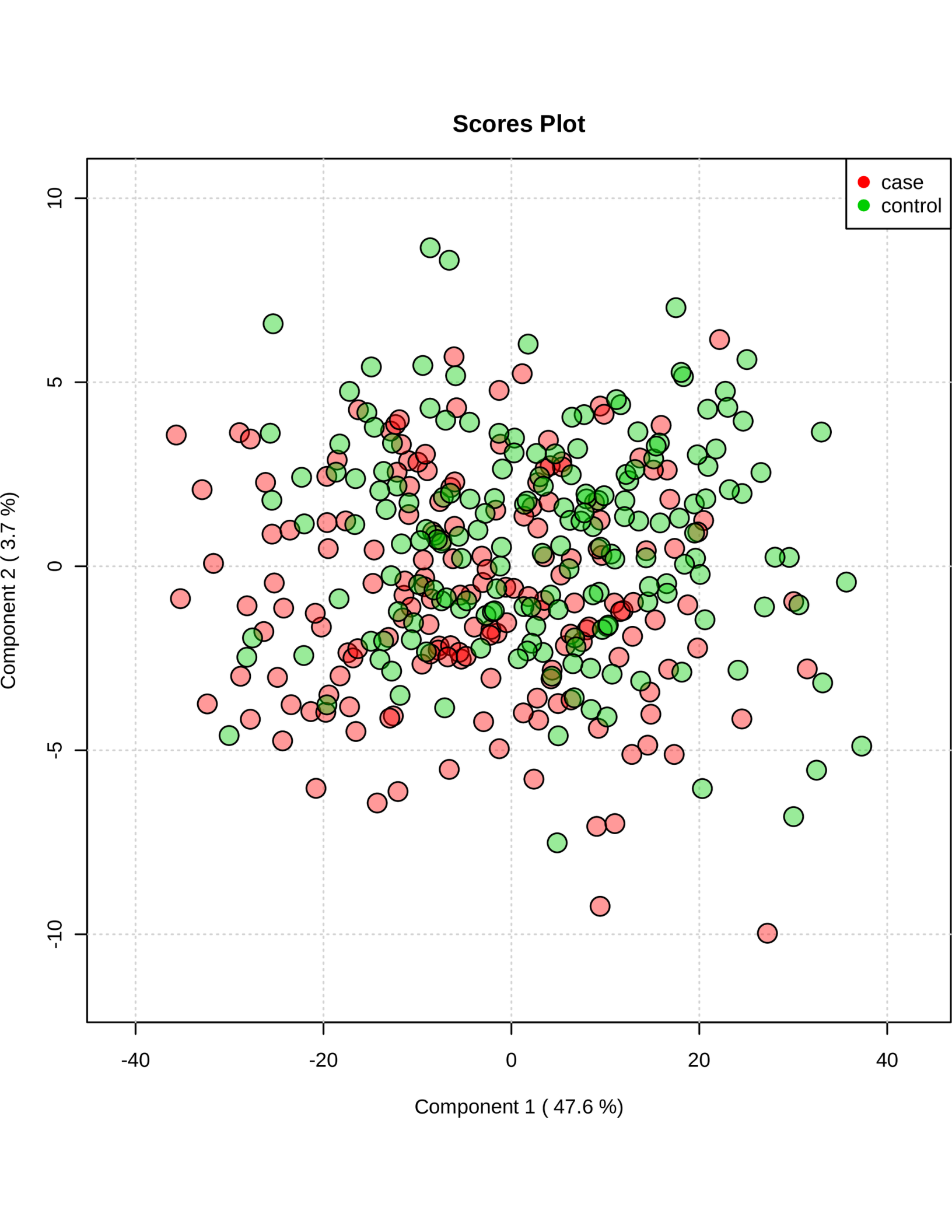

A
Sphingolipid metabolism
Linoleic acid metabolism
alpha-Linolenic acid metabolism
Glycerolipid metabolism
4.68
1.79
1.38
1.99
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
-log10(p-value)
B
DHAP
Glucose
P<0.001
Fatty acid CoA
Serine
3SA
SA
LPI
PI
CDP-DAG
PA
LPA
G3P
LPS
PS
DAG
P<0.2
FFA
P<0.2
DCER
Membrane Lipids
LPE
PE
TAG
P<0.01
S1P
SP
CER
SM
P<0.2
P<0.01
LPC
PC
DAG
CE
P<0.2
P<0.05
HCER
P<0.01
P<0.2
P<0.05
CHOL
MAG
P<0.2
Storage Lipids
LCER
P<0.001
Phospholipids Metabolism
Neutral Lipids Metabolism
Sphingolipids Metabolism

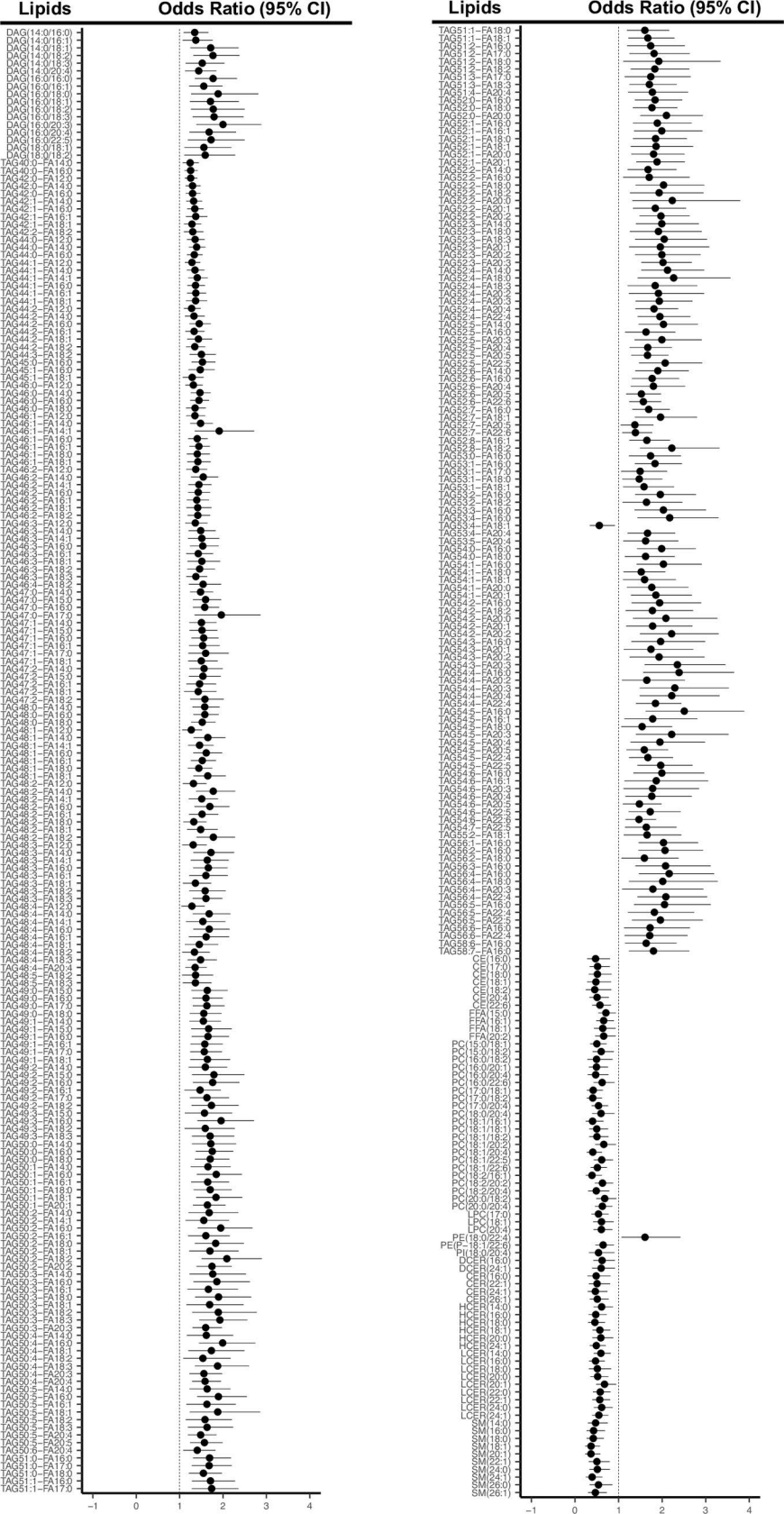
A

Lipids	Odds Ratio	95%CI-Lower	95%CI-Upper	FDR
PC(17:0/18:1)	0.42	0.27	0.64	6.21E-04
TAG42:1(14:0)	1.32	1.15	1.52	7.51E-04
TAG44:0(16:0)	1.34	1.17	1.53	3.42E-04
TAG46:0(14:0)	1.47	1.26	1.71	1.96E-04
TAG47:0(16:0)	1.58	1.30	1.91	2.33E-04
TAG47:1(15:0)	1.52	1.23	1.87	8.21E-04
TAG48:0(14:0)	1.58	1.29	1.92	2.74E-04
TAG48:1(18:1)	1.65	1.32	2.06	3.19E-04
TAG48:2(16:0)	1.70	1.34	2.14	3.19E-04
TAG52:1(20:1)	1.88	1.41	2.52	3.72E-04
TAG52:4(14:0)	2.12	1.52	2.97	3.15E-04

B




Scores Plot
case
control
10
5
0
-5
-10
Component 2 (3.7 %)
-40
-20
0
20
40
Component 1 (47.6 %)


Lipids
Odds Ratio (95% CI)
DAG(14:0/16:0)
DAG(14:0/16:1)
DAG(14:0/18:1)
DAG(14:0/18:2)
DAG(14:0/18:3)
DAG(14:0/20:4)
DAG(16:0/16:0)
DAG(16:0/16:1)
DAG(16:0/18:0)
DAG(16:0/18:1)
DAG(16:0/18:2)
DAG(16:0/18:3)
DAG(16:0/20:3)
DAG(16:0/20:4)
DAG(18:0/22:5)
DAG(18:0/18:1)
DAG(18:0/18:2)
TAG40:0 – FA14:0
TAG40:0 – FA16:0
TAG42:0 – FA12:0
TAG42:0 – FA14:0
TAG42:0 – FA16:0
TAG42:1 – FA14:0
TAG42:1 – FA16:0
TAG42:1 – FA16:1
TAG42:1 – FA18:1
TAG42:2 – FA18:2
TAG43:0 – FA17:0
TAG44:0 – FA14:0
TAG44:0 – FA16:0
TAG44:1 – FA12:0
TAG44:1 – FA14:0
TAG44:1 – FA14:1
TAG44:1 – FA16:0
TAG44:1 – FA16:1
TAG44:1 – FA18:1
TAG44:2 – FA12:0
TAG44:2 – FA14:0
TAG44:2 – FA16:1
TAG44:2 – FA18:1
TAG44:2 – FA18:2
TAG44:3 – FA18:2
TAG45:0 – FA17:0
TAG45:1 – FA16:0
TAG45:1 – FA18:1
TAG46:0 – FA12:0
TAG46:0 – FA14:0
TAG46:0 – FA16:0
TAG46:0 – FA18:0
TAG46:1 – FA12:0
TAG46:1 – FA14:0
TAG46:1 – FA14:1
TAG46:1 – FA16:0
TAG46:1 – FA16:1
TAG46:1 – FA18:0
TAG46:1 – FA18:1
TAG46:2 – FA12:0
TAG46:2 – FA14:0
TAG46:2 – FA14:1
TAG46:2 – FA16:0
TAG46:2 – FA16:1
TAG46:2 – FA18:1
TAG46:2 – FA18:2
TAG46:3 – FA12:0
TAG46:3 – FA14:0
TAG46:3 – FA14:1
TAG46:3 – FA16:0
TAG46:3 – FA16:1
TAG46:3 – FA18:1
TAG46:3 – FA18:2
TAG46:3 – FA18:3
TAG46:4 – FA18:2
TAG47:0 – FA14:0
TAG47:0 – FA15:0
TAG47:0 – FA16:0
TAG47:0 – FA17:0
TAG47:1 – FA14:0
TAG47:1 – FA15:0
TAG47:1 – FA16:0
TAG47:1 – FA16:1
TAG47:1 – FA17:0
TAG47:1 – FA18:1
TAG47:2 – FA14:0
TAG47:2 – FA15:0
TAG47:2 – FA16:1
TAG47:2 – FA18:1
TAG47:2 – FA18:2
TAG48:0 – FA14:0
TAG48:0 – FA16:0
TAG48:0 – FA18:0
TAG48:1 – FA12:0
TAG48:1 – FA14:0
TAG48:1 – FA14:1
TAG48:1 – FA16:0
TAG48:1 – FA16:1
TAG48:1 – FA18:0
TAG48:1 – FA18:1
TAG48:2 – FA12:0
TAG48:2 – FA14:0
TAG48:2 – FA14:1
TAG48:2 – FA16:0
TAG48:2 – FA16:1
TAG48:2 – FA18:0
TAG48:2 – FA18:1
TAG48:2 – FA18:2
TAG48:3 – FA15:0
TAG48:3 – FA14:0
TAG48:3 – FA14:1
TAG48:3 – FA16:0
TAG48:3 – FA16:1
TAG48:3 – FA18:1
TAG48:3 – FA18:2
TAG48:3 – FA18:3
TAG48:4 – FA14:0
TAG48:4 – FA14:1
TAG48:4 – FA16:0
TAG48:4 – FA16:1
TAG48:4 – FA18:2
TAG48:4 – FA18:3
TAG48:4 – FA20:4
TAG48:5 – FA18:2
TAG48:5 – FA18:3
TAG49:0 – FA15:0
TAG49:0 – FA16:0
TAG49:0 – FA17:0
TAG49:0 – FA18:0
TAG49:1 – FA14:0
TAG49:1 – FA15:0
TAG49:1 – FA16:0
TAG49:1 – FA16:1
TAG49:1 – FA17:0
TAG49:1 – FA18:1
TAG49:2 – FA14:0
TAG49:2 – FA15:0
TAG49:2 – FA16:0
TAG49:2 – FA16:1
TAG49:2 – FA17:0
TAG49:2 – FA18:2
TAG49:3 – FA15:0
TAG49:3 – FA16:0
TAG49:3 – FA18:2
TAG49:3 – FA18:3
TAG50:0 – FA14:0
TAG50:0 – FA16:0
TAG50:0 – FA18:0
TAG50:1 – FA14:0
TAG50:1 – FA16:0
TAG50:1 – FA16:1
TAG50:1 – FA18:0
TAG50:1 – FA18:1
TAG50:1 – FA20:1
TAG50:2 – FA14:0
TAG50:2 – FA14:1
TAG50:2 – FA16:0
TAG50:2 – FA16:1
TAG50:2 – FA18:0
TAG50:2 – FA18:1
TAG50:2 – FA18:2
TAG50:2 – FA20:2
TAG50:3 – FA14:0
TAG50:3 – FA16:0
TAG50:3 – FA16:1
TAG50:3 – FA18:0
TAG50:3 – FA18:1
TAG50:3 – FA18:2
TAG50:3 – FA18:3
TAG50:3 – FA20:3
TAG50:4 – FA14:0
TAG50:4 – FA16:0
TAG50:4 – FA18:1
TAG50:4 – FA18:2
TAG50:4 – FA18:3
TAG50:4 – FA20:3
TAG50:4 – FA20:4
TAG50:5 – FA14:0
TAG50:5 – FA16:0
TAG50:5 – FA16:1
TAG50:5 – FA18:2
TAG50:5 – FA18:3
TAG50:5 – FA20:4
TAG50:5 – FA20:5
TAG50:6 – FA20:4
TAG51:0 – FA16:0
TAG51:0 – FA17:0
TAG51:1 – FA16:0
TAG51:1 – FA17:0
–1
0
1
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Lipids
Odds Ratio (95% CI)
TAG51:1 – FA18:0
TAG51:2 – FA18:1
TAG51:2 – FA18:2
TAG51:2 – FA17:0
TAG51:2 – FA18:0
TAG51:2 – FA18:2
TAG51:3 – FA17:0
TAG51:3 – FA18:3
TAG51:4 – FA20:4
TAG52:0 – FA16:0
TAG52:0 – FA18:0
TAG52:0 – FA20:0
TAG52:1 – FA16:0
TAG52:1 – FA16:1
TAG52:1 – FA18:0
TAG52:1 – FA18:1
TAG52:1 – FA20:0
TAG52:2 – FA20:1
TAG52:2 – FA14:0
TAG52:2 – FA16:0
TAG52:2 – FA18:0
TAG52:2 – FA18:2
TAG52:2 – FA20:0
TAG52:2 – FA20:1
TAG52:3 – FA20:2
TAG52:3 – FA14:0
TAG52:3 – FA18:0
TAG52:3 – FA18:3
TAG52:3 – FA20:1
TAG52:3 – FA20:2
TAG52:3 – FA20:3
TAG52:4 – FA14:0
TAG52:4 – FA18:0
TAG52:4 – FA18:3
TAG52:4 – FA20:3
TAG52:4 – FA20:4
TAG52:4 – FA22:4
TAG52:5 – FA14:0
TAG52:5 – FA16:0
TAG52:5 – FA20:3
TAG52:5 – FA20:4
TAG52:5 – FA20:5
TAG52:5 – FA22:5
TAG52:6 – FA14:0
TAG52:6 – FA16:0
TAG52:6 – FA20:4
TAG52:6 – FA20:5
TAG52:6 – FA22:6
TAG52:7 – FA16:0
TAG52:7 – FA18:1
TAG52:7 – FA20:5
TAG52:7 – FA22:6
TAG52:8 – FA16:1
TAG52:8 – FA18:2
TAG53:0 – FA16:0
TAG53:1 – FA16:0
TAG53:1 – FA17:0
TAG53:1 – FA18:0
TAG53:1 – FA18:1
TAG53:2 – FA16:0
TAG53:2 – FA18:2
TAG53:3 – FA16:0
TAG53:4 – FA18:0
TAG53:4 – FA18:1
TAG53:4 – FA20:4
TAG53:5 – FA20:4
TAG54:0 – FA16:0
TAG54:0 – FA18:0
TAG54:1 – FA16:0
TAG54:1 – FA18:0
TAG54:1 – FA18:1
TAG54:1 – FA20:0
TAG54:1 – FA20:1
TAG54:2 – FA16:0
TAG54:2 – FA18:2
TAG54:2 – FA20:0
TAG54:2 – FA20:1
TAG54:2 – FA20:2
TAG54:3 – FA16:0
TAG54:3 – FA20:1
TAG54:3 – FA20:2
TAG54:3 – FA20:3
TAG54:4 – FA16:0
TAG54:4 – FA20:2
TAG54:4 – FA20:3
TAG54:4 – FA20:4
TAG54:4 – FA22:4
TAG54:5 – FA16:0
TAG54:5 – FA18:1
TAG54:5 – FA18:2
TAG54:5 – FA20:3
TAG54:5 – FA20:4
TAG54:5 – FA20:5
TAG54:5 – FA22:4
TAG54:5 – FA22:5
TAG54:6 – FA16:0
TAG54:6 – FA16:1
TAG54:6 – FA20:3
TAG54:6 – FA20:4
TAG54:6 – FA20:5
TAG54:6 – FA22:5
TAG54:7 – FA22:5
TAG55:2 – FA18:1
TAG56:0 – FA16:0
TAG56:2 – FA16:0
TAG56:2 – FA18:0
TAG56:3 – FA16:0
TAG56:4 – FA18:0
TAG56:4 – FA18:0
TAG56:4 – FA20:3
TAG56:4 – FA22:4
TAG56:5 – FA16:0
TAG56:5 – FA22:4
TAG56:6 – FA22:5
TAG56:6 – FA16:0
TAG56:6 – FA22:4
TAG58:5 – FA16:0
TAG58:7 – FA16:0
CE(16:0)
CE(17:0)
CE(18:0)
CE(18:1)
CE(18:2)
CE(20:4)
CE(22:6)
FFA(15:0)
FFA(18:1)
FFA(18:1)
FFA(20:2)
PC(15:0/18:1)
PC(15:0/18:2)
PC(16:0/18:2)
PC(16:0/20:1)
PC(16:0/20:4)
PC(16:0/22:6)
PC(17:0/18:1)
PC(17:0/18:2)
PC(17:0/20:4)
PC(18:0/20:4)
PC(18:1/18:1)
PC(18:1/18:2)
PC(18:1/18:2)
PC(18:1/20:4)
PC(18:1/22:5)
PC(18:1/22:6)
PC(18:2/16:1)
PC(18:2/20:2)
PC(18:2/20:4)
PC(20:0/18:2)
PC(20:0/20:4)
LPC(17:0)
LPC(18:1)
LPC(20:4)
PE(18:0/22:6)
PE(P-18:1/22:6)
PI(18:0/20:4)
DCER(16:0)
DCER(24:1)
CER(16:0)
CER(22:1)
CER(24:1)
CER(26:1)
HCER(14:0)
HCER(16:0)
HCER(18:0)
HCER(18:1)
HCER(20:0)
HCER(24:1)
LCER(14:0)
LCER(16:0)
LCER(18:0)
LCER(20:0)
LCER(20:1)
LCER(22:0)
LCER(22:1)
LCER(24:0)
LCER(24:1)
SM(14:0)
SM(16:0)
SM(18:0)
SM(18:1)
SM(20:1)
SM(22:1)
SM(24:0)
SM(24:1)
SM(26:0)
SM(26:1)
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0
1
2
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