Identification of Shared and Unique Serum Lipid Profiles in Diabetes Mellitus and Myocardial Infarction

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Background—Diabetes mellitus (DM) and cardiovascular disease are associated with dyslipidemia, but the detailed lipid molecular pattern in both diseases remains unknown.

Methods and Results—We used shotgun mass spectrometry to determine serum levels of 255 molecular lipids in 316 controls, 171 DM, and 99 myocardial infarction (MI) events from a cohort derived from the Malmö Diet and Cancer study. Orthogonal projections to latent structures analyses were conducted between the lipids and clinical parameters describing DM or MI. Fatty acid desaturases (FADS) and elongation of very long chain fatty acid protein 5 (ELOVL5) activities were estimated by calculating product to precursor ratios of polyunsaturated fatty acids in complex lipids. *FADS* genotypes encoding these desaturases were then tested for association with lipid levels and ratios. Differences in the levels of lipids belonging to the phosphatidylcholine and triacylglyceride (TAG) classes contributed the most to separating DM from controls. TAGs also played a dominating role in discriminating MI from controls. Levels of C18:2 fatty acids in complex lipids were lower both in DM and MI versus controls (DM, $P=0.004$; MI, $P=6.0E-06$) at least due to an acceleration in the metabolic flux from C18:2 to C20:4 (eg, increased estimated ELOVL5: DM, $P=0.02$; MI, $P=0.04$, and combined elongase-desaturase activities: DM, $P=3.0E-06$; MI, $P=2.0E-06$). Minor allele carriers of *FADS* genotypes were associated with increased levels of C18:2 ($P\leq0.007$) and lower desaturase activity ($P\leq0.002$).

Conclusions—We demonstrate a possible relationship between decreased levels of C18:2 in complex lipids and DM or MI. We thereby highlight the importance of molecular lipids in the pathogenesis of both diseases. (*J Am Heart Assoc*. 2016;5:e004503 doi: 10.1161/JAHA.116.004503)

Key Words: diabetes mellitus • fatty acid desaturase • genotype • lipid metabolites • myocardial infarction

I t is well known that both diabetes mellitus (DM) and cardiovascular disease (CVD) are associated with dyslipidemia. Typically, DM is characterized by high total

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Accompanying Tables S1 through S8 and Figures S1, S2 are available at http://jaha.ahajournals.org/content/5/12/e004503/DC1/embed/inline-supplementary-material-1.pdf

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triacylglyceride (TAG) levels and low high-density lipoprotein (HDL) cholesterol[1] while CVD is associated with elevated low-density lipoprotein (LDL) and low HDL cholesterol levels.[2] There are also strong emerging evidences of a causal role of TAG levels in CVD.[3–6] In addition, DM is one of the major risk factors for CVD[7] and thus both may have similar determinants. The reason DM is a strong risk factor for CVD is still unknown; however, shared etiological disturbances of lipid metabolism are a possibility, and, if proven true, would open up novel treatment targets for both diseases.

Although used in clinical assessments for decades, total plasma TAGs as well as LDL and HDL cholesterol levels are sum measurements of numerous lipid molecular species and are only part of the different lipid classes present in the circulation.[8] Lipidomics-based studies have now identified specific molecular lipids associated with DM[9,10] and CVD,[11] but the overall detailed high coverage lipid molecular pattern in both diseases is still largely unknown. We thus aimed to define fingerprints of lipid molecular species in DM and CVD in fasted serum. A mass spectrometry (MS)–based shotgun lipidomics approach was used. In contrast to our previous study,[12] we have extended the lipid coverage down to the

molecular lipid species level with identification of individual fatty acids (ie, chain length and degree of saturation) for the majority of the lipid classes analyzed.

Methods

Study Participants and Data Collection

The MDC-CC (Malmö Diet and Cancer Cardiovascular Cohort) is a Swedish cohort designed to study the epidemiology of carotid artery disease with baseline data recorded from 1991 through 1996.[13,14] All individuals who were alive and still living in Sweden were invited to participate in a reexamination between 2007 and 2012, of whom 3734 attended. Participants in the reinvestigation cohort underwent a medical history assessment, a physical examination, and a laboratory assessment of DM and cardiovascular risk factors, including measurement of the common carotid artery intima-media thickness by ultrasound.[14,15] The MDC-CC was approved by the ethics committee at Lund University, and all participants provided written informed consent.

DM was defined as the following: fasting plasma glucose level of ≥7.0 mmol/L, or a 120-minute value post-oral glucose tolerance test plasma glucose level of >11.0 mmol/L, or a history of physician diagnosis of DM, or taking antidiabetic medication, or having been registered in local or national diabetes mellitus registries.[16] Myocardial infarction (MI) was defined as fatal or nonfatal MI on the basis of the *International Classification of Disease—9th Revision* (ICD-9) code 410 (ie, acute MI) and *ICD-10* code I21 (ie, ST-segment elevation and non—ST-segment elevation MI), or death due to ischemic heart disease according to *ICD-9* codes 412 (ie, old MI) and 414 (ie, other forms of chronic ischemic heart disease), or *ICD-10* codes I22 (ie, subsequent ST-segment elevation and non—ST-segment elevation MI), I23 (ie, certain current complications following ST-segment elevation and non—ST-segment elevation MI), or I25 (ie, chronic ischemic heart disease) and was ascertained from local or national records as previously described.[14]

From the MDC-CC reexamination were randomly selected 316 controls (ie, individuals without DM or MI), 171 DM events (101 prevalent cases and 54 incident cases), and 99 MI events (79 prevalent cases and 11 incident cases). A total of 10 individuals displayed both end points. Clinical characteristics of the study samples are presented in Tables 1 and 2.

Serum Lipidomics

Lipid extraction of never thawed, overnight fasted serum samples stored at −80°C upon collection was performed at Lipotype GmbH using high throughput Shotgun Lipidomics

Table 1. Y Variables in the DM Model

	Controls	DM
No.	293	155
Incidents, %	0	34.8
Age, y	72.8±5.8	72.6±5.3
Women, %	67.9	54.8
BMI, kg/m^2	26.4±4.2	27.9±4.8
Waist, cm	90.0±12.3	96.3±12.9
Waist to hip ratio	0.9±0.08	0.9±0.09
Total triglycerides, mmol/L	1.1±0.6	1.3±0.6
Total cholesterol, mmol/L	5.4±1.1	4.7±1.0
HDL cholesterol, mmol/L	1.5±0.5	1.3±0.4
LDL cholesterol, mmol/L	3.4±1.0	2.9±0.9
Fasting glucose, mmol/L	5.7±0.6	7.4±1.4
2-h glucose (OGTT), mmol/L	6.8±2.1	10.2±3.2
U-albumin, g/L	0.02±0.1	0.02±0.06
U-creatinine, mmol/L	8.2±4.0	8.6±3.9
Statin, %	20.1	49
Antihypertensive medicine, %	39.2	72.3
Diabetes medicine, %	0	33

Values are mean±SD or percentage. BMI indicates body mass index; DM, diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test.

(Dresden, Germany) technology as previously described.[17] In short, 2 μL of blood serum were extracted with MTBE/MetOH 7:2 with a fully automated liquid handling station (Hamilton) in a 96 well format. Shotgun MS analysis was conducted on a QExactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled to a TriVersa NanoMate robotic nanoflow ion source (Advion BioSciences, Ithaca, NY).[17] Lipids were identified and quantified using the proprietary LipotypeXplorer software, which is based on LipidXplorer.[18] Lipid intensities were normalized to lipid class–specific internal standards and data reported as molar amounts. Analytical quality was assessed by the inclusion of reference and blank samples. Data were corrected for batch effects and drift based on reference samples. Median technical variation was 7.2% (coefficient of variation). A total of 357 lipid species belonging to 16 major lipid classes were identified and quantified. Lipid species present in <50% of all samples were excluded, leaving 255 lipid species for further analysis.

Multivariate Data Analysis

Orthogonal projections to latent structures (OPLS) methods were adopted to analyze the correlation between lipid molecular species and clinical parameters. OPLS is a

Table 2. Y Variables in the MI Model

	Controls	MI
No.	293	90
Incidents, %	0	11
Age, y	72.8±5.8	75.1±4.9
Women, %	67.9	33.3
BMI, kg/m^2	26.4±4.2	26.6±3.9
Waist, cm	90.0±12.3	94.3±12.0
Waist to hip ratio	0.9±0.08	0.9±0.08
Systolic blood pressure, mm Hg	143.0±18.6	143.6±18.1
Diastolic blood pressure, mm Hg	83.2±9.9	80.2±9.0
Heart rate	67.7±11.1	65.3±10.4
Total triglycerides, mmol/L	1.1±0.6	1.1±0.5
Total cholesterol, mmol/L	5.4±1.1	4.2±0.9
HDL cholesterol, mmol/L	1.5±0.5	1.3±0.4
LDL cholesterol, mmol/L	3.4±1.0	2.5±0.8
Fasting glucose, mmol/L	5.7±0.6	6.0±0.8
2-h glucose (OGTT), mmol/L	6.8±2.1	7.5±1.9
Plaque score	3.1±1.8	4.0±1.4
Plaque area, mm^2	20.8±11.8	23.0±12.4
Blood flow velocity, max	0.7±0.3	0.7±0.4
Blood flow velocity, diastolic	0.2±0.1	0.2±0.1
Lumen diameter, mean CCA	6.3±0.8	6.6±0.9
Lumen diameter, max CCA	6.6±0.8	6.9±0.9
Stenosis	1.9±10.8	5.5±17.4
IMT, mean CCA	0.9±0.2	1.0±0.3
IMT, max CCA	1.0±0.2	1.2±0.5
IMT, max CCA bifurcation	1.8±0.7	2.1±0.7
Statins, %	20.1	80
Antihypertensive medication, %	39.2	89
Diabetes medication, %	0	3.3

Values are mean±SD or percentage. BMI indicates body mass index; CCA, common carotid artery; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein; MI, myocardial infarction; OGTT, oral glucose tolerance test.

supervised multivariate method where the systematic variation in X matrix is divided into two parts—one that is linearly related to Y (and therefore predictive) and one that is unrelated (orthogonal) to Y.[17,18] Data were mean-centered and unit variance scaled prior to the analysis. The model complexity was estimated according to cross-validation.[19] The software used for multivariate models was Simca p+14x64 (Umetrics). OPLS has previously been used to analyze gene/isoform expression and metabolomics as well as protein expression data.[20–22]

In the present study, two separate OPLS analyses were performed: one with 16 clinical parameters as Y matrix for DM (Table 1) and one with 27 clinical parameters as Y matrix for MI (Table 2). A total of 487 and 415 individuals were originally included in the DM and MI analysis, respectively. Individuals with >50% missing data were excluded, resulting in 448 and 383 individuals in the DM and MI groups, respectively, in the final analyses. For the controls and MI events, there were no differences in any of the clinical characteristics between the included individuals and the one excluded (Tables S1 and S2). For DM events, all clinical characteristics between included and excluded individuals were similar, except for urinary creatinine, which was lower in the excluded versus the included individuals, and the percentage of individuals taking antihypertensive medicine, which was higher in the excluded versus the included individuals (Table S3).

Additional OPLS analyses were performed for both DM and MI, excluding the 10 individuals displaying both DM and MI. The results show very similar OPLS model parameters compared with the analyses with the 10 individuals included (Table S4). Therefore, the 10 individuals were subsequently included in the analyses. The significance of lipid species related to correlation between lipid species levels and clinical parameters in DM and MI separately was analyzed by means of the predictive loadings in the OPLS models. An OPLS model including several Y vectors will in most cases result in several predictive components (showing correlation to the Y vectors) and maybe one or several orthogonal components (showing systematic variation in the X matrix that is not related to Y). In order to analyze the importance of each X variable for all the predictive projections resulting from the performed OPLS analyses, a variable influence on projection (VIP) parameter was used, which summarizes the importance of the predictive principal components calculated by cross-validation.[18,23] Variables with VIP >1 were considered to be significant. All parameters from both the DM and MI models derived from 7-fold cross-validation are shown in Table S4. Calculated VIPs from the OPLS models for DM and MI are shown in Tables S5 through S7.

Estimation of Desaturases and Elongase Activities

Delta-5 desaturase (D5D), encoded by *FADS1*, delta-6 desaturase (D6D), encoded by *FADS2,* and elongation of very long chain fatty acid 5 (ELOVL5), encoded by *ELOVL5*, are required for the de novo synthesis of long-chain polyunsaturated fatty acids (PUFAs). We calculated product to precursor ratios of PUFAs contained in complex lipids (glycerophospholipids and diacylglycerides [DAGs]) from the DM or MI model, respectively, as a surrogate measure for these desaturases and elongase activities,[24] which reflects both transcription level and catalytic capacity of these enzymes. The ratio of C20:4 to C20:3 was used to estimate D5D activity as previously

performed,[25] ratio of C18:3 to C18:2 for D6D activity, ratio of C20:4 to C18:2 for combined elongase-desaturase activity,[26] and ratio of C20:3 to C18:3 for ELOVL5 activity.

SNP Selection

Genotype information on 11 *FADS* single nucleotide polymorphisms (SNPs), which were previously genotyped in the current dataset using an Illumina Infinium HumanOmniExpressExome BeadChip array (San Diego, CA) and that were previously shown to associate with DM at nominal significance (0.002≤*P*≤0.02) in DIAGRAM v3 genome-wide association study (GWAS) meta-analysis,[27] was available for 525 of the participants (list given in Table S8). Eight of the 11 gene variants were in linkage disequilibrium (r^2>0.8 and D′>0.9) and rs174550 was used as representative of the linkage disequilibrium block, which gave 4 gene variants left.

Statistical Methods

Additional statistical analyses were conducted in SPSS version 22 (SPSS Inc, Chicago, IL). Welch's *t* test was used to test whether the product to precursor ratios of PUFAs contained in glycerophospholipids and DAGs differed between DM and controls or MI and controls because of the uneven sample size between groups.

We tested the association of *FADS1-2* gene variants with *Z* scores of complex lipid levels or ratios using linear regression models adjusted for age and sex. We used Bonferroni correction to handle false discovery rates from multiple testing and set a cutoff at *P*<0.0025 (0.05/[4 SNPs×5 phenotypes]). For other tests, data were considered significant if *P*<0.05.

Figures with lipid molecular species were constructed in GraphPad Prism version 6 (GraphPad Software, La Jolla, CA).

Results

A PCs and TAGs Signature Associates With DM

OPLS was applied to simultaneously analyze the correlations between 255 lipid molecular species belonging to 12 major lipid classes (X variables) and 16 clinical parameters describing DM (Y variables) in 448 individuals, of which 155 were DM and 293 were controls. The clinical parameters included measurements of blood glucose after overnight fasting and at 120 minutes post-oral glucose tolerance test, as well as additional known DM risk factors that were used as covariates (Table 1). OPLS resulted in 7 predictive components as well as one orthogonal component covering 72% and 12%, respectively, of the variation between the lipid species and the clinical parameters (Table S3). Figure 1 shows OPLS score

and loading plots illustrating predictive components 1 to 3 (pq 1–pq 3) in the DM model. The score and loading plots are dependent on each other and are interpreted simultaneously. A trend for separation between DM and controls is observed in the score plots (Figure 1A and 1B). In order to identify the lipid species significantly contributing to the separation between DM and controls, the VIP variable that summarizes the loadings from all the predictive components of the DM model was used. A total of 138 lipid species were found to significantly contribute to the separation between DM and controls (ie, VIP >1) (Table S5). The top 36 of the significant lipid species showing the highest VIP values (ie, VIP ≥1.2) belong mainly to the lipid classes phosphatidylcholine (PC) and TAG (Figure 2A). PCs and TAGs containing saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) were elevated in DM versus controls, while PCs and TAGs containing fatty acids with 2 double bounds were decreased (Figure 2B). All palmitic acid (C16:0) and all stearic acid (C18:0) containing VIP PCs were more abundant in DM versus controls except for the PC species containing a linoleic acid (C18:2) as the second acyl chain, which were decreased (Figure 2C). In the OPLS loading plots, PC and TAG lipid species cluster close to each other, respectively (Figure 1C and 1D), and TAG lipid species correlate well with total TAGs concentration.

A TAGs Signature Associates With MI

Similarly, OPLS was applied to simultaneously analyze correlations between 255 lipid molecular species (X variables) belonging to 12 major lipid classes and 27 clinical parameters describing MI (Y variables) in 383 individuals, of which 90 were MI and 293 controls. The clinical parameters were comprised of known risk factors for MI, including measurements of the intima-media thickness in the common carotid artery and bifurcation (Table 2). OPLS resulted in 6 predictive components explaining 50% of the variation between the lipid species and the clinical parameters (Table S4). Figure 3 shows OPLS score and loading plots illustrating the predictive components 1 to 3 (pq 1–pq 3) in the MI model. A trend for separation between MI and controls is observed in the score plots (Figure 3A and 3B). A total of 143 lipid species were found to significantly contribute to the separation between MI and controls (ie, VIP >1) (Table S6). Among the lipid species with the highest VIP values (ie, VIP ≥1.2), 29 of 31 are TAG lipid species (Figure 4A). The majority of the top VIP TAGs display higher levels in MI versus controls (Figure 4A) and are mainly composed of SFAs or MUFAs (ie, total double bound number ≤3) (Figure 4B). In the OPLS loading plots, the TAGs cluster together (Figure 3C and 3D, colored in green) and exhibit a good correlation with the traditionally measured total TAGs concentration.
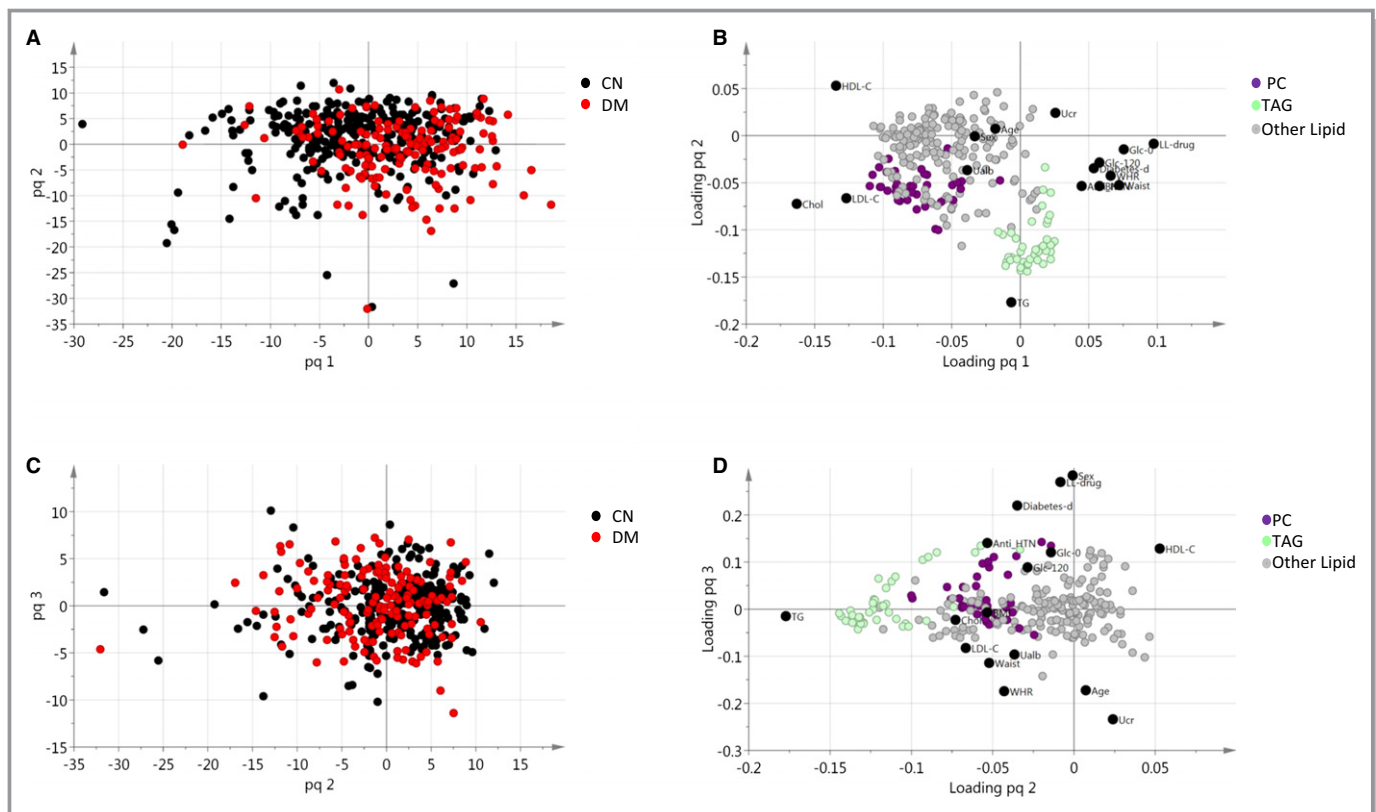
Figure 1. Orthogonal projections to latent structures score (A through C) and loading (B through D) plots of lipid species and clinical parameters in diabetes mellitus (DM). The analyses were performed on 448 individuals, 255 lipid species, and 16 clinical parameters. Two-dimensional score plots showing predictive component 1 to 2 (pq1–pq2) and predictive component 2 to 3 (pq2–pq3) (A through C). The black color indicates individuals who did not develop diabetes mellitus (controls [CN]) and the red color indicates individuals with DM. The analysis was performed on lipid species as X and clinical parameters describing DM as Y matrix. Two-dimensional loading plots showing predictive loading components 1 to 2 and 2 to 3 (Loading pq 1–Loading pq 2, and Loading pq 2–Loading pq 3) (B through D). All lipid species are marked in grey filled circles except triacylglyceride (TAG) (green filled circles) and phosphatidylcholine (PC) (violet filled circles). Clinical parameters are marked in black filled circles with each individual abbreviated clinical parameter name denoted.

Altered PUFAs Metabolism in DM and MI

In the DM model, all top VIP PCs that contained a C18:2 fatty acid were less abundant in DM versus controls (Figure 2C). This could reflect dietary intake and/or altered endogenous PUFAs metabolism. In a next step, we thus investigated the de novo synthesis of long-chain PUFAs by calculating product to precursor ratios of PUFAs contained in complex lipids from the DM model to estimate desaturase and elongase activities. Levels of C18:2 in complex lipids were lower in DM versus controls ($P=0.004$) and levels of C20:4 were higher but not statistically significant ($P=0.12$) (Table 3). The ratio of C18:3 to C18:2 was unchanged (D6D activity) ($P=0.48$) while the ratio of C20:3 to C18:3 (ELOVL5 activity) was higher in DM ($P=0.02$) and the ratio of C20:4 to C20:3 (D5D activity) was also higher in DM but not statistically significant ($P=0.17$). Combined elongase-desaturase activity as estimated by the C20:4 to C18:2 ratio was significantly increased in DM versus controls ($P=3.0E-06$).

Product to precursor ratios of PUFAs incorporated in glycerophospholipids and DAGs from the MI model were also calculated in MI and controls. Levels of C18:2 in complex lipids were lower in MI versus controls ($P=6.0E-06$) but levels of C20:4 were similar ($P=0.66$) (Table 3). Estimated D6D activity was unchanged ($P=0.79$) while ELOVL5 activity was higher in MI ($P=0.04$), and D5D activity was also higher in MI but only marginally statistically significant ($P=0.07$). Combined elongase-desaturase activity was significantly increased in MI versus controls ($P=2.0E-06$).

FADS Genotypes are Associated With Altered Levels of PUFAs in Serum Complex Lipids and Altered Desaturase Activity

Next, we examined the association of 4 genetic variants in the *FADS* gene cluster previously shown to associate with DM in the Diagram GWAS[27] (Table S8) with the levels and the product to precursor ratios of serum glycerophospholipids and DAGs PUFAs. rs174550 and rs174611 minor alleles were associated with increased levels of C18:2 ($P=0.007$ and

Figure 2. Characteristics of the top loadings from the diabetes mellitus (DM) model. Ratios in DM vs controls for the lipid species that contribute the most to the separation between DM and controls (variable influence on projection ≥1.2) (A and B). Graph of lipid classes and fatty acid acyl chain length (A). Graph of lipid classes and fatty acid acyl chain double bound number (B). Acyl chain characteristics of the significant phosphatidylcholine lipid species. Fatty acids less abundant in DM vs controls are indicated in blue and more abundant in DM vs controls are indicated in red (C). LPE indicates lysophosphatidylethanolamine; PC, phosphatidylcholine; PC O, phosphatidylcholine ether; SE, steryl ester; TAG, triacylglyceride.

0.001) (Table 4). Rs174550 and rs174570 minor allele were associated with significantly decreased levels of C20:4 (P=9.5E-07 and 1.2E-05). Only the rs174570 minor allele was significantly associated with decreased C18:3 to C18:2 ratio (D6D activity) (P=0.001). The minor allele for all 4 SNPs was associated with decreased ratio of C20:4 to C20:3 (D5D activity) (9.1E-16≤P≤0.002) and decreased C20:4 to C18:2 ratio (combined elongase-desaturase activity) (2.4E-21≤P≤6.0E-06) (Table 4).

Lipidomic Profile Shows Differences and Similarities Between DM and MI

We next compared the lipid species contributing to the DM model with the ones important for the MI model (ie, lipids with VIP >1). Some lipid species are unique for DM (17 lipid species) or MI (19 lipid species). Many are common to both models (123 lipid species), of which one third displayed

opposite regulation between the DM and the MI models and two thirds are similarly regulated (Figure 5).

The majority of the lipid species unique for DM are glycerophospholipids, including phosphatidylinositols and plasmalogen glycerophospholipids (PC-O and PE-O) (Figures S1A and S2A), while the lipid species that are unique for MI are mainly sphingolipids, including ceramides, sphingomyelins, and LysoPC16:0 (Figures S1D and S2D). Among the common discordant lipid species, several TAGs that are decreased in DM versus controls are increased in MI versus controls while several PCs and sphingomyelins that are increased in DM versus controls are decreased in MI versus controls (Figure S1B and S1E).

Discussion

In the present study, we used a lipidomics approach combined with OPLS to establish the detailed high coverage lipid
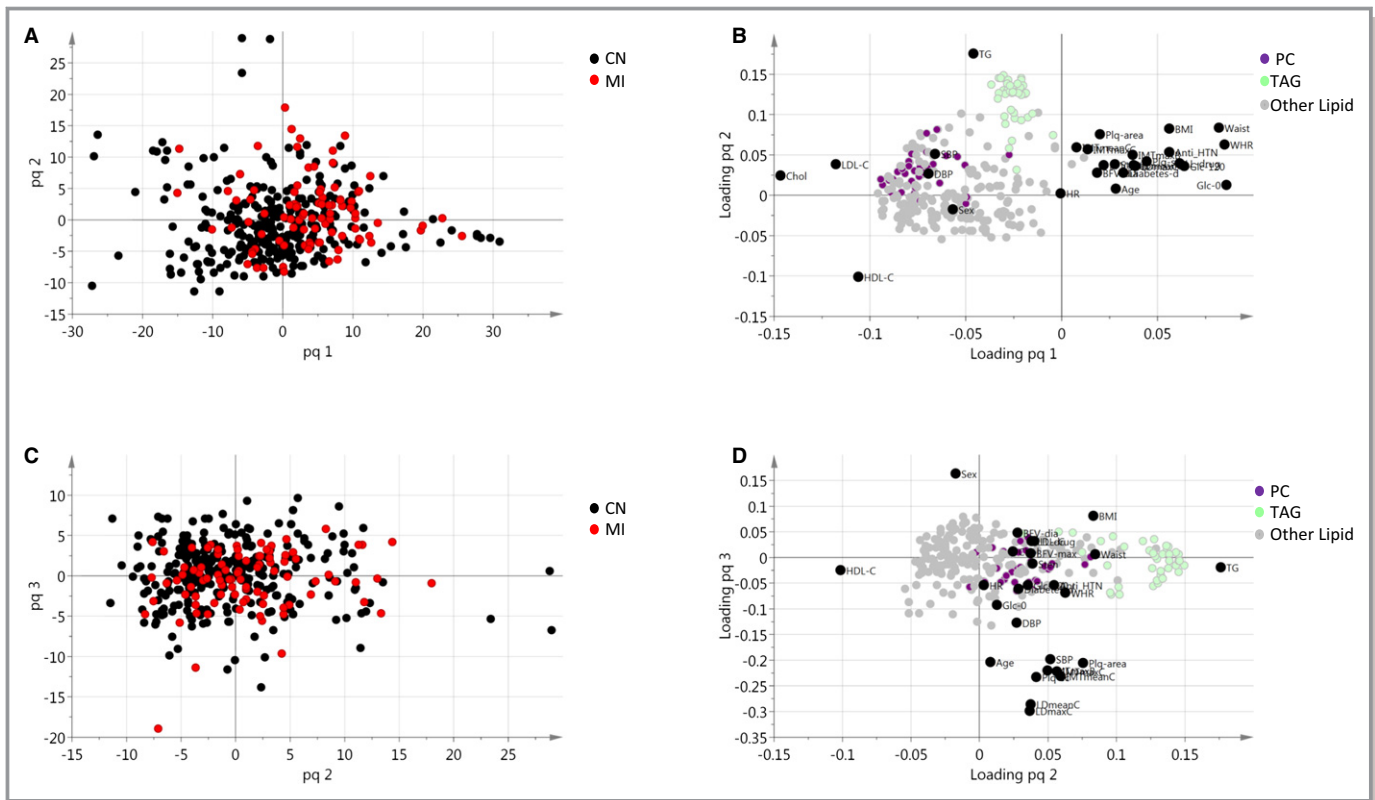
Figure 3. Orthogonal projections to latent structures score (A through C) and loading (B through D) plots of lipid species and clinical parameters in myocardial infarction (MI). The analyses were performed on 383 individuals, 255 lipid species, and 27 clinical parameters. Two-dimensional score plots showing predictive component 1 to 2 (pq1–pq2) and predictive component 2 to 3 (pq 2–pq 3) (A through C). The black color indicates controls (CN) and the red color indicates individuals with MI. The analysis was performed on lipid species as X and clinical parameters describing MI as Y matrix. Two-dimensional loading plots showing predictive loading components 1 to 2 and 2 to 3 (Loading pq 1–Loading pq 2, and Loading pq 2–Loading pq 3) (B through D). All lipid species are marked in grey filled circles except triacylglyceride (TAG) (green filled circles) and phosphatidylcholine (PC) (violet filled circles). Clinical parameters are marked in black filled circles with each individual abbreviated clinical parameter name denoted.

molecular pattern associated with DM and MI in serum. Both DM and MI were associated with changes in lipid classes as well as in lipid species. The lipid classes PC and TAG contributed the most to discriminate DM from controls. The

TAG lipid class as a whole has previously been shown to positively significantly associate with the presence of DM and prediabetes in the AusDiab (Australian Diabetes, Obesity and Lifestyle) study cohort but no significant association was seen
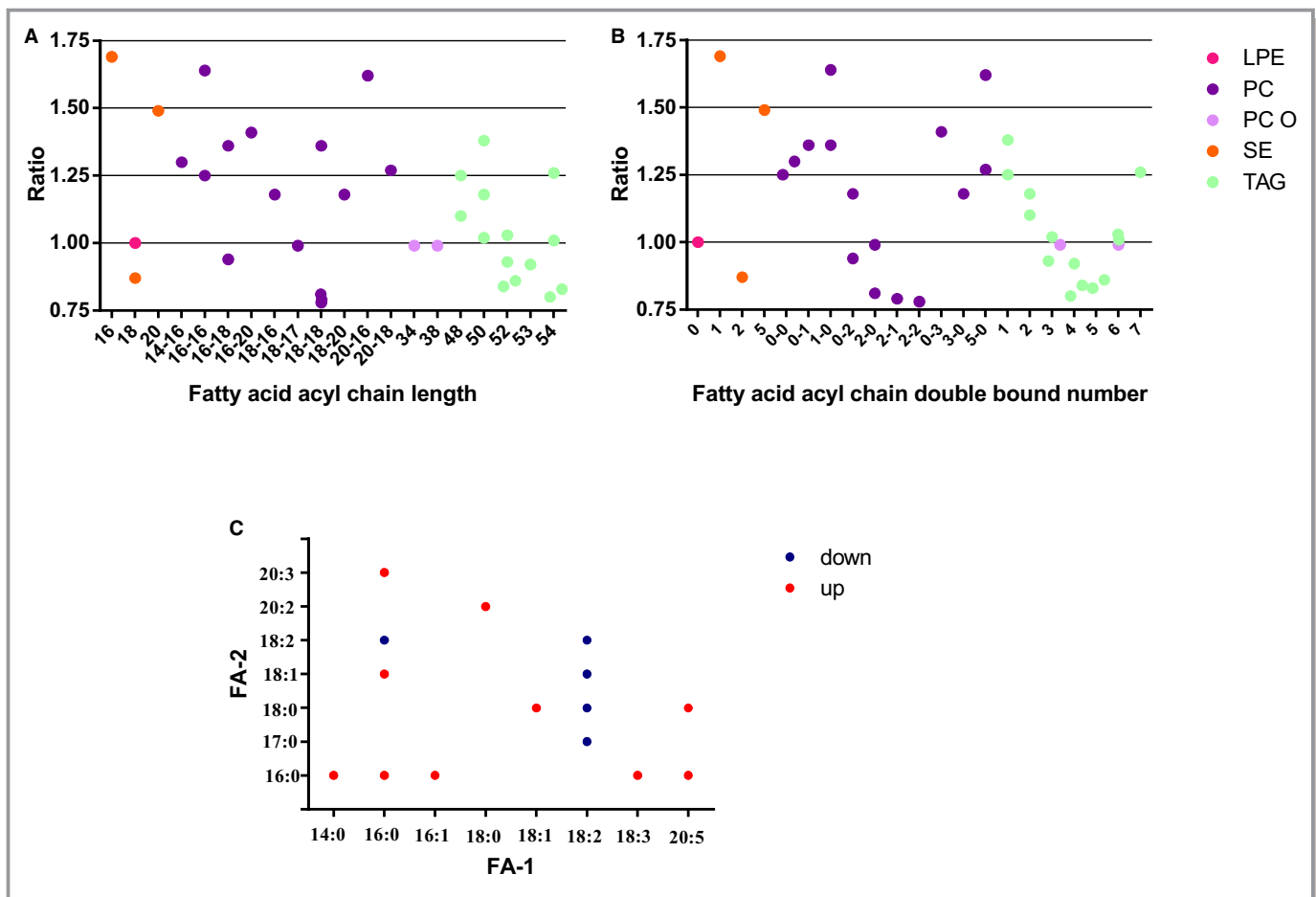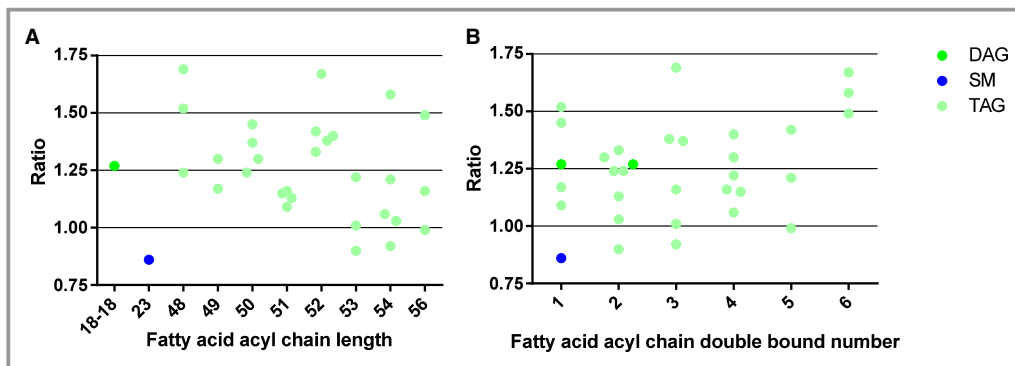

Figure 4. Characteristics of the top loadings from the myocardial infarction (MI) model. Ratios in MI vs controls for the lipid species that contribute the most to the separation between MI and controls (variable influence on projection ≥1.2) (A and B). Graph of lipid classes and fatty acid acyl chain length (A). Graph of lipid classes and fatty acid acyl chain double bound number (B). DAG indicates diacylglyceride; SM, sphingomyelin; TAG, triacylglyceride.

Table 3. PUFA Metabolism in DM and MI

	Controls (n=293)	DM (n=155)	*P* Value	Controls (n=293)	MI (n=90)	*P* Value
C18:2, nmol	1.79±0.66	1.61±0.60	0.004*	1.78±0.66	1.46±0.54	6.0E-06*
C20:4, nmol	0.51±0.20	0.54±0.23	0.12	0.52±0.20	0.53±0.21	0.66
C18:3/C18:2 (D6D)	0.02±0.01	0.02±0.01	0.48	0.02±0.01	0.02±0.01	0.79
C20:3/C18:3 (ELOVL5)	7.30±4.29	8.18±3.65	0.02*	7.30±4.29	8.20±3.34	0.04*
C20:4/C20:3 (D5D)	2.74±0.92	2.87±1.00	0.17	2.80±0.92	3.00±0.90	0.07
C20:4/C18:2 (combined elongase-desaturase)	0.30±0.10	0.36±0.14	3.0E-06*	0.30±0.10	0.38±0.13	2.0E-06*

Absolute levels or ratios of polyunsaturated fatty acids (PUFAs) contained in glycerophospholipids and diacylglycerides. D5D indicates delta-5 desaturase; D6D, delta-6 desaturase; DM, diabetes mellitus; ELOVL5, elongation of very long chain fatty acid protein 5; MI, myocardial infarction.
*Statistically significant associations (*P*<0.05) by Welch's *t* test.

for the PC lipid class. However, both the TAG and PC lipid classes were significantly positively associated with DM and prediabetes in the SAFHS (San Antonio Family Heart Study) cohort, which has a more similar sex structure to our MDC-CC subcohort than to the AusDiab cohort.[9]

Palmitic acid (C16:0) and stearic acid (C18:0) containing discriminating PC species were elevated in serum from DM versus controls, except for the one containing linoleic acid (C18:2) as the second fatty acid, which were decreased. In a small cohort of 20 individuals per group, a similar relative increase of PC containing C16:0 and C18:0 in isolated very LDL (VLDL) and LDL particles from dyslipidemic type 2 diabetic women versus controls was previously reported.[28] PC species with C18:2 were not measured in this study but several C18:2 containing TAG species were decreased in VLDL and LDL particles from dyslipidemic type 2 diabetic individuals.

We also showed that lipid species belonging to the TAG lipid class played a dominating role to separate MI from controls and that the majority of the discriminating TAG lipid species were upregulated in MI versus controls. This, together with recent genetic evidence in support of a causal role of raised circulating concentrations of TAGs in CVD,[3–6] further strengthens the role of TAGs in the pathogenesis of CVD.

Levels of C18:2 containing serum glycerophospholipids and diacylglycerides were lower in DM versus controls. Prospective studies have previously shown an inverse association between the proportion of linoleic acid in plasma and erythrocyte membrane phospholipids and diabetes mellitus risk[29,30] and our data indicate that this imbalance remains with overt DM. A decrease of the levels of C18:2 containing serum glycerophospholipids and diacylglycerides was also observed in MI versus controls. This is consistent with results from the Verona Heart Study where lower concentrations of linoleic acid (C18:2) in serum phospholipids and in red blood cell membranes were reported in coronary artery disease (CAD) patients versus controls.[26]

Table 4. FADS Gene Variants Associated With DM Associate With Serum Complex Lipid PUFA Levels

	rs174550		rs174570		rs174593		rs174611	
	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
C18:2	0.18 (0.06)	0.007	0.10 (0.09)	0.23	0.11 (0.07)	0.12	0.23 (0.07)	0.001*
C20:4	−0.32 (0.06)	9.5E-07*	−0.37 (0.08)	1.2E-05*	−0.15 (0.07)	0.04	−0.09 (0.07)	0.17
C18:3/C18:2 (D6D)	−0.08 (0.07)	0.23	−0.30 (0.09)	0.001*	−0.02 (0.08)	0.83	−0.08 (0.07)	0.25
C20:4/C20:3 (D5D)	−0.53 (−0.06)	9.1E-16*	−0.38 (0.09)	1.2E-05*	−0.30 (0.08)	9.0E-05*	−0.22 (0.07)	0.002*
C20:4/C18:2 (combined elongase-desaturase)	−0.62 (−0.06)	2.4E-21*	−0.59 (0.09)	1.3E-11*	−0.35 (0.08)	6.0E-06*	−0.36 (0.07)	3.1E-07*

Linear regressions were performed with *Z* scores of levels or ratios of polyunsaturated fatty acids (PUFAs) contained in glycerophospholipids and diacylglycerides adjusting for age and sex (n=525). β-correlation coefficients represent the change in PUFA levels or ratios, expressed as multiples of 1-SD, per effect allele. D5D indicates delta-5 desaturase; D6D, delta-6 desaturase; DM, diabetes mellitus; FADS, fatty acid desaturases.
*Statistically significant associations (*P*<0.0025).
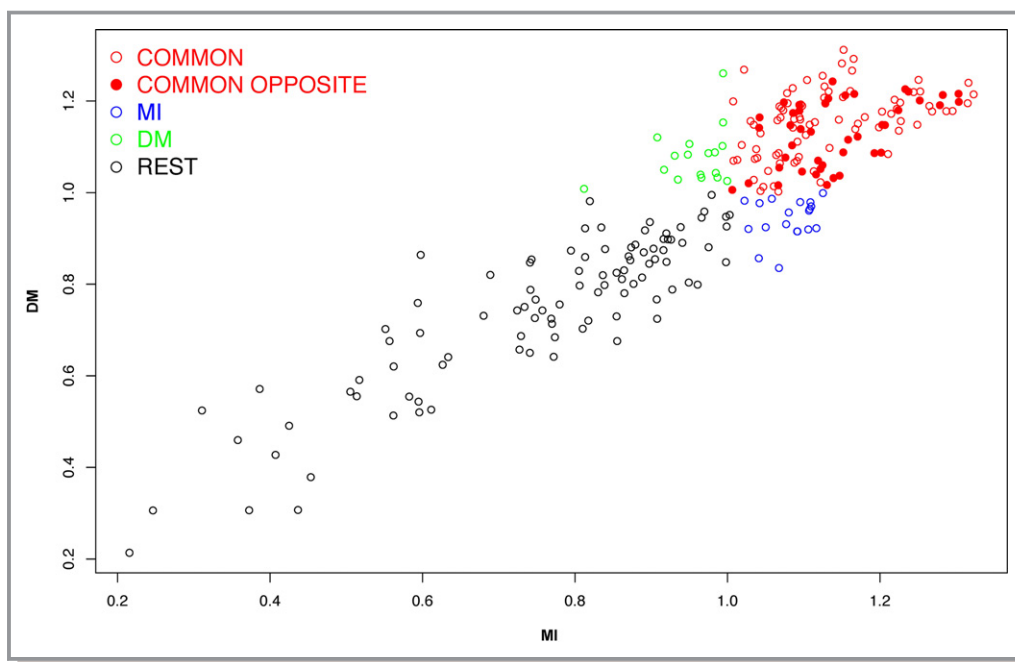
Figure 5. Comparison between diabetes mellitus (DM) and myocardial infarction (MI) loadings. Significant loadings (variable influence on projection >1) are colored according to common for DM and MI (red circles) and unique for DM and MI (green and blue circles, respectively). Red filled circles indicate common discordant loadings according to fold change. Red empty circles indicate common concordant loadings according to fold change.

Next, we investigated whether an abnormality of PUFA levels in complex lipids was due to alterations in the pathway for de novo synthesis of PUFAs. Our data indicate that elongation of PUFAs is accelerated both in DM and MI patients and there was also a tendency for an accelerated desaturation process through increased D5D activity both in DM and MI patients, albeit not statistically significant. Overall, our study suggests that there was an acceleration in the metabolic flux from C18:2 to C20:4 in DM and MI.

Because polymorphisms in the *FADS* gene cluster have been shown to associate with PUFA levels in plasma and erythrocyte membrane phospholipids,[31,32] we next tested whether *FADS* gene variants have a functional effect on desaturase activity. Minor allele carriers of the investigated *FADS* genotypes tended to be associated with increased levels of C18:2 containing serum glycerophospholipids and DAGs and decreased levels of C20:4 containing lipid species, all were significantly associated with lower estimated D5D activity, as previously reported.[25,31,33]

A negative correlation of D5D activity with diabetes mellitus risk has previously been reported.[29,30] However, in a meta-analyses of GWAS, *FADS1* rs174550 major allele was shown to be associated with increased fasting glucose at genome-wide significance (P=8.3E-09)[34] and increased DM risk at nominal significance (P=2.9E-03 and 2.3E-04),[27,34] thus indicating that the genetically determined high D5D

activity is associated with increased DM risk, which could support a causal relationship between decreased levels of C18:2 containing complex lipids and DM in our study.

No significant association between *FADS* gene variants and CAD has been identified in GWAS ($0.16 \leq P \leq 0.7$).[35] However, by using an additive model of *FADS* SNPs, *FADS* haplotypes, or both, a significant effect on a person's susceptibility to CAD was reported. Carriers of a higher number of unfavorable *FADS* gene variants, ie, associated with an elevated desaturase activity, more often experienced CAD than those with less unfavorable alleles.[26] This could indicate a possible causal relationship between decreased levels of C18:2 containing complex lipids and MI in our study.

Because of the close relationship between DM and MI, we compared the discriminating lipid species pattern for both end points. Of interest, LysoPC16:0, for which we previously found low levels to predict the risk of future CVD,[12] was only important in the MI model and was decreased in MI versus controls in the current study. Although several TAG lipid species were similarly increased in the DM and MI models, there were nonetheless several TAG lipid species that were decreased in DM while increased in MI in the respective models, indicating opposite roles of these TAG species in the pathogenesis of DM and MI. Of note, we observed similar cholesteryl ester profiles in the DM and MI models despite the well-known role of cholesterol in MI development. Most

probably, this is because a large proportion of individuals with MI take statins medication.

Study Limitations

The present study has several limitations. Although all of the patients with DM were older than 50 years, we did not have data to specifically exclude individuals with type 1 DM. Thus, there may be sporadic cases of type 1 DM among the DM group. In addition, with the technique used, it is not possible to identify the position of the double bounds on the acyl chains; hence, we are not able to distinguish between omega-6 PUFAs and omega-3 PUFAs, and measured their sum only. However, since it is well known that omega-6 fatty acids predominate in the contemporary Western diet,[36] the impact of omega-3 PUFAs on our results should be minimal. Moreover, by estimating desaturases and elongase activities by calculating product to precursor ratios, we cannot distinguish between activity changes due to altered transcription levels and changes due to modified intrinsic enzymatic capacity.

As expected, MI patients and, to a lesser extent, DM patients were more commonly taking statin therapy than controls; therefore, there are limitations to what we can conclude about cholesteryl esters. However, our study is representative of current DM and MI patients.

Conclusions

Using shotgun lipidomics and OPLS, we were able to identify a PC and TAG lipid molecular species signature in DM patients and a TAGs-based signature in MI patients. We also report altered PUFAs metabolism both in DM and MI patients with an increased flux from C18:2 to C20:4. We confirm a genetic role, via the *FADS* gene cluster, in affecting serum PUFA levels through modulation of desaturase activity. In addition, we demonstrate a possible causal relationship between decreased levels of C18:2 containing complex lipids and DM or MI. Overall, our study highlights the importance of lipid molecular species as potential etiological factors and treatment targets in DM and MI patients, although further investigations are needed to establish the pathophysiological consequences of lipid profile alterations.

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Disclosures

None.

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Table S1. Clinical characteristics of the study samples.

Clinical characteristics of the controls

	Included Controls	Excluded Controls
n	293	23
Age	72.8 ± 5.8	73.0 ±5.0
Women (%)	67.9	75.0
BMI (kg/m2)	26.4 ±4.2	25.4 ± 3.6
Systolic blood pressure (mm Hg)	143.0 ± 18.6	138.3 ± 18.5
Total Triglycerides (mmol/L)	1.1 ± 0.6	1.0 ± 0.4
Total cholesterol (mmol/L)	5.4 ± 1.1	5.4 ± 0.9
HDL cholesterol (mmol/L)	1.5 ± 0.5	1.5 ± 0.4
LDL cholesterol (mmol/L)	3.4 ± 1.0	3.4 ± 0.7
Fasting glucose (mmol/L)	5.7 ± 0.6	5.8 ± 0.7
Waist (cm)	90.0 ± 12.3	86.5 ± 11.6
Waist to hip ratio	0.9 ± 0.1	0.8 ± 0.1
2-h glucose (OGTT) (mmol/L)	6.8 ± 2.1	7.4 ± 2.8
U-Albumin (g/L)	0.02 ± 0.1	0.01 ± 0.01
U-Creatinine (mmol/L)	8.2 ± 4.0	7.8 ± 4.0
Diastolic blood pressure (mm Hg)	83.2 ± 9.9	79.8 ± 7.1
Heart rate	67.7 ± 11.1	67.3 ± 9.9
Plaque score	3.1 ± 1.8	2.4 ± 1.9
Plaque area (mm2)	20.8 ± 11.8	16.0 ± 4.9
Blood flow velocity. max	0.7 ± 0.3	0.6 ± 0.1
Blood flow velocity. diastolic	0.2 ± 0.1	0.2 ± 0.1
Lumen diameter. mean CCA	6.3 ± 0.8	6.3 ± 0.7
Lumen diameter. max CCA	6.6 ± 0.8	6.6± 0.8
Stenosis	1.8 ± 10.8	0.0 ± 0.0
IMT mean CCA	0.9 ± 0.2	0.9 ± 0.1
IMT max CCA	1.0 ± 0.2	1.0 ± 0.2
IMT max CCA bifurcation	1.8 ± 0.7	1.6 ± 0.5
% statin therapy	20.1	16.7
% anti-hypertensive medicine	39.2	30.4
% diabetes medicine	0.0	0.0

Values are mean ± standard deviation or percentage. P values were calculated by comparing included controls versus excluded controls, using a t test for continuous variables and Pearson Chi-Square for binary variables. BMI, body mass index; CCA, common carotid artery; HDL, high-density lipoproteins; IMT, intima media thickness; LDL, low-density lipoproteins; OGTT, oral glucose tolerance test.

Table S2. Clinical characteristics of the MI events

	Included MI	Excluded MI
n	90	9
Age	75.1 ± 5.0	72.1 ± 7.7
Women (%)	33.3	33.3
BMI (kg/m2)	26.6 ± 3.9	24.6 ± 3.2
Systolic blood pressure (mm Hg)	143.6 ± 18.1	140.3 ± 15.6
Total Triglycerides (mmol/L)	1.1 ± 0.5	1.0 ± 0.4
Total cholesterol (mmol/L)	4.2 ± 0.9	3.9 ± 0.6
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.2
LDL cholesterol (mmol/L)	2.5 ± 0.8	2.1 ± 0.4
Fasting glucose (mmol/L)	6.0 ± 0.8	6.1 ± 0.7
Waist (cm)	94.3 ± 12.0	91.9 ± 9.1
Waist to hip ratio	0.9 ± 0.08	0.9 ± 0.1
2-h glucose (OGTT) (mmol/L)	7.5 ± 1.9	8.4 ± 1.6
Diastolic blood pressure (mm Hg)	80.2 ± 9.0	80.0 ± 9.8
Heart rate	65.3 ± 10.4	59.7 ± 11.6
Plaque score	4.0 ± 1.4	3.9 ± 1.2
Plaque area (mm2)	23.0 ± 12.4	25.0 ± 24.0
Blood flow velocity. max	0.7 ± 0.4	0.6 ± 0.2
Blood flow velocity. diastolic	0.2 ± 0.16	0.2 ± 0.1
Lumen diameter. mean CCA	6.6 ± 0.9	6.8 ± 0.5
Lumen diameter. max CCA	6.9 ± 0.9	7.0 ± 0.6
Stenosis	5.5 ± 17.4	0.0 ± 0.0
IMT mean CCA	1.0 ± 0.3	1.0 ± 0.2
IMT max CCA	1.2 ± 0.5	1.2 ± 0.3
IMT max CCA bifurcation	2.1 ± 0.7	2.0 ± 0.4
% statin therapy	80.0	89.0
% anti-hypertensive medicine	89.0	89.0
% diabetes medicine	3.3	0.0

Values are mean ± standard deviation or percentage. P values were calculated by comparing included MI versus excluded MI, using a t test for continuous variables and Pearson Chi-Square for binary variables. BMI, body mass index; CCA, common carotid artery; HDL, high-density lipoproteins; IMT, intima media thickness; LDL, low-density lipoproteins; OGTT, oral glucose tolerance test.

Table S3. Clinical characteristics of the DM events

	Included DM	Excluded DM
n	155	16
Age	72.6 ± 5.3	72.8 ± 5.3
Women (%)	54.8	75.0
BMI (kg/m2)	27.9 ± 4.8	29.5 ±6.6
Systolic blood pressure (mm Hg)	147.8 ± 16.7	141.9 ± 19.1
Total Triglycerides (mmol/L)	1.3 ± 0.6	1.1 ± 0.4
Total cholesterol (mmol/L)	4.7 ± 1.0	4.5 ± 0.5
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.3
LDL cholesterol (mmol/L)	2.9 ± 0.9	2.8 ± 0.5
Fasting glucose (mmol/L)	7.4 ± 1.4	8.0 ± 2.4
Waist (cm)	96.3 ± 12.9	97.9 ± 17.1
Waist to hip ratio	0.9 ± 0.09	0.9 ± 0.09
2-h glucose (OGTT) (mmol/L)	10.2 ± 3.2	9.2 ± 2.6
U-Albumin (g/L)	0.02 ± 0.06	0.01 ± 0.01
U-Creatinine (mmol/L)	8.6 ± 3.9	6.3 ± 2.9 *
% statin therapy	49.0	50.0
% anti-hypertensive medicine	72.3	100 *
% diabetes medicine	33.0	37.5

Values are mean ± standard deviation or percentage. P values were calculated by comparing included DM versus excluded DM excluded, using a t test for continuous variables and Pearson Chi-Square for binary variables. BMI, body mass index; HDL, high-density lipoproteins; LDL, low-density lipoproteins; OGTT, oral glucose tolerance test.

Table S4. Model quality parameters tables.

OPLS models for DM and MI with clinical parameters as Y. Number of principal components (PCs) refers to the optimal number of PCs for a particular multivariate data model according to cross-validation. R_X^2 accounts for the explained variance while Q_Y^2 accounts for the cumulative fraction of the total variation of Y that can be predicted by the model. R_Y^2 accounts for the explained variance for the Y component. Predictive components explain the variation accounted for by each predictive component. Orthogonal variation explains variation for each orthogonal component where applicable.

DM (n=448)

Principal components	R_X^2	Q_Y^2	R_Y^2
Model summary	0,637	0,243	0,724
Predictive	0,519	0,243	0,724
P1	0,19	0,106	0,236
P2	0,343	0,173	0,364
P3	0,383	0,191	0,471
P4	0,415	0,197	0,545
P5	0,464	0,215	0,607
P6	0,494	0,227	0,675
P7	0,519	0,243	0,724
Orthogonal in X	0,117		
O1	0,117		

DM (n=438) without 10 individuals displaying both DM and MI

Principal components	R_X^2	Q_Y^2	R_Y^2
Model summary	0,639	0,235	0,726
Predictive	0,521	0,235	0,726
P1	0,199	0,0969	0,232
P2	0,347	0,164	0,362
P3	0,387	0,182	0,471
P4	0,419	0,189	0,543
P5	0,466	0,202	0,604
P6	0,495	0,219	0,674
P7	0,521	0,235	0,726
Orthogonal in X	0,117		
O1	0,117		

MI (n=383)

Principal components	R_X^2	Q_Y^2	R_Y^2
Model summary	0,604	0,113	0,503
Predictive	0,604	0,113	0,503
P1	0,304	0,0384	0,15
P2	0,435	0,0816	0,243
P3	0,492	0,0833	0,325
P4	0,532	0,084	0,403
P5	0,576	0,0959	0,441
P6	0,604	0,113	0,503

MI (n=373) without individuals displaying both DM and MI

Principal components	R_X^2	Q_Y^2	R_Y^2
Model summary	0,605	0,113	0,521
Predictive	0,605	0,113	0,521
P1	0,315	0,0366	0,151
P2	0,44	0,0808	0,248
P3	0,492	0,0781	0,331
P4	0,535	0,0849	0,416
P5	0,576	0,0973	0,453
P6	0,605	0,113	0,521

Table S5. Lipid species with significant VIP in the DM model

Lipid specie	Total length	Total db	Total oh	Ratio DM vs CN	VIP value DM Model
PC-38:2;0(18:0;0-20:2;0)	38	2	0	1,18	1,31
PC-35:2;0(18:2;0-17:0;0)	35	2	0	0,99	1,29
PC-36:3;0(18:2;0-18:1;0)	36	3	0	0,79	1,28
PC-36:5;0(20:5;0-16:0;0)	36	5	0	1,62	1,27
PC-32:1;0(16:1;0-16:0;0)	32	1	0	1,64	1,27
PC-38:5;0(20:5;0-18:0;0)	38	5	0	1,27	1,26
SE-43:2;0(27:1;0-16:1;0)	43	2	0	1,69	1,25
TAG-48:1;0	48	1	0	1,25	1,25
PC-36:2;0(18:2;0-18:0;0)	36	2	0	0,81	1,25
PC-36:1;0(18:1;0-18:0;0)	36	1	0	1,36	1,24
TAG-50:2;0	50	2	0	1,18	1,24
PC-34:2;0(16:0;0-18:2;0)	34	2	0	0,94	1,23
PC-30:0;0(14:0;0-16:0;0)	30	0	0	1,30	1,23
TAG-54:4;0	54	4	0	0,80	1,23
TAG-54:7;0	54	7	0	1,26	1,22
PC O--34:3;0	34	3	0	0,99	1,22
TAG-48:2;0	48	2	0	1,10	1,22
TAG-54:5;0	54	5	0	0,83	1,22
TAG-50:1;0	50	1	0	1,38	1,22
PC-36:4;0(18:2;0-18:2;0)	36	4	0	0,78	1,22
TAG-53:4;0	53	4	0	0,92	1,22
PC-34:1;0(16:0;0-18:1;0)	34	1	0	1,36	1,22
TAG-50:3;0	50	3	0	1,02	1,21
TAG-52:4;0	52	4	0	0,84	1,21
LPE-18:2;0(18:2;0)	18	2	0	1,00	1,21
SE-45:3;0(27:1;0-18:2;0)	45	3	0	0,87	1,21
PC-36:3;0(16:0;0-20:3;0)	36	3	0	1,41	1,21
PC-32:0;0(16:0;0-16:0;0)	32	0	0	1,25	1,21
TAG-52:6;0	52	6	0	1,03	1,20
TAG-52:5;0	52	5	0	0,86	1,20
PC-36:3;0(18:3;0-18:0;0)	36	3	0	0,89	1,20
TAG-52:3;0	52	3	0	0,93	1,20
PC-34:3;0(18:3;0-16:0;0)	34	3	0	1,18	1,20
TAG-54:6;0	54	6	0	1,01	1,20
PC O--38:6;0	38	6	0	0,99	1,20
SE-47:6;0(27:1;0-20:5;0)	47	6	0	1,49	1,20
TAG-51:3;0	51	3	0	1,04	1,19
PC-32:1;0(14:0;0-18:1;0)	32	1	0	1,36	1,19

PC O--34:2;0	34	2	0	0,87	1,19
PC-35:1;0(17:0;0-18:1;0)	35	1	0	1,40	1,19
TAG-50:4;0	50	4	0	0,92	1,19
PC O--36:3;0	36	3	0	0,71	1,19
TAG-49:2;0	49	2	0	1,14	1,19
PC-38:3;0(20:3;0-18:0;0)	38	3	0	1,44	1,19
PI-34:1;0(18:1;0-16:0;0)	34	1	0	1,40	1,18
TAG-49:1;0	49	1	0	1,26	1,18
TAG-56:8;0	56	8	0	1,33	1,18
DAG-36:3;0(18:1;0-18:2;0)	36	3	0	0,84	1,18
PC O--34:1;0	34	1	0	1,20	1,18
TAG-51:2;0	51	2	0	1,23	1,18
TAG-53:3;0	53	3	0	1,01	1,18
TAG-52:2;0	52	2	0	1,10	1,18
TAG-54:3;0	54	3	0	0,98	1,18
PC-34:3;0(16:1;0-18:2;0)	34	3	0	1,04	1,17
TAG-56:4;0	56	4	0	1,00	1,17
TAG-56:7;0	56	7	0	1,44	1,16
PC-36:2;0(18:1;0-18:1;0)	36	2	0	0,90	1,16
PC-38:4;0(18:1;0-20:3;0)	38	4	0	1,28	1,16
SE-41:1;0(27:1;0-14:0;0)	41	1	0	1,48	1,16
SE-44:2;0(27:1;0-17:1;0)	44	2	0	1,46	1,16
PC-32:2;0(14:0;0-18:2;0)	32	2	0	1,13	1,16
LPC-18:2;0(18:2;0)	18	2	0	0,85	1,16
SE-45:4;0(27:1;0-18:3;0)	45	4	0	1,41	1,16
PC-38:6;0(16:0;0-22:6;0)	38	6	0	1,22	1,16
TAG-54:2;0	54	2	0	1,04	1,16
PC-40:5;0(22:5;0-18:0;0)	40	5	0	1,09	1,15
PI-36:1;0(18:0;0-18:1;0)	36	1	0	1,01	1,15
SE-43:1;0(27:1;0-16:0;0)	43	1	0	1,09	1,15
TAG-53:2;0	53	2	0	0,98	1,15
SE-45:2;0(27:1;0-18:1;0)	45	2	0	1,20	1,15
TAG-58:8;0	58	8	0	1,47	1,15
TAG-48:3;0	48	3	0	0,91	1,15
TAG-51:4;0	51	4	0	0,86	1,15
SM-32:1;2	32	1	2	1,16	1,15
TAG-51:1;0	51	1	0	1,21	1,14
TAG-48:0;0	48	0	0	1,09	1,14
PC O--40:7;0	40	7	0	1,29	1,14
PC-34:2;0(16:1;0-18:1;0)	34	2	0	1,21	1,14
PE-36:2;0(18:2;0-18:0;0)	36	2	0	1,13	1,14

TAG-56:5;0	56	5	0	0,95	1,13
TAG-46:0;0	46	0	0	0,90	1,13
PC-34:0;0(16:0;0-18:0;0)	34	0	0	0,95	1,13
TAG-58:7;0	58	7	0	1,45	1,13
SM-33:1;2	33	1	2	1,09	1,12
TAG-58:9;0	58	9	0	1,03	1,12
TAG-50:5;0	50	5	0	0,80	1,12
PC-38:5;0(16:0;0-22:5;0)	38	5	0	1,17	1,11
LPE-20:5;0(20:5;0)	20	5	0	1,63	1,11
PI-34:2;0(18:2;0-16:0;0)	34	2	0	1,64	1,10
TAG-46:1;0	46	1	0	0,93	1,10
SE-47:4;0(27:1;0-20:3;0)	47	4	0	1,27	1,10
SM-34:1;2	34	1	2	0,97	1,10
LPC-17:2;0(17:2;0)	17	2	0	1,05	1,09
SM-40:1;2	40	1	2	0,98	1,09
PC-40:6;0(22:6;0-18:0;0)	40	6	0	1,02	1,09
SM-41:2;2	41	2	2	1,12	1,09
PE O--38:7;0	38	7	0	1,46	1,09
SM-40:2;2	40	2	2	1,03	1,09
PC O--36:2;0	36	2	0	0,77	1,09
TAG-56:6;0	56	6	0	1,06	1,08
SE-45:5;0(27:1;0-18:4;0)	45	5	0	2,36	1,08
PC-38:5;0(20:4;0-18:1;0)	38	5	0	0,95	1,08
PE O--36:6;0	36	6	0	1,79	1,08
SM-41:1;2	41	1	2	0,89	1,08
PC O--32:1;0	32	1	0	1,20	1,08
PC-36:4;0(16:0;0-20:4;0)	36	4	0	0,96	1,08
PC O--36:4;0	36	4	0	0,95	1,07
PC-38:4;0(20:4;0-18:0;0)	38	4	0	0,86	1,07
SM-39:1;2	39	1	2	1,12	1,07
LPC-20:5;0(20:5;0)	20	5	0	1,53	1,07
Cer-42:1;2	42	1	2	1,12	1,07
ST-27:1;0	27	1	0	1,14	1,07
PC O--38:5;0	38	5	0	0,89	1,06
Cer-40:1;2	40	1	2	1,15	1,06
LPE-18:1;0(18:1;0)	18	1	0	1,00	1,05
TAG-46:2;0	46	2	0	0,79	1,05
PI-36:2;0(18:0;0-18:2;0)	36	2	0	1,10	1,05
PE O--40:8;0	40	8	0	1,18	1,05
PC-37:4;0(20:4;0-17:0;0)	37	4	0	1,33	1,05
Cer-41:1;2	41	1	2	1,21	1,05

SM-32:2;2	32	2	2	0,99	1,04
LPC-18:1;0(18:1;0)	18	1	0	1,09	1,04
SE-42:1;0(27:1;0-15:0;0)	42	1	0	1,06	1,04
SM-38:1;2	38	1	2	1,16	1,04
PC O--36:1;0	36	1	0	0,97	1,03
SE-43:3;0(27:1;0-16:2;0)	43	3	0	2,20	1,03
SM-39:2;2	39	2	2	1,31	1,03
PC O--38:4;0	38	4	0	0,74	1,03
PE O--36:3;0	36	3	0	0,76	1,03
PC O--40:6;0	40	6	0	0,83	1,03
SM-34:2;2	34	2	2	0,97	1,02
PI-38:3;0(18:0;0-20:3;0)	38	3	0	1,31	1,02
SM-35:1;2	35	1	2	1,04	1,02
SM-40:3;2	40	3	2	1,01	1,02
PE O--40:7;0	40	7	0	1,23	1,01
PC O--32:2;0	32	2	0	1,11	1,01
PI-36:2;0(18:1;0-18:1;0)	36	2	0	0,86	1,01
PI-36:4;0(20:4;0-16:0;0)	36	4	0	2,03	1,01

In bold, lipid species with VIP $\geq$ 1.2. CN, controls; db, double bound. Cer, ceramide; DAG, diacylglyceride; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPE O, lysophosphatidylethanolamine ether; PC, phosphatidylcholine; PC O, phosphatidylcholine ether; PE, phosphatidylethanolamine; PE O, phosphatidylethanolamine ether; PI, phosphatidylinositol; SE, steryl ester; SM, sphingomyelin; TAG, triacylglyceride.

Table S6. Lipid species with significant VIP in the MI model

Lipid specie	Total length	Total db	Total oh	Ratio MI vs CN	VIP value MI Model
TAG-50:3;0	50	3	0	1,37	1,32
TAG-50:2;0	50	2	0	1,24	1,32
TAG-51:3;0	51	3	0	1,16	1,32
TAG-52:3;0	52	3	0	1,38	1,30
TAG-53:4;0	53	4	0	1,22	1,30
TAG-51:2;0	51	2	0	1,13	1,30
TAG-53:3;0	53	3	0	1,01	1,29
TAG-52:4;0	52	4	0	1,40	1,28
TAG-50:4;0	50	4	0	1,30	1,28
TAG-52:2;0	52	2	0	1,33	1,27
TAG-49:2;0	49	2	0	1,30	1,26
TAG-48:2;0	48	2	0	1,24	1,25
TAG-52:5;0	52	5	0	1,42	1,25
TAG-48:1;0	48	1	0	1,52	1,25
TAG-53:2;0	53	2	0	0,90	1,25
TAG-50:1;0	50	1	0	1,45	1,24
TAG-54:5;0	54	5	0	1,21	1,24
TAG-54:4;0	54	4	0	1,06	1,23
TAG-54:2;0	54	2	0	1,03	1,23
TAG-54:6;0	54	6	0	1,58	1,23
TAG-56:5;0	56	5	0	0,99	1,22
DAG-36:3;0(18:1;0-18:2;0)	36	3	0	1,27	1,22
TAG-49:1;0	49	1	0	1,17	1,22
TAG-52:6;0	52	6	0	1,67	1,22
TAG-56:4;0	56	4	0	1,16	1,21
TAG-56:6;0	56	6	0	1,49	1,21
TAG-51:4;0	51	4	0	1,15	1,21
TAG-48:3;0	48	3	0	1,69	1,20
TAG-54:3;0	54	3	0	0,92	1,20
SM-41:2;2	41	2	2	0,86	1,20
TAG-51:1;0	51	1	0	1,09	1,20
SM-40:2;2	40	2	2	0,82	1,19
TAG-56:7;0	56	7	0	1,86	1,18
SE-43:1;0(27:1;0-16:0;0)	43	1	0	1,01	1,17
SM-33:1;2	33	1	2	0,80	1,17
PE-36:2;0(18:2;0-18:0;0)	36	2	0	1,11	1,17
PC-34:1;0(16:0;0-18:1;0)	34	1	0	0,93	1,17
PC-35:2;0(18:2;0-17:0;0)	35	2	0	0,83	1,17

PC-32:1;0(16:1;0-16:0;0)	32	1	0	1,22	1,16
TAG-54:7;0	54	7	0	1,80	1,16
TAG-50:5;0	50	5	0	1,32	1,16
LPE-18:2;0(18:2;0)	18	2	0	0,94	1,15
PC-38:2;0(18:0;0-20:2;0)	38	2	0	1,17	1,15
SM-40:1;2	40	1	2	1,01	1,15
SE-45:3;0(27:1;0-18:2;0)	45	3	0	0,93	1,15
PC-36:3;0(18:2;0-18:1;0)	36	3	0	0,63	1,15
SM-38:1;2	38	1	2	0,90	1,15
LPC-18:2;0(18:2;0)	18	2	0	0,90	1,15
SM-39:2;2	39	2	2	0,97	1,14
PC-36:1;0(18:1;0-18:0;0)	36	1	0	0,86	1,14
SM-34:1;2	34	1	2	0,73	1,13
PC O--34:3;0	34	3	0	0,76	1,13
PC-32:0;0(16:0;0-16:0;0)	32	0	0	0,76	1,13
SM-35:1;2	35	1	2	0,78	1,13
PC-32:1;0(14:0;0-18:1;0)	32	1	0	0,94	1,13
PC O--38:6;0	38	6	0	0,87	1,13
PC-34:2;0(16:0;0-18:2;0)	34	2	0	0,76	1,13
PC-36:3;0(16:0;0-20:3;0)	36	3	0	1,03	1,13
SM-36:1;2	36	1	2	0,78	1,13
SE-43:2;0(27:1;0-16:1;0)	43	2	0	1,34	1,12
Cer-40:1;2	40	1	2	0,87	1,12
TAG-46:2;0	46	2	0	1,28	1,12
SM-34:2;2	34	2	2	0,77	1,12
SM-39:1;2	39	1	2	0,87	1,12
Cer-42:2;2	42	2	2	0,84	1,12
LPC-18:1;0(18:1;0)	18	1	0	0,93	1,12
PC-40:5;0(22:5;0-18:0;0)	40	5	0	1,16	1,11
PC-37:4;0(20:4;0-17:0;0)	37	4	0	1,16	1,11
SM-37:1;2	37	1	2	0,81	1,11
TAG-46:0;0	46	0	0	2,46	1,11
SM-41:3;2	41	3	2	0,90	1,11
Cer-40:2;2	40	2	2	0,75	1,11
SE-45:2;0(27:1;0-18:1;0)	45	2	0	1,03	1,11
PE-38:4;0(20:4;0-18:0;0)	38	4	0	1,08	1,11
SM-38:2;2	38	2	2	0,82	1,11
PC-36:2;0(18:2;0-18:0;0)	36	2	0	0,67	1,10
TAG-58:7;0	58	7	0	1,50	1,10
PC O--36:3;0	36	3	0	0,55	1,10
Cer-41:1;2	41	1	2	0,89	1,10

PC O--34:2;0	34	2	0	0,90	1,10
PC-34:2;0(16:1;0-18:1;0)	34	2	0	0,83	1,10
PC-32:2;0(14:0;0-18:2;0)	32	2	0	1,38	1,10
SM-42:3;2	42	3	2	0,77	1,10
PC-36:2;0(18:1;0-18:1;0)	36	2	0	0,50	1,09
PC O--34:1;0	34	1	0	0,80	1,09
PC-35:1;0(17:0;0-18:1;0)	35	1	0	0,92	1,09
SM-41:1;2	41	1	2	0,86	1,09
SM-36:2;2	36	2	2	0,76	1,09
PC-38:5;0(16:0;0-22:5;0)	38	5	0	1,26	1,09
Cer-41:2;2	41	2	2	0,77	1,09
Cer-42:1;2	42	1	2	1,04	1,09
ST-27:1;0	27	1	0	1,09	1,09
SE-44:2;0(27:1;0-17:1;0)	44	2	0	1,07	1,09
PC-34:3;0(16:1;0-18:2;0)	34	3	0	0,78	1,09
TAG-48:0;0	48	0	0	2,03	1,09
PC-30:0;0(14:0;0-16:0;0)	30	0	0	1,04	1,09
TAG-46:1;0	46	1	0	1,34	1,08
SM-32:1;2	32	1	2	0,58	1,08
SM-42:2;2	42	2	2	0,88	1,08
SE-47:6;0(27:1;0-20:5;0)	47	6	0	1,88	1,08
PC-36:4;0(18:2;0-18:2;0)	36	4	0	0,58	1,08
LPE-18:0;0(18:0;0)	18	0	0	1,00	1,08
PC O--32:1;0	32	1	0	0,81	1,08
PC-34:3;0(18:3;0-16:0;0)	34	3	0	0,93	1,07
TAG-56:8;0	56	8	0	1,90	1,07
PI-34:1;0(18:1;0-16:0;0)	34	1	0	1,41	1,07
SE-41:1;0(27:1;0-14:0;0)	41	1	0	1,10	1,07
PC O--38:5;0	38	5	0	0,76	1,07
PC-38:3;0(20:3;0-18:0;0)	38	3	0	1,06	1,07
LPE-18:1;0(18:1;0)	18	1	0	0,81	1,07
Cer-42:3;2	42	3	2	0,83	1,07
PE O--38:7;0	38	7	0	1,52	1,07
PE O--38:6;0	38	6	0	1,23	1,07
SM-40:3;2	40	3	2	0,76	1,07
SE-45:4;0(27:1;0-18:3;0)	45	4	0	1,15	1,07
PC-38:5;0(20:4;0-18:1;0)	38	5	0	0,88	1,06
PE O--40:7;0	40	7	0	1,07	1,06
PC O--37:5;0	37	5	0	0,65	1,06
PE O--40:8;0	40	8	0	1,15	1,05
Cer-38:1;2	38	1	2	0,80	1,05

PC O--32:2;0	32	2	0	1,08	1,05
PC-34:0;0(16:0;0-18:0;0)	34	0	0	0,79	1,04
SE-49:6;0(27:1;0-22:5;0)	49	6	0	1,31	1,04
PC-38:4;0(18:1;0-20:3;0)	38	4	0	0,89	1,04
LPC-18:0;0(18:0;0)	18	0	0	0,84	1,04
PC O--40:7;0	40	7	0	0,86	1,04
LPC-16:0;0(16:0;0)	16	0	0	0,88	1,04
PC-36:4;0(16:0;0-20:4;0)	36	4	0	0,82	1,04
LPC-17:2;0(17:2;0)	17	2	0	1,35	1,04
PC O--36:4;0	36	4	0	0,72	1,04
TAG-58:8;0	58	8	0	1,37	1,03
PE O--36:3;0	36	3	0	0,71	1,03
PC-38:6;0(16:0;0-22:6;0)	38	6	0	1,10	1,03
PI-38:3;0(18:0;0-20:3;0)	38	3	0	0,93	1,03
Cer-39:1;2	39	1	2	0,85	1,03
SM-42:1;2	42	1	2	1,29	1,02
PC-36:5;0(20:5;0-16:0;0)	36	5	0	1,63	1,02
PI-34:2;0(18:2;0-16:0;0)	34	2	0	1,03	1,02
PC-38:4;0(20:4;0-18:0;0)	38	4	0	0,88	1,01
LPC-20:5;0(20:5;0)	20	5	0	1,72	1,01
PC-36:3;0(18:3;0-18:0;0)	36	3	0	0,72	1,01
PI-36:4;0(20:4;0-16:0;0)	36	4	0	0,55	1,01

In bold, lipid species with VIP ≥ 1.2. In bold and italic, lipid species with saturated or monounsaturated aliphatic fatty acid chains and VIP ≥ 1.2. CN, controls; db, double bound. er, ceramide; DAG, diacylglyceride; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPE O, lysophosphatidylethanolamine ether; PC, phosphatidylcholine; PC O, phosphatidylcholine ether; PE, phosphatidylethanolamine; PE O, phosphatidylethanolamine ether; PI, phosphatidylinositol; SE, steryl ester; SM, sphingomyelin; TAG, triacylglyceride.

Table S7. All lipid species analyzed and ratios in DM versus controls or MI versus controls and respective VIP values obtained from the DM OPLS model and the MI OPLS model

Lipid specie	Total length	Total db	Total oh	FA1 length	FA1 db	FA2 length	FA2 db	Ratio DM vs CN	VIP value DM Model	Ratio MI vs CN	VIP value MI Model
Cer-32:1;2	32	1	2	14	0			1,12	0,69	0,81	0,60
Cer-33:1;2	33	1	2	15	0			1,39	0,64	0,76	0,63
Cer-34:1;2	34	1	2	16	0			1,13	0,65	1,17	0,74
Cer-34:2;2	34	2	2	16	1			0,83	0,75	0,74	0,73
Cer-36:1;2	36	1	2	18	0			1,30	0,85	0,86	0,92
Cer-36:2;2	36	2	2	18	1			1,63	0,52	1,70	0,31
Cer-37:1;2	37	1	2	19	0			1,77	0,53	1,13	0,61
Cer-38:1;2	38	1	2	20	0			1,37	0,92	0,80	1,05
Cer-39:1;2	39	1	2	21	0			1,46	0,92	0,85	1,03
Cer-40:1;2	40	1	2	22	0			1,15	1,06	0,87	1,12
Cer-40:2;2	40	2	2	22	1			1,20	0,96	0,75	1,11
Cer-41:1;2	41	1	2	23	0			1,21	1,05	0,89	1,10
Cer-41:2;2	41	2	2	23	1			1,25	0,92	0,77	1,09
Cer-42:1;2	42	1	2	24	0			1,12	1,07	1,04	1,09
Cer-42:2;2	42	2	2	24	1			1,12	0,92	0,84	1,12
Cer-42:3;2	42	3	2	24	2			1,19	0,84	0,83	1,07
DAG-32:0;0(16:0;0-16:0;0)	32	0	0	16	0	16	0	0,90	0,57	2,91	0,39
DAG-34:0;0(18:0;0-16:0;0)	34	0	0	18	0	16	0	0,73	0,68	1,54	0,56
DAG-34:1;0(18:1;0-16:0;0)	34	1	0	18	1	16	0	0,97	0,59	1,36	0,52
DAG-34:2;0(18:2;0-16:0;0)	34	2	0	18	2	16	0	1,24	0,72	1,92	0,91
DAG-36:2;0(18:1;0-18:1;0)	36	2	0	18	1	18	1	0,94	0,62	1,22	0,56
DAG-36:3;0(18:1;0-18:2;0)	36	3	0	18	1	18	2	0,84	1,18	1,27	1,22
DAG-36:4;0(18:2;0-18:2;0)	36	4	0	18	2	18	2	0,76	0,72	1,62	0,82
HexCer-34:1;2	34	1	2	16	0			0,93	0,88	0,82	0,84
HexCer-40:1;2	40	1	2	22	0			0,53	0,90	0,86	0,93
HexCer-42:1;2	42	1	2	24	0			0,62	0,89	1,08	0,88
HexCer-42:2;2	42	2	2	24	1			0,44	0,86	0,45	0,87
LPA-16:0;0(16:0;0)	16	0	0	16	0			1,22	0,98	0,97	0,82
LPA-16:1;0(16:1;0)	16	1	0	16	1			0,90	0,49	1,12	0,42
LPA-18:1;0(18:1;0)	18	1	0	18	1			0,77	0,66	0,56	0,73
LPA-18:2;0(18:2;0)	18	2	0	18	2			0,92	0,77	0,88	0,75
LPA-20:4;0(20:4;0)	20	4	0	20	4			1,01	0,31	1,32	0,44
LPC-14:0;0(14:0;0)	14	0	0	14	0			1,24	0,81	1,01	0,86

LPC-15:0;0(15:0;0)	15	0	0	15	0			1,14	0,79	1,21	0,93
LPC-16:0;0(16:0;0)	16	0	0	16	0			0,98	0,86	0,88	1,04
LPC-16:1;0(16:1;0)	16	1	0	16	1			1,35	0,89	1,20	0,94
LPC-17:1;0(17:1;0)	17	1	0	17	1			1,43	0,76	1,38	0,78
LPC-17:2;0(17:2;0)	17	2	0	17	2			1,05	1,09	1,35	1,04
LPC-18:0;0(18:0;0)	18	0	0	18	0			0,92	0,98	0,84	1,04
LPC-18:1;0(18:1;0)	18	1	0	18	1			1,09	1,04	0,93	1,12
LPC-18:2;0(18:2;0)	18	2	0	18	2			0,85	1,16	0,90	1,15
LPC-18:3;0(18:3;0)	18	3	0	18	3			1,11	0,96	0,85	0,97
LPC-19:3;0(19:3;0)	19	3	0	19	3			0,90	0,82	0,94	0,69
LPC-20:3;0(20:3;0)	20	3	0	20	3			1,72	0,85	1,21	0,74
LPC-20:4;0(20:4;0)	20	4	0	20	4			0,92	0,72	1,01	0,77
LPC-20:5;0(20:5;0)	20	5	0	20	5			1,53	1,07	1,72	1,01
LPC-22:6;0(22:6;0)	22	6	0	22	6			1,19	0,80	1,25	0,96
LPE-16:0;0(16:0;0)	16	0	0	16	0			1,08	0,88	1,03	0,98
LPE-18:0;0(18:0;0)	18	0	0	18	0			1,02	0,93	1,00	1,08
LPE-18:1;0(18:1;0)	18	1	0	18	1			1,00	1,05	0,81	1,07
LPE-18:2;0(18:2;0)	18	2	0	18	2			1,00	1,21	0,94	1,15
LPE-20:2;0(20:2;0)	20	2	0	20	2			0,53	0,88	0,45	0,87
LPE-20:3;0(20:3;0)	20	3	0	20	3			1,44	0,81	0,97	0,89
LPE-20:4;0(20:4;0)	20	4	0	20	4			0,96	0,78	0,90	0,83
LPE-20:5;0(20:5;0)	20	5	0	20	5			1,63	1,11	2,28	0,95
LPE-22:5;0(22:5;0)	22	5	0	22	5			1,13	0,80	0,60	0,88
LPE-22:6;0(22:6;0)	22	6	0	22	6			1,34	0,78	1,12	0,86
LPE O--16:1;0(16:1;0)	16	1	0	16	1			0,99	0,31	0,52	0,25
LPE O--18:1;0(18:1;0)	18	1	0	18	1			0,61	0,43	0,35	0,41
LPI-18:0;0(18:0;0)	18	0	0	18	0			0,78	0,54	0,64	0,59
LPI-18:1;0(18:1;0)	18	1	0	18	1			1,34	0,79	1,48	0,74
LPI-18:2;0(18:2;0)	18	2	0	18	2			1,43	0,57	1,02	0,51
LPI-20:3;0(20:3;0)	20	3	0	20	3			1,58	0,52	1,40	0,60
LPI-20:4;0(20:4;0)	20	4	0	20	4			1,21	0,62	0,95	0,63
PC-30:0;0(14:0;0-16:0;0)	30	0	0	14	0	16	0	1,30	1,23	1,04	1,09
PC-31:0;0(14:0;0-17:0;0)	31	0	0	14	0	17	0	1,51	0,55	0,67	0,58
PC-32:0;0(16:0;0-16:0;0)	32	0	0	16	0	16	0	1,25	1,21	0,76	1,13
PC-32:1;0(14:0;0-18:1;0)	32	1	0	14	0	18	1	1,36	1,19	0,94	1,13
PC-32:1;0(16:1;0-16:0;0)	32	1	0	16	1	16	0	1,64	1,27	1,22	1,16
PC-32:2;0(14:0;0-18:2;0)	32	2	0	14	0	18	2	1,13	1,16	1,38	1,10
PC-33:1;0(16:0;0-17:1;0)	33	1	0	16	0	17	1	1,27	0,73	2,70	0,68
PC-34:0;0(16:0;0-18:0;0)	34	0	0	16	0	18	0	0,95	1,13	0,79	1,04
PC-34:1;0(16:0;0-18:1;0)	34	1	0	16	0	18	1	1,36	1,22	0,93	1,17
PC-34:2;0(16:0;0-18:2;0)	34	2	0	16	0	18	2	0,94	1,23	0,76	1,13

PC-34:2;0(16:1;0-18:1;0)	34	2	0	16	1	18	1	1,21	1,14	0,83	1,10
PC-34:3;0(16:1;0-18:2;0)	34	3	0	16	1	18	2	1,04	1,17	0,78	1,09
PC-34:3;0(18:3;0-16:0;0)	34	3	0	18	3	16	0	1,18	1,20	0,93	1,07
PC-35:1;0(17:0;0-18:1;0)	35	1	0	17	0	18	1	1,40	1,19	0,92	1,09
PC-35:2;0(18:2;0-17:0;0)	35	2	0	18	2	17	0	0,99	1,29	0,83	1,17
PC-36:1;0(16:0;0-20:1;0)	36	1	0	16	0	20	1	1,37	0,92	2,58	0,89
PC-36:1;0(18:1;0-18:0;0)	36	1	0	18	1	18	0	1,36	1,24	0,86	1,14
PC-36:2;0(16:0;0-20:2;0)	36	2	0	16	0	20	2	0,86	0,86	0,71	0,81
PC-36:2;0(18:1;0-18:1;0)	36	2	0	18	1	18	1	0,90	1,16	0,50	1,09
PC-36:2;0(18:2;0-18:0;0)	36	2	0	18	2	18	0	0,81	1,25	0,67	1,10
PC-36:3;0(16:0;0-20:3;0)	36	3	0	16	0	20	3	1,41	1,21	1,03	1,13
PC-36:3;0(18:2;0-18:1;0)	36	3	0	18	2	18	1	0,79	1,28	0,63	1,15
PC-36:3;0(18:3;0-18:0;0)	36	3	0	18	3	18	0	0,89	1,20	0,72	1,01
PC-36:4;0(16:0;0-20:4;0)	36	4	0	16	0	20	4	0,96	1,08	0,82	1,04
PC-36:4;0(18:2;0-18:2;0)	36	4	0	18	2	18	2	0,78	1,22	0,58	1,08
PC-36:5;0(20:5;0-16:0;0)	36	5	0	20	5	16	0	1,62	1,27	1,63	1,02
PC-37:4;0(20:4;0-17:0;0)	37	4	0	20	4	17	0	1,33	1,05	1,16	1,11
PC-38:2;0(18:0;0-20:2;0)	38	2	0	18	0	20	2	1,18	1,31	1,17	1,15
PC-38:3;0(20:3;0-18:0;0)	38	3	0	20	3	18	0	1,44	1,19	1,06	1,07
PC-38:4;0(18:1;0-20:3;0)	38	4	0	18	1	20	3	1,28	1,16	0,89	1,04
PC-38:4;0(20:4;0-18:0;0)	38	4	0	20	4	18	0	0,86	1,07	0,88	1,01
PC-38:5;0(16:0;0-22:5;0)	38	5	0	16	0	22	5	1,17	1,11	1,26	1,09
PC-38:5;0(20:4;0-18:1;0)	38	5	0	20	4	18	1	0,95	1,08	0,88	1,06
PC-38:5;0(20:5;0-18:0;0)	38	5	0	20	5	18	0	1,27	1,26	1,41	0,99
PC-38:6;0(16:0;0-22:6;0)	38	6	0	16	0	22	6	1,22	1,16	1,10	1,03
PC-40:5;0(22:5;0-18:0;0)	40	5	0	22	5	18	0	1,09	1,15	1,16	1,11
PC-40:6;0(22:6;0-18:0;0)	40	6	0	22	6	18	0	1,02	1,09	0,99	0,98
PC O--30:0;0	30	0	0					1,67	0,80	1,57	0,84
PC O--30:1;0	30	1	0					1,15	0,91	0,64	0,92
PC O--32:0;0	32	0	0					1,30	0,90	1,00	0,92
PC O--32:1;0	32	1	0					1,20	1,08	0,81	1,08
PC O--32:2;0	32	2	0					1,11	1,01	1,08	1,05
PC O--34:1;0	34	1	0					1,20	1,18	0,80	1,09
PC O--34:2;0	34	2	0					0,87	1,19	0,90	1,10
PC O--34:3;0	34	3	0					0,99	1,22	0,76	1,13
PC O--35:3;0	35	3	0					0,65	0,84	0,84	0,90
PC O--35:4;0	35	4	0					0,76	0,90	0,83	0,92
PC O--36:1;0	36	1	0					0,97	1,03	0,49	0,99
PC O--36:2;0	36	2	0					0,77	1,09	0,71	0,98
PC O--36:3;0	36	3	0					0,71	1,19	0,55	1,10
PC O--36:4;0	36	4	0					0,95	1,07	0,72	1,04

PC O--37:5;0	37	5	0					0,95	0,99	0,65	1,06
PC O--38:4;0	38	4	0					0,74	1,03	0,61	0,93
PC O--38:5;0	38	5	0					0,89	1,06	0,76	1,07
PC O--38:6;0	38	6	0					0,99	1,20	0,87	1,13
PC O--40:5;0	40	5	0					1,33	0,88	0,54	0,90
PC O--40:6;0	40	6	0					0,83	1,03	0,68	1,00
PC O--40:7;0	40	7	0					1,29	1,14	0,86	1,04
PC O--42:5;0	42	5	0					0,80	0,74	0,46	0,76
PE-34:2;0(16:0;0-18:2;0)	34	2	0	16	0	18	2	1,19	0,95	1,21	0,97
PE-36:2;0(18:1;0-18:1;0)	36	2	0	18	1	18	1	0,54	0,21	0,56	0,22
PE-36:2;0(18:2;0-18:0;0)	36	2	0	18	2	18	0	1,13	1,14	1,11	1,17
PE-38:4;0(20:4;0-18:0;0)	38	4	0	20	4	18	0	1,14	0,96	1,08	1,11
PE O--34:2;0	34	2	0					0,77	0,82	0,72	0,84
PE O--34:3;0	34	3	0					0,80	0,92	0,68	0,94
PE O--36:2;0	36	2	0					0,43	0,68	0,43	0,77
PE O--36:3;0	36	3	0					0,76	1,03	0,71	1,03
PE O--36:4;0	36	4	0					0,76	0,82	0,64	0,85
PE O--36:5;0	36	5	0					0,99	0,77	0,97	0,91
PE O--36:6;0	36	6	0					1,79	1,08	2,36	0,93
PE O--38:4;0	38	4	0					0,85	0,80	0,66	0,81
PE O--38:5;0	38	5	0					0,63	0,73	1,15	0,85
PE O--38:6;0	38	6	0					1,16	1,00	1,23	1,07
PE O--38:7;0	38	7	0					1,46	1,09	1,52	1,07
PE O--39:7;0	39	7	0					0,89	0,83	1,58	0,81
PE O--40:5;0	40	5	0					0,96	0,64	1,18	0,77
PE O--40:6;0	40	6	0					1,24	0,87	0,92	0,92
PE O--40:7;0	40	7	0					1,23	1,01	1,07	1,06
PE O--40:8;0	40	8	0					1,18	1,05	1,15	1,05
PI-34:1;0(18:1;0-16:0;0)	34	1	0	18	1	16	0	1,40	1,18	1,41	1,07
PI-34:2;0(18:2;0-16:0;0)	34	2	0	18	2	16	0	1,64	1,10	1,03	1,02
PI-36:1;0(18:0;0-18:1;0)	36	1	0	18	0	18	1	1,01	1,15	0,67	0,99
PI-36:2;0(18:0;0-18:2;0)	36	2	0	18	0	18	2	1,10	1,05	0,94	0,92
PI-36:2;0(18:1;0-18:1;0)	36	2	0	18	1	18	1	0,86	1,01	0,37	0,81
PI-36:3;0(18:1;0-18:2;0)	36	3	0	18	1	18	2	0,65	0,86	0,42	0,60
PI-36:4;0(20:4;0-16:0;0)	36	4	0	20	4	16	0	2,03	1,01	0,55	1,01
PI-38:3;0(18:0;0-20:3;0)	38	3	0	18	0	20	3	1,31	1,02	0,93	1,03
PI-38:4;0(18:0;0-20:4;0)	38	4	0	18	0	20	4	0,88	0,85	0,75	1,00
SE-41:1;0(27:1;0-14:0;0)	41	1	0	27	1	14	0	1,48	1,16	1,10	1,07
SE-41:2;0(27:1;0-14:1;0)	41	2	0	27	1	14	1	1,12	0,87	2,25	0,79
SE-42:1;0(27:1;0-15:0;0)	42	1	0	27	1	15	0	1,06	1,04	0,68	0,96
SE-43:1;0(27:1;0-16:0;0)	43	1	0	27	1	16	0	1,09	1,15	1,01	1,17

SE-43:2;0(27:1;0-16:1;0)	43	2	0	27	1	16	1	1,69	1,25	1,34	1,12
SE-43:2;0(27:2;0-16:0;0)	43	2	0	27	2	16	0	2,38	0,51	0,71	0,56
SE-43:3;0(27:1;0-16:2;0)	43	3	0	27	1	16	2	2,20	1,03	4,09	0,97
SE-44:1;0(27:1;0-17:0;0)	44	1	0	27	1	17	0	0,98	0,94	0,40	0,90
SE-44:2;0(27:1;0-17:1;0)	44	2	0	27	1	17	1	1,46	1,16	1,07	1,09
SE-45:1;0(27:1;0-18:0;0)	45	1	0	27	1	18	0	1,16	0,99	1,02	0,98
SE-45:2;0(27:1;0-18:1;0)	45	2	0	27	1	18	1	1,20	1,15	1,03	1,11
SE-45:3;0(27:1;0-18:2;0)	45	3	0	27	1	18	2	0,87	1,21	0,93	1,15
SE-45:4;0(27:1;0-18:3;0)	45	4	0	27	1	18	3	1,41	1,16	1,15	1,07
SE-45:4;0(27:2;0-18:2;0)	45	4	0	27	2	18	2	1,14	0,69	0,92	0,73
SE-45:5;0(27:1;0-18:4;0)	45	5	0	27	1	18	4	2,36	1,08	3,22	0,95
SE-46:2;0(27:1;0-19:1;0)	46	2	0	27	1	19	1	1,30	0,76	0,78	0,59
SE-46:2;0(28:1;0-18:1;0)	46	2	0	28	1	18	1	0,59	0,70	0,75	0,55
SE-46:3;0(27:1;0-19:2;0)	46	3	0	27	1	19	2	1,54	0,31	2,75	0,37
SE-46:3;0(28:1;0-18:2;0)	46	3	0	28	1	18	2	0,35	0,92	1,83	0,83
SE-46:4;0(27:1;0-19:3;0)	46	4	0	27	1	19	3	1,10	0,56	1,84	0,51
SE-46:4;0(28:2;0-18:2;0)	46	4	0	28	2	18	2	0,27	0,73	2,52	0,75
SE-47:2;0(27:1;0-20:1;0)	47	2	0	27	1	20	1	1,49	0,46	1,61	0,36
SE-47:3;0(27:1;0-20:2;0)	47	3	0	27	1	20	2	1,76	0,85	1,38	0,90
SE-47:3;0(29:1;0-18:2;0)	47	3	0	29	1	18	2	0,71	0,92	2,83	0,81
SE-47:4;0(27:1;0-20:3;0)	47	4	0	27	1	20	3	1,27	1,10	1,16	0,99
SE-47:5;0(27:1;0-20:4;0)	47	5	0	27	1	20	4	0,90	0,93	1,03	1,00
SE-47:6;0(27:1;0-20:5;0)	47	6	0	27	1	20	5	1,49	1,20	1,88	1,08
SE-49:6;0(27:1;0-22:5;0)	49	6	0	27	1	22	5	1,04	1,00	1,31	1,04
SHE-36:1;0	36	1	0					0,78	0,38	0,74	0,45
SM-32:1;2	32	1	2	14	0			1,16	1,15	0,58	1,08
SM-32:2;2	32	2	2	14	1			0,99	1,04	0,49	0,98
SM-33:1;2	33	1	2	15	0			1,09	1,12	0,80	1,17
SM-33:2;2	33	2	2	15	1			1,39	0,71	0,95	0,77
SM-34:0;2	34	0	2	16	0			1,12	0,85	1,55	0,87
SM-34:1;2	34	1	2	16	0			0,97	1,10	0,73	1,13
SM-34:1;3	34	1	3	16	0			0,94	0,74	0,61	0,72
SM-34:2;2	34	2	2	16	1			0,97	1,02	0,77	1,12
SM-35:1;2	35	1	2	17	0			1,04	1,02	0,78	1,13
SM-35:2;2	35	2	2	17	1			1,42	0,68	1,18	0,86
SM-36:1;2	36	1	2	18	0			1,06	1,00	0,78	1,13
SM-36:2;2	36	2	2	18	1			0,97	0,92	0,76	1,09
SM-36:3;2	36	3	2	18	2			0,80	0,83	0,47	0,86
SM-37:1;2	37	1	2	19	0			1,15	0,97	0,81	1,11
SM-37:2;2	37	2	2	19	1			1,04	0,80	1,07	0,95
SM-38:1;2	38	1	2	20	0			1,16	1,04	0,90	1,15

SM-38:2;2	38	2	2	20	1			1,03	0,92	0,82	1,11
SM-39:1;2	39	1	2	21	0			1,12	1,07	0,87	1,12
SM-39:2;2	39	2	2	21	1			1,31	1,03	0,97	1,14
SM-40:1;2	40	1	2	22	0			0,98	1,09	1,01	1,15
SM-40:2;2	40	2	2	22	1			1,03	1,09	0,82	1,19
SM-40:3;2	40	3	2	22	2			1,01	1,02	0,76	1,07
SM-41:1;2	41	1	2	23	0			0,89	1,08	0,86	1,09
SM-41:2;2	41	2	2	23	1			1,12	1,09	0,86	1,20
SM-41:3;2	41	3	2	23	2			1,15	0,98	0,90	1,11
SM-42:1;2	42	1	2	24	0			1,07	0,98	1,29	1,02
SM-42:2;2	42	2	2	24	1			1,03	0,96	0,88	1,08
SM-42:3;2	42	3	2	24	2			0,98	0,98	0,77	1,10
SM-42:4;2	42	4	2	24	3			0,84	0,95	0,61	1,00
SM-43:2;2	43	2	2	25	1			1,13	0,95	0,76	1,00
SM-43:3;2	43	3	2	25	2			1,46	0,70	1,07	0,81
ST-27:1;0	27	1	0					1,14	1,07	1,09	1,09
TAG-46:0;0	46	0	0					0,90	1,13	2,46	1,11
TAG-46:1;0	46	1	0					0,93	1,10	1,34	1,08
TAG-46:2;0	46	2	0					0,79	1,05	1,28	1,12
TAG-48:0;0	48	0	0					1,09	1,14	2,03	1,09
TAG-48:1;0	48	1	0					1,25	1,25	1,52	1,25
TAG-48:2;0	48	2	0					1,10	1,22	1,24	1,25
TAG-48:3;0	48	3	0					0,91	1,15	1,69	1,20
TAG-49:1;0	49	1	0					1,26	1,18	1,17	1,22
TAG-49:2;0	49	2	0					1,14	1,19	1,30	1,26
TAG-50:1;0	50	1	0					1,38	1,22	1,45	1,24
TAG-50:2;0	50	2	0					1,18	1,24	1,24	1,32
TAG-50:3;0	50	3	0					1,02	1,21	1,37	1,32
TAG-50:4;0	50	4	0					0,92	1,19	1,30	1,28
TAG-50:5;0	50	5	0					0,80	1,12	1,32	1,16
TAG-51:1;0	51	1	0					1,21	1,14	1,09	1,20
TAG-51:2;0	51	2	0					1,23	1,18	1,13	1,30
TAG-51:3;0	51	3	0					1,04	1,19	1,16	1,32
TAG-51:4;0	51	4	0					0,86	1,15	1,15	1,21
TAG-52:2;0	52	2	0					1,10	1,18	1,33	1,27
TAG-52:3;0	52	3	0					0,93	1,20	1,38	1,30
TAG-52:4;0	52	4	0					0,84	1,21	1,40	1,28
TAG-52:5;0	52	5	0					0,86	1,20	1,42	1,25
TAG-52:6;0	52	6	0					1,03	1,20	1,67	1,22
TAG-53:2;0	53	2	0					0,98	1,15	0,90	1,25
TAG-53:3;0	53	3	0					1,01	1,18	1,01	1,29

TAG-53:4;0	53	4	0					0,92	1,22	1,22	1,30
TAG-54:2;0	54	2	0					1,04	1,16	1,03	1,23
TAG-54:3;0	54	3	0					0,98	1,18	0,92	1,20
TAG-54:4;0	54	4	0					0,80	1,23	1,06	1,23
TAG-54:5;0	54	5	0					0,83	1,22	1,21	1,24
TAG-54:6;0	54	6	0					1,01	1,20	1,58	1,23
TAG-54:7;0	54	7	0					1,26	1,22	1,80	1,16
TAG-56:2;0	56	2	0					0,80	0,87	1,05	0,89
TAG-56:3;0	56	3	0					1,24	0,85	1,32	0,74
TAG-56:4;0	56	4	0					1,00	1,17	1,16	1,21
TAG-56:5;0	56	5	0					0,95	1,13	0,99	1,22
TAG-56:6;0	56	6	0					1,06	1,08	1,49	1,21
TAG-56:7;0	56	7	0					1,44	1,16	1,86	1,18
TAG-56:8;0	56	8	0					1,33	1,18	1,90	1,07
TAG-58:7;0	58	7	0					1,45	1,13	1,50	1,10
TAG-58:8;0	58	8	0					1,47	1,15	1,37	1,03
TAG-58:9;0	58	9	0					1,03	1,12	1,32	0,91

CN, controls; db, double bound. Cer, ceramide; DAG, diacylglyceride; HexCer, very long chain monoglycosylated ceramide; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPE O, lysophosphatidylethanolamine ether; LPI, lysophosphatidylinositol; PC, phosphatidylcholine; PC O, phosphatidylcholine ether; PE, phosphatidylethanolamine; PE O, phosphatidylethanolamine ether; PI, phosphatidylinositol; SE, steryl ester; SM, sphingomyelin; TAG, triacylglyceride.

Table S8. FADS gene variants genotyped in MDC-CC and results for the association with DM in DIAGRAM (1)

SNP	Gene	Ch	Genotype MDC-CC	Risk Allele	Other Allele	*P*	OR	OR_95L	OR_95U
rs174550	FADS1	11	231/238/54	T	C	2.9E-03	1.06	1.02	1.10
rs174546	FADS1	11	233/238/54	C	T	3.2E-03	1.06	1.02	1.10
rs174547	FADS1	11	233/238/54	T	C	3.3E-03	1.05	1.02	1.09
rs174548	FADS1	11	255/223/46	C	G	1.6E-03	1.06	1.02	1.10
rs1535	FADS2	11	232/238/53	A	G	3.0E-03	1.06	1.02	1.10
rs174576	FADS2	11	233/237/55	C	A	4.2E-03	1.06	1.02	1.10
rs174577	FADS2	11	233/237/55	C	A	1.1E-02	1.05	1.01	1.08
rs174583	FADS2	11	233/234/56	C	T	6.5E-03	1.05	1.01	1.09
rs174570	FADS2	11	387/125/12	C	T	1.1E-02	1.06	1.01	1.12
rs174593	FADS2	11	303/199/23	T	C	2.1E-02	1.06	1.01	1.11
rs174611	FADS2	11	260/226/39	T	C	1.3E-02	1.05	1.01	1.09

Gene variants in italic are in LD with the gene variant in bold ($r^2 >0.8$).

1.Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, Prokopenko I, Kang HM, Dina C, Esko T, Fraser RM, Kanoni S, Kumar A, Lagou V, Langenberg C, Luan J, Lindgren CM, Müller-Nurasyid M, Pechlivanis S, Rayner NW, Scott LJ, Wiltshire S, Yengo L, Kinnunen L, Rossin EJ, Raychaudhuri S, Johnson AD, Dimas AS, Loos RJ, Vedantam S, Chen H, Florez JC, Fox C, Liu CT, Rybin D, Couper DJ, Kao WH, Li M, Cornelis MC, Kraft P, Sun Q, van Dam RM, Stringham HM, Chines PS, Fischer K, Fontanillas P, Holmen OL, Hunt SE, Jackson AU, Kong A, Lawrence R, Meyer J, Perry JR, Platou CG, Potter S, Rehnberg E, Robertson N, Sivapalaratnam S, Stančáková A, Stirrups K, Thorleifsson G, Tikkanen E, Wood AR, Almgren P, Atalay M, Benediktsson R, Bonnycastle LL, Burtt N, Carey J, Charpentier G, Crenshaw AT, Doney AS, Dorkhan M, Edkins S, Emilsson V, Eury E, Forsen T, Gertow K, Gigante B, Grant GB, Groves CJ, Guiducci C, Herder C, Hreidarsson AB, Hui J, James A, Jonsson A, Rathmann W, Klopp N, Kravic J, Krjutškov K, Langford C, Leander K, Lindholm E, Lobbens S, Männistö S, Mirza G, Mühleisen TW, Musk B, Parkin M, Rallidis L, Saramies J, Sennblad B, Shah S, Sigurðsson G, Silveira A, Steinbach G, Thorand B, Trakalo J, Veglia F, Wennauer R, Winckler W, Zabaneh D, Campbell H, van Duijn C, Uitterlinden AG, Hofman A, Sijbrands E, Abecasis GR, Owen KR, Zeggini E, Trip MD, Forouhi NG, Syvänen AC, Eriksson JG, Peltonen L, Nöthen MM, Balkau B, Palmer CN, Lyssenko V, Tuomi T, Isomaa B, Hunter DJ, Qi L; Wellcome Trust Case Control Consortium; Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) Investigators; Genetic Investigation of ANthropometric Traits (GIANT) Consortium; Asian Genetic Epidemiology Network–Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium, Shuldiner AR, Roden M, Barroso I, Wilsgaard T, Beilby J, Hovingh K, Price JF, Wilson JF, Rauramaa R, Lakka TA, Lind L, Dedoussis G, Njølstad I, Pedersen NL, Khaw KT, Wareham NJ, Keinanen-Kiukaanniemi SM, Saaristo TE, Korpi-Hyövälti E, Saltevo J, Laakso M, Kuusisto J, Metspalu A, Collins FS, Mohlke KL, Bergman RN, Tuomilehto J, Boehm BO, Gieger C, Hveem K, Cauchi S, Froguel P, Baldassarre D, Tremoli E, Humphries SE, Saleheen D, Danesh J, Ingelsson E, Ripatti S, Salomaa V, Erbel R, Jöckel KH, Moebus S, Peters A, Illig T, de Faire U, Hamsten A, Morris AD, Donnelly PJ, Frayling TM, Hattersley AT, Boerwinkle E, Melander O, Kathiresan S, Nilsson PM, Deloukas P, Thorsteinsdottir U, Groop LC, Stefansson K, Hu F, Pankow JS, Dupuis J, Meigs JB, Altshuler D, Boehnke M, McCarthy MI; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat Genet*. 2012;44:981-90.
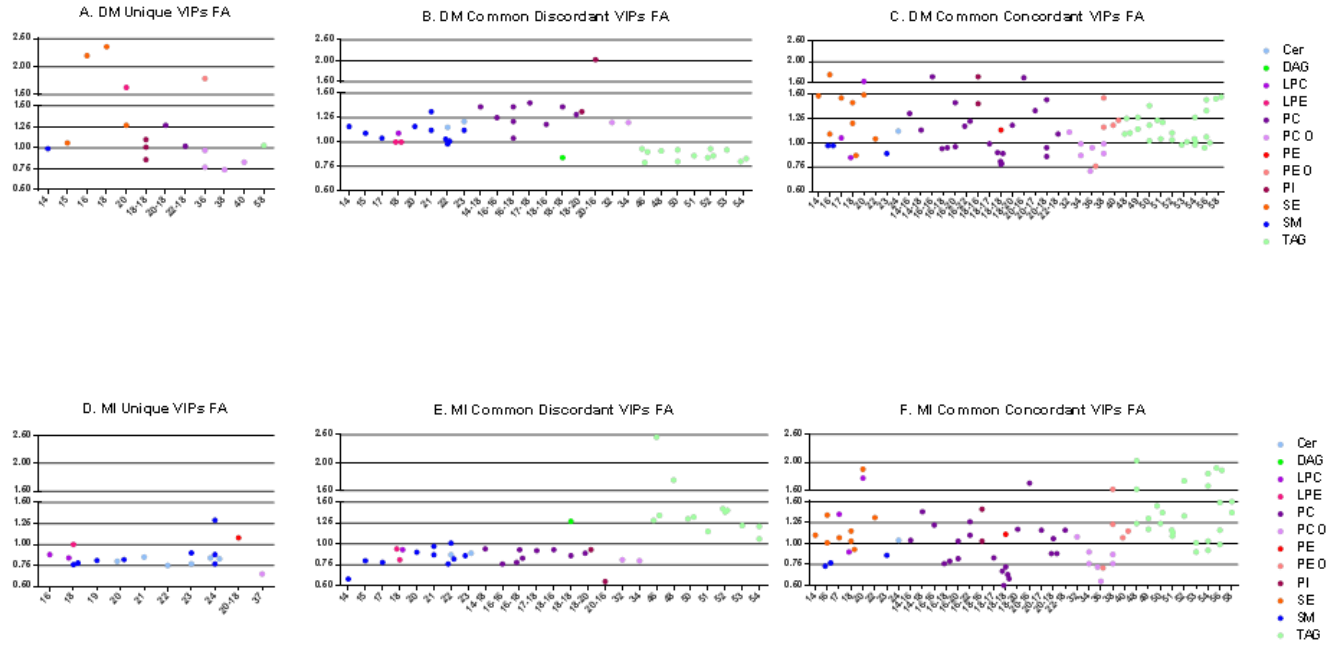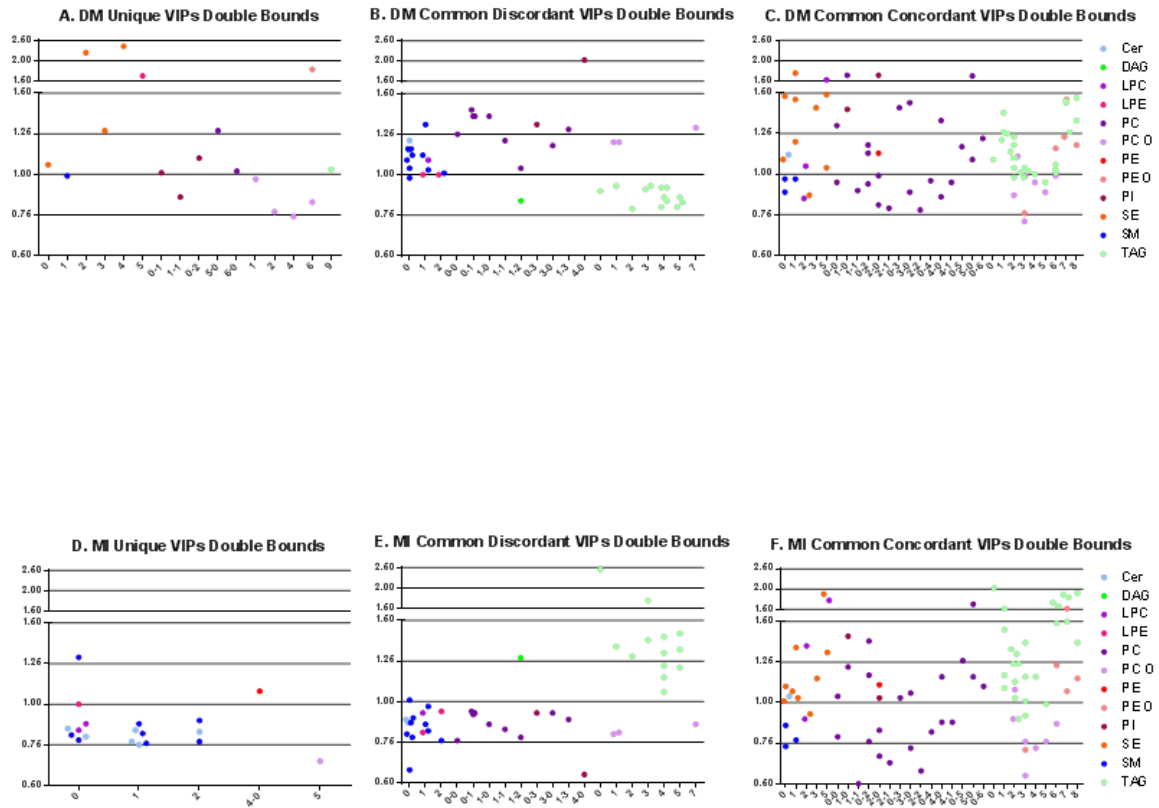
Figure S1.

Figure S2.

Supplemental Figure Legends:

Figure S1. Comparison between significant loadings acyl chain length for DM and MI from OPLS models. Lipid species unique for DM (A), for MI (D); lipid species common for DM and MI but with discordant loadings according to fold change (B-E); lipid species common for DM and MI but with concordant loadings according to fold change (C-F).

Figure S2. Comparison between significant loadings acyl chain double bound numbers for DM and MI from OPLS models. Lipid species unique for DM (A), for MI (D); lipid species common for DM and MI but with discordant loadings according to fold change (B-E); lipid species common for DM and MI but with concordant loadings according to fold change (C-F).