Supplementary Text

BGM Methods

Plasma samples from the FHS and PESA were stored and maintained at -80°C prior to analysis. In the FHS, lipid measurements were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring (MRM). This discovery LC/MS approach measured all detectable lipids with reproducible quality control data using a 4000QTrap instrument (AB/SCIEX, Concord, Ontario, Canada). Plasma sample aliquots (10 µL) were extracted with v/v 1:120 extraction solvent of 10:25:65 dichloromethane:methanol:isoproanol. The extraction solvent contains three internal standards: LPE 14:0, PC 20:0/20:0, and TAG 17:0/17:0/17:0 at concentrations of 4 µg/mL, 0.2 µg /mL and 0.2 µg /mL, respectively. The extracts were vortexed for 10 seconds before being centrifuged to pellet the proteins thoroughly (3000 rpm for 10 minutes at room temperature). Approximately 900 □L of the supernatant is transferred to autosampler vials.

HPLC Analysis

Lipids are separated on a Gemini C6-phenyl (2.1x150 mm 3 µm, 110 Ā) HPLC column held constant at 37 degrees C. The injected sample amount is 5 µL. The HPLC solvents used for the separation are: Solvent A: 30% methanol, 0.1% formic acid, 1mM ammonium acetate; Solvent B: 100% methanol, 0.1% formic acid, 1mM ammonium acetate; Solvent C: 100% ethylacetate, 0.1% formic acid, 1mM ammonium acetate. Gradient conditions are:

Time	Solvent	Flow (µl/min)
0	65% A, 35% B, 0% C	300
3	15% A, 85% B, 0% C	300
15	1% A, 99% B, 0% C	575
19	0% A, 99% B, 1% C	600
22	0% A, 80% B, 20% C	600

Under these solvent conditions (lyso)phosphatidylcholines and sphyngomyelins, (L)PC and SM, are detected in their native positive state (through their quaternary ammonium group); (lyso)phosphatidylethanolamines, (L)PEs, are detected as protonated ions; cholesterol esters (CE), di- and triglycerides (DG, TG) are detected as ammonium

adducts. Complete table of MRM transitions for lipid analysis is shown in Supplemental table 2A. Other source parameters for the QTrap4000 instrument are listed below:

Parameter	Setting
Curtain Gas	20 psi
Collision Gas	Medium
Spray Voltage	4500 V
Source Temperature	350K
Gas 1	40 psi
Gas 2	30 psi
Heater	On
Entrance Potential	15V
Exit Potential	10V
MRM Cycle Time	1 s
MRM Detection Window	40 s

Class-specific transitions were used to measure most of the lipid components: the m/z 369 fragment for cholesterol esters (CEs); the m/z 184 fragment for lysophosphatidyl- and phosphatidyl-cholines (LPCs and PCs) and sphingomyelins (SMs); the 141-Da neutral loss for lysophosphatidyl- and phosphatidylethanolamines (LPEs and PEs); and the 184-Da neutral loss for phosphatidylserines (PSs). Fatty acyl chain losses were monitored from diacyl- and triacylglycerols (DAGs and TAGs) and select PCs. For ceramides (Cers) and select SMs, the characteristic fragment from the sphingoid base (d18:1, d18:0, etc.) was used. Each ion was monitored in positive ion mode. Supplementary Table 2 provides MRM transitions for all measured lipid species. Using these transitions, the effective dynamic range for detection approached five orders of magnitude. Normalization and integration of MRM peaks were completed with MultiQuant v2 software (AB/SCIEX, Concord, Ontario, Canada). Each peak area for a target lipid component was normalized to the median peak area of the same component, as detected in nine uniformly distributed quality control replicates. Raw quantitative data were evaluated and batch/trend-corrected on a component-by-component basis if a statistically significant bias was identified.

Analysis of FHS samples was carried out in 12 data acquisition batches, each consisting of 64 samples (56 primary and eight quality control). The primary samples were arranged in 82 blocks of eight. The samples in each block were matched for age, sex, and cohort type; together, they represented all eight metabolic risk factor groups. All samples from a single block were analyzed in the same batch. Otherwise, the samples were assayed in a random order with quality control samples interleaved at regular intervals. For the PESA samples, the standards, consisting of isotopically-labeled lipids, were used for external standardization (i.e., lipid family assignment) and internal

standardization (i.e., adjustment of potential inter- and intra-assay variances) as shown in Supplementary Table 7. Stock solutions were prepared by dissolving lipid standards in methyl tert-butyl ether (MTBE) at a concentration of 1 mg/mL, and working solutions were diluted to 2.5 μg/mL in MTBE.

The median coefficient of variation (CV) was 8.4% across all lipid analytes, with CVs <10% for 125 species. Only one analyte (PC 36:0) had a CV >20% due to signal saturation and was removed from analysis. Seven lipids contained missing values: SM 36:0 had missing values for two participants; DAG 50:0, PS 36:0, SM (d17:1/14:0), SM 43:1, and TAG 50:5 had 56 missing values; and SM (d18:2/16:0) had 112 missing values. Because the missing values for the latter six were clustered in batches (batch size=56), no imputation was performed. Testing for batch and run order revealed no significant effects.

PESA Methods

Lipidomic analysis of the PESA samples was conducted based on a previously validated method1. Briefly, in order to precipitate the protein samples 5 μL of miliQ water and 20 μl of methanol were added to 10 μl of plasma sample. After the addition, the samples were vigorously agitated for 2 minutes. Then, for lipid extraction, 250 μl of MTBE (containing internal lipid standards, see Supplementary Table 7) were added and the samples were immersed in a water bath (ATU Ultrasonidos, Valencia, Spain) with an ultrasound frequency of 40 kHz and power of 100 W at 10°C for 30 minutes. Then, 75 μL of MilliQ water were added to the mixture, and the organic phase was separated by centrifugation at 1400 g for 10 min at 10°C.

Lipid extracts were subjected to liquid chromatography coupled to mass-spectrometry using an Agilent UPLC 1290 coupled to the Q-TOF MS/MS 6520 (Agilent Technologies, Barcelona, Spain) based on a previously published method2. Sample compartment was refrigerated at 4°C, and, for each sample, 10 μL of lipid extract were applied onto 1.8 μm particle 100 x 2.1 mm i.d. Waters Acquity HSS T3 column (Waters, Mildord, MA) heated to 55°C. The flow rate was 400 μl/min for both solvent A, composed of 10 mM ammonium acetate in acetonitrile-water (40:60, v/v), and solvent B, composed of 10 mM ammonium acetate in acetonitrile-isopropanol (10:90, v/v). The gradient started at 40% B and reached 100% B at 10 min where it held for 2 min. Finally, the system was switched back to 60% B and equilibrated for 3 min. Duplicate runs of the samples were performed to collect positive and negative electrospray ionized lipid species in a TOF mode, operated in full-scan mode at 100 to 3000 m/z in an extended dynamic range (2 GHz), using N2 as nebulizer gas (5 L/min, 350°C). The capillary voltage was set to 3500 V

with a scan rate of 1 scan/s. Continuous infusion using a double spray with masses 121.050873, 922.009798 (positive ion mode) and 119.036320, 966.000725 (negative ion mode) was used for in-run calibration of the mass spectrometer. Lipid species identity was confirmed by MS/MS as previously described3.

The MassHunter Data Analysis Software (Agilent Technologies, Barcelona, Spain) was used to collect the results, and to obtain the molecular features of the samples, which represented different co-migrating ionic species of a given molecular entity (i.e., ion adducts) using the Molecular Feature Extractor algorithm (Agilent Technologies, Barcelona, Spain)4. This algorithm uses the accuracy of the mass measurements to group related ions (based on charge-state envelope, isotopic distribution, and/or the presence of different adducts and dimers/trimers) and assign multiple species (ions) to a single compound referred to as a feature. Finally, the MassHunter Mass Profiler Professional Software (Agilent Technologies, Barcelona, Spain) was used to perform a non-targeted lipidomic analysis of the extracted features. Only common features (found in at least 50% of the samples of the same condition) were taken into account to correct for individual bias. Multivariate statistics (PCA and PLS-DA analyses) were done using this software. The masses representing significant differences by ANOVA ($p < 0.05$ with Benjamini-Hochberg Multiple Testing Correction) were searched against the LIPID MAPS database (The LIPID MAPS Lipidomics Gateway, http://www.lipidmaps.org/, May 2014) (exact mass ppm < 20). The identities obtained were then compared to retention time and MS/MS spectrum of the authentic standards added.

Supplementary Tables

Supplementary Table 1. Clinical Characteristics of the Longitudinal Sample

Clinical Characteristics	Baseline	Follow-up
Sample size (#)	650	554
Age (years)	52.8±13.1	58.6±12.2
Women (%)	50	50
BMI (kg/m^2)	29.9±5.7	30.4±6.0
Glucose (mg/dL)*	102±19	103±21
HDL-C (mg/dL)†	53±16	56±18
TAG (mg/dL)†	130±94	130±95

*Values for glucose level at follow-up were imputed for those who reported use of diabetes medications at follow-up (n=30); glucose was raised by 10% or to a maximum of 126 mg/dL.

†Values for HDL-C and TAG at follow-up were imputed for those who reported use of lipid-lowering medications at follow-up (n=133); HDL-C was lowered by 10% and TAG was raised by 20%.

Supplementary Table 2. Replication of lipid cross-sectional associations with obesity in the ERF study, SAFHS, and PESA cohort

		Discovery (FHS)			Replication (ERF)						Replication (SAFHS)		Replication (PESA)	
		LCMS			LCMS		Biocrates		ESI-MS		ESI-MS		LCMS	
Lipid Species	Class	Beta	SE	P	Beta	P	Beta	P	Beta	P	Beta	P	Beta	P
LPC 17:0	LPC	-0.618	0.072	1.05E-16			-0.263	1.73E-15						
LPC 18:2	LPC	-0.524	0.069	1.67E-13	-0.204	2.86E-24	-0.249	6.17E-14	-0.169	6.28E-06			-0.152	2.60E-04
LPC 18:0p	LPC	-0.503	0.07	2.28E-12	0.022	2.74E-01	-0.155	4.47E-06	0.013	7.38E-01				
LPC 18:1	LPC	-0.51	0.072	3.89E-12	-0.183	1.45E-19	-0.270	2.76E-16	-0.138	2.41E-04			-0.154	2.03E-04
LPC 18:0e	LPC	-0.503	0.071	3.95E-12	0.022	2.74E-01	-0.155	4.47E-06	0.013	7.38E-01				
PC 16:2e	PC	-0.448	0.067	4.52E-11										
SM 36:0	SM	0.493	0.075	7.81E-11										
LPC 22:5	LPC	-0.462	0.073	5.08E-10					-0.039	3.00E-01				
PC 40:8	PC	-0.46	0.074	8.80E-10										
LPC 17:1	LPC	-0.447	0.073	1.97E-09							-1.64	1.45E-10		
LPC 15:0	LPC	-0.439	0.076	1.06E-08					-0.043	2.58E-01	-1.35	2.09E-07		
LPC 18:0	LPC	-0.423	0.074	1.34E-08	0.022	2.74E-01	-0.155	4.47E-06	0.013	7.38E-01				
LPC 22:6	LPC	-0.427	0.075	2.26E-08	-0.076	1.89E-04			-0.014	7.20E-01	-1.11	7.60E-06		
PC 34:0	PC	-0.407	0.074	6.45E-08			-0.115	7.21E-04	-0.070	6.30E-02				
LPC 16:0	LPC	-0.387	0.072	1.31E-07	-0.005	8.02E-01	-0.198	3.69E-09	-0.029	4.49E-01				
LPC 20:4	LPC	-0.365	0.071	3.30E-07	-0.070	6.64E-04	-0.161	1.76E-06	0.011	7.72E-01				
LPC 18:1e	LPC	-0.373	0.073	4.35E-07	-0.183	1.45E-19	-0.270	2.76E-16	-0.138	2.41E-04				
TAG 50:1	TAG	0.329	0.065	6.44E-07	0.235	6.78E-32							0.319	2.69E-06
TAG 50:2	TAG	0.323	0.065	7.76E-07	0.231	5.42E-31							0.283	1.58E-05
LPC 18:3	LPC	-0.369	0.076	1.40E-06					-0.038	3.12E-01				
PC 38:3	PC	0.362	0.075	1.66E-06	0.262	4.07E-40	0.224	1.87E-11	0.256	3.73E-12				
SM (d18:0/24:0)	SM	0.347	0.075	4.86E-06										
LPE 16:0	LPE	-0.352	0.078	7.17E-06							-0.96	5.45E-05		
TAG 48:0	TAG	0.31	0.069	8.65E-06	0.207	8.02E-24								
LPE 18:1	LPE	-0.343	0.077	1.04E-05							-0.99	8.19E-06		
LPC 16:0e	LPC	-0.317	0.072	1.16E-05			-0.198	3.69E-09	-0.029	4.49E-01				
LPC 16:1e	LPC	-0.31	0.072	1.86E-05			-0.010	7.69E-01	0.097	1.00E-02				
DAG 32:0	DAG	0.28	0.067	3.04E-05										

LPC 18:2e	LPC	-0.299	0.074	5.24E-05	-0.204	2.86E-24	-0.249	6.17E-14	-0.169	6.28E-06				
TAG 48:1	TAG	0.276	0.069	6.40E-05	0.222	3.49E-28							0.371	1.65E-05
PC 40:7	PC	-0.302	0.075	6.85E-05	-0.065	1.55E-03			-0.002	9.68E-01				
PC 36:3e	PC	-0.283	0.072	1.01E-04	0.066	1.30E-03	0.024	4.84E-01	0.001	9.75E-01				
LPC 20:5	LPC	-0.296	0.076	1.04E-04										
TAG 52:1	TAG	0.251	0.066	1.51E-04	0.242	8.34E-34								
LPE 18:2	LPE	-0.276	0.078	4.26E-04							-0.76	1.18E-03		
PC 34:1e	PC	-0.249	0.071	4.58E-04	-0.014	4.82E-01	-0.038	2.67E-01	0.004	9.18E-01				
PC 36:2	PC	-0.27	0.077	4.85E-04	0.036	8.21E-02	0.040	2.41E-01	0.037	3.35E-01				
PC 36:0	PC	-0.27	0.077	5.13E-04			-0.143	2.64E-05	-0.004	9.17E-01			0.164	7.09E-04
DAG 34:1	DAG	0.221	0.064	5.48E-04										

Full replication results for lipid species associated with obesity in the FHS. Estimated β coefficients represent the mean differences in standardized lipid measures between participants with and without obesity. The p-value threshold for discovery in the FHS was determined by Bonferroni correction for the number of principal components and number of analyses at $p<5.7x10^{-4}$ [0.05/(22x4)]. The p-value threshold for replication was also determined by Bonferroni correction for the number of lipid species available for replication at $1.56x10^{-3}$ (0.05/32). Blanks represent lipid species not available for replication.

Abbreviations: DAG=diacylglycerol; LPC=lysophosphatidylcholine; LPE=lysophosphatidylethanolamine; PC=phosphatidylcholine; SM=sphingomyelin; TAG=triacylglycerol.

Supplementary Table 2a. MRM Transitions table

Lipid Species*	Q1†	Q3†	Transition	LIPID MAPS ID‡
LPC 17:0	510.355	184.076	LPC 17:0 (sn-1) □ PCho	LMGP01050024
LPE 18:2	478.293	337.274	LPE 18:2 (sn-1) - 141	LMGP02050011
LPC 18:0p	508.376	184.076	LPC 18:0p □ PCho	LMGP01070009
LPC 18:1	522.355	184.076	LPC 18:1 (sn-1) □ PCho	LMGP01050029
LPC 18:0e	510.390	184.076	LPC 18:0e □ PCho	LMGP01060014
PC 32:0	734.569	184.076	PC 32:0 □ PCho	LMGP01010564
SM 36:0	733.622	184.076	SM (d18:0) 18:0 □ PCho	LMSP03010020; LMSP03010054
LPC 22:5	570.356	184.076	LPC 22:5 (sn-1, sn-2) □ PCho	Unknown
PC 40:8	830.569	184.076	PC 40:8 □ PCho	Multiple components
LPC 17:1	508.34	184.076	LPC 17:1 (sn-1) □ PCho	LMGP01050002; LMGP01050126
LPC 15:0	482.324	184.076	LPC 15:0 (sn-1) □ PCho	LMGP01050016
LPC 18:0	524.371	184.076	LPC 18:0 (sn-1) □ PCho	LMGP01050026
LPC 22:6	568.34	184.076	LPC 22:6 (sn-1) □ PCho	LMGP01050056
PC 34:0	762.601	184.076	PC 34:0 □ PCho	LMGP01010573; LMGP01010742
LPC 16:0	496.34	184.076	LPC 16:0 (sn-1) □ PCho	LMGP01050018
LPC 20:4	544.34	184.076	LPC 20:4 (sn-1) □ PCho	LMGP01050048
LPC 18:1e	508.376	184.076	LPC 18:1e □ PCho	LMGP01060034; LMGP01060039
TAG 50:1	850.785	577.49	TAG 50:1 - C16:0	LMGL03010005; LMGL03010006
TAG 50:2	848.77	575.503	TAG 50:2 - C16:0	LMGL03010044; LMGL03010043
LPC 18:3	518.324	184.076	LPC 18:3 (sn-1) □ PCho	LMGP01050038; LMGP01050128
PC 38:3	812.616	546.36	PC 38:3 - C18:0	Multiple components
SM (d18:0/24:0)	817.716	184.076	SM (d18:0) 24:0 □ PCho	LMSP03010024
LPE 16:0	454.293	313.274	LPE 16:0 (sn-1) - 141	LMGP02050002
TAG 48:0	824.77	551.503	TAG 48:0 - C16:0	LMGL03010001; LMGL03014225
LPE 18:1	480.308	339.29	LPE 18:1 (sn-1) - 141	LMGP02050004
LPC 16:0e	482.360	184.076	LPC 16:0e □ PCho	LMGP01060010
LPC 16:1e	480.345	184.076	LPC 16:1e □ PCho	LMGP01060028; LMGP01060029;
DAG 32:0	586.54	313.280	DG 32:0 - C16:0	LMGL02010009; LMGL02010001
LPC 18:2e	506.361	184.076	LPC 18:2e □ PCho	LMGP01070012
TAG 48:1	822.754	549.487	TAG 48:1 - C16:0	LMGL03014226; LMGL03010017
PC 40:7	832.585	184.076	PC 40:7 □ PCho	Multiple components
PE 36:3e (or 36:2p)	728.559	392.35	PE 36:3e - 141	Unknown
LPC 20:5	542.330	184.076	LPC 20:5 □ PCho	LMGP01050050
TAG 52:1	878.817	605.55	TAG 52:1 - C16:0	LMGL03010085
LPE 18:2	478.293	337.274	LPE 18:2 (sn-1) - 141	LMGP02050011
PC 34:1e	746.606	184.076	PC 34:1e □ PCho	LMGP01020152
PC 36:2	786.601	522.36	PC 36:2 - C18:1	Multiple components
PC 36:0	790.632	524.5	PC 36:0 - C18:0	LMGP01010006
DAG 34:1	612.556	313.273	DAG 34:1 - C18:1	LMGL02010004; LMGL02010005;

Supplementary Table 3. Replication of lipid cross-sectional associations with dysglycemia in the ERF study

		Discovery (FHS)			Replication (ERF)					
		LCMS			LCMS		Biocrates		ESI-MS	
Lipid Species	Lipid Class	Beta	SE	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Cer (d18:0/24:0)	Cer	0.28	0.07	6.59E-05	0.045	2.88E-02				
LPC 18:1e	LPC	-0.279	0.073	1.50E-04	-0.057	5.61E-03				
LPC 17:0	LPC	-0.271	0.073	1.98E-04			-0.263	1.69E-15		
SM 36:0	SM	0.275	0.075	2.43E-04						
LPC 18:0e	LPC	-0.26	0.071	2.80E-04	0.039	5.87E-02	-0.160	2.26E-06	-0.009	8.15E-01
LPC 16:1e	LPC	-0.254	0.072	4.48E-04	0.058	4.83E-03	-0.029	4.00E-01	0.078	4.02E-02
SM (d18:0/24:0)	SM	0.265	0.075	4.79E-04						
LPC 18:0p	LPC	-0.244	0.07	5.48E-04	0.039	5.87E-02	-0.160	2.26E-06	-0.009	8.15E-01

Full replication results for lipid species associated with dysglycemia in the FHS. Estimated β coefficients represent the mean differences in standardized lipid measures between participants with and without dysglycemia. The p-value threshold for discovery in the FHS was determined by Bonferroni correction for the number of principal components and number of analyses at $p<5.7x10^{-4}$ [0.05/(22x4)]. The p-value threshold for replication of the lipid cross-sectional associations with dysglycemia was determined by Bonferroni correction for the number of lipid species available for replication at $p<8.33x10^{-3}$ (0.05/6). Blanks represent lipid species not available for replication.

Abbreviations: Cer=ceramide; LPC=lysophosphatidylcholine; SM=sphingomyelin.

Supplementary Table 4. Cross-Sectional Lipid Associations with Dyslipidemia in FHS and PESA

		Discovery (FHS)			Replication (PESA)		
Lipid Species	**Class**	**Estimated β***	**SE**	**P-value†**	**Estimated β***	**SE**	**P-value†**
TAG 52:3	TAG	1.195	0.061	9.76E-68	0.508	0.053	**2.36E-20**
TAG 52:2	TAG	1.133	0.062	4.65E-60	0.507	0.055	**3.88E-19**
DAG 36:2	DAG	1.133	0.063	2.59E-58			
DAG 36:3	DAG	1.132	0.064	8.17E-58			
TAG 54:3	TAG	1.109	0.063	6.51E-57	0.416	0.051	**1.51E-15**
TAG 50:3	TAG	1.122	0.064	7.75E-57	0.610	0.066	**3.20E-19**
DAG 34:1	DAG	1.110	0.063	1.60E-56			
TAG 54:2	TAG	1.083	0.063	2.13E-54	0.497	0.059	**2.40E-16**
TAG 54:4	TAG	1.085	0.064	9.18E-54	0.422	0.051	**6.98E-16**
TAG 50:4	TAG	1.093	0.065	6.79E-53			
TAG 52:4	TAG	1.081	0.065	2.06E-52	0.585	0.061	**1.87E-20**
TAG 56:5	TAG	1.063	0.064	5.72E-52			
TAG 50:2	TAG	1.057	0.064	6.34E-51	0.581	0.065	**5.30E-18**
TAG 52:5	TAG	1.060	0.065	7.50E-50	0.567	0.069	**1.21E-15**
DAG 36:1	DAG	1.024	0.065	2.84E-47			
TAG 56:4	TAG	1.017	0.066	2.94E-46	0.489	0.058	**1.45E-16**
TAG 50:1	TAG	0.993	0.065	3.91E-45	0.576	0.067	**1.04E-16**
TAG 48:3	TAG	1.021	0.067	8.88E-45			
TAG 54:5	TAG	0.993	0.066	1.38E-43	0.459	0.057	**2.92E-15**
TAG 48:2	TAG	0.999	0.067	1.82E-43	0.661	0.081	**1.79E-15**
DAG 32:0	DAG	0.983	0.066	5.12E-43			
TAG 52:1	TAG	0.965	0.066	1.70E-42			
DAG 36:4	DAG	0.986	0.068	1.53E-41			
TAG 56:3	TAG	0.964	0.067	3.89E-41	0.452	0.052	**4.87E-17**
TAG 50:5	TAG	0.979	0.071	1.09E-37			
TAG 56:6	TAG	0.912	0.067	1.26E-37	0.084	0.040	3.60E-02
TAG 48:1	TAG	0.915	0.068	2.11E-36	0.603	0.086	**5.42E-12**
TAG 54:1	TAG	0.897	0.067	6.40E-36			
PC 36:2e	PC	-0.878	0.067	2.13E-35			
DAG 38:5	DAG	0.871	0.069	3.61E-33			
TAG 50:0	TAG	0.833	0.069	1.21E-30			
TAG 46:1	TAG	0.847	0.070	2.40E-30			
TAG 48:0	TAG	0.824	0.069	5.11E-30			
TAG 52:6	TAG	0.837	0.070	6.85E-30	0.183	0.044	**3.44E-05**
TAG 54:6	TAG	0.837	0.070	8.19E-30	0.475	0.058	**1.17E-15**
TAG 46:2	TAG	0.829	0.071	1.07E-28			
TAG 46:0	TAG	0.801	0.070	1.63E-27	0.357	0.048	**6.04E-13**
TAG 58:6	TAG	0.796	0.070	3.06E-27			
TAG 58:8	TAG	0.795	0.071	7.06E-27			

		Discovery (FHS)			Replication (PESA)		
Lipid Species	**Class**	**Estimated β***	**SE**	**P-value†**	**Estimated β***	**SE**	**P-value†**
TAG 44:0	TAG	0.793	0.071	1.20E-26			
TAG 54:7	TAG	0.777	0.072	2.24E-25	0.117	0.039	3.00E-03
PE 36:2	PE	0.774	0.071	2.50E-25			
PC 34:1e	PC	-0.726	0.070	3.01E-23			
TAG 56:7	TAG	0.728	0.071	7.09E-23	0.332	0.073	**6.80E-06**
TAG 60:8	TAG	0.718	0.071	1.81E-22			
PC 36:3e	PC	-0.718	0.072	7.34E-22			
PE 38:4	PE	0.705	0.071	1.97E-21			
PE 34:1	PE	0.698	0.073	2.08E-20			
SM (d18:2/24:1)	SM	-0.620	0.068	9.79E-19	-0.043	0.038	2.57E-01
Cer (d18:1/22:0)	Cer	0.638	0.072	4.65E-18			
DAG 34:0	DAG	0.669	0.076	1.41E-17			
PE 36:3	PE	0.634	0.073	4.17E-17			
PE 34:2	PE	0.626	0.073	6.56E-17			
PE 40:6	PE	0.590	0.071	6.39E-16			
SM 42:2	SM	-0.588	0.071	7.50E-16			
PC 34:2e	PC	-0.563	0.075	1.82E-13			
TAG 56:8	TAG	0.550	0.074	3.01E-13			
PE 36:4	PE	0.530	0.074	2.30E-12			
SM C41:2	SM	-0.472	0.069	1.65E-11			
Cer (d18:0/24:0)	Cer	0.471	0.069	2.36E-11			
SM (d18:1/16:0)	SM	-0.475	0.072	7.95E-11	-0.046	0.027	9.40E-02
PE 40:7e	PE	-0.438	0.073	2.56E-09			
Cer (d18:1/24:1)	Cer	0.434	0.072	3.29E-09			
PC 38:3	PC	0.431	0.074	1.03E-08			
CE 16:2	CE	0.426	0.075	2.29E-08			
PC 40:4	PC	0.422	0.076	3.62E-08			
PC 38:5e	PC	-0.415	0.075	4.74E-08			
PE 38:6	PE	0.394	0.073	8.34E-08			
LPC 18:2	LPC	-0.373	0.069	9.40E-08	-0.103	0.042	1.40E-02
SM (d18:0/16:0)	SM	-0.383	0.073	2.11E-07			
LPC 18:0p	LPC	-0.360	0.070	3.48E-07			
LPC 16:1e	LPC	-0.353	0.072	1.04E-06			
PE 36:3e	PE	-0.373	0.076	1.08E-06			
CE 14:0	CE	0.376	0.077	1.22E-06			
SM 43:2	SM	-0.343	0.076	8.74E-06			
Cer (d18:1/24:0)	Cer	0.325	0.074	1.38E-05			
SM (d18:2/16:0)	SM	-0.325	0.074	1.47E-05			
LPC 22:6	LPC	-0.327	0.075	1.58E-05			
SM 33:1	SM	-0.323	0.075	1.77E-05			
SM (d18:2/22:0)	SM	-0.304	0.071	2.38E-05			

		Discovery (FHS)			Replication (PESA)		
Lipid Species	**Class**	**Estimated β***	**SE**	**P-value†**	**Estimated β***	**SE**	**P-value†**
PC 36:4e	PC	-0.322	0.076	2.38E-05			
CE 15:0	CE	0.326	0.077	2.77E-05			
LPC 18:2e	LPC	-0.301	0.073	4.33E-05			
TAG 60:10	TAG	0.308	0.076	5.31E-05			
TAG 58:9	TAG	0.310	0.077	6.40E-05			
PS 36:0	PS	-0.316	0.079	7.32E-05			
PC 40:8	PC	-0.270	0.074	2.56E-04			
SM (d18:1/24:0)	SM	-0.267	0.075	4.11E-04	-0.068	0.046	1.40E-01
PE 38:5e	PE	-0.265	0.075	4.76E-04			

*Estimated β coefficients represent the mean differences in standardized lipid measures between participants with and without dyslipidemia.

†The p-value threshold for significance was determined by the Bonferroni method and based on the number of principle components. In discovery, the p-value threshold was $p<5.7x10^{-4}$ [0.05/(22x4)]. The replication p-value threshold was $p<9.6x10^{-4}$ [0.05/(4x13)]; significant p-values in replication are shown in **bold**.

Abbreviations: CE=cholesteryl ester; Cer=ceramide; DAG=diacylglycerol; LPC=lysophosphatidylcholine; PC=phosphatidylcholine; PE=phosphatidylethanolamine; PS=phosphatidylserine; SM=sphingomyelin; TAG=triacylglycerol.

Supplementary Table 5. Shared Lipid Markers for Obesity and Dysglycemia in FHS

Lipid		Obesity		Dysglycemia	
Species	**Class**	**Estimated β†**	**P-value‡**	**Estimated β†**	**P-value‡**
LPC 17:0	LPC	-0.618	1.05E-16	-0.271	1.98E-04
LPC 18:0p	LPC	-0.503	2.28E-12	-0.244	5.48E-04
LPC 18:0e	LPC	-0.503	3.95E-12	-0.260	2.80E-04
SM 36:0	SM	0.493	7.81E-11	0.275	2.43E-04
LPC 18:1e	LPC	-0.373	4.35E-07	-0.279	1.50E-04
SM (d18:0/24:0)	SM	0.347	4.86E-06	0.265	4.79E-04
LPC 16:1e	LPC	-0.310	1.86E-05	-0.254	4.48E-04

*Estimated β coefficients represent the mean differences in standardized lipid measures between participants with and without the stated metabolic risk factor.

†The p-value threshold for significance ($p<5.7x10^{-4}$) was determined by the Bonferroni method [0.05/(22x4)].

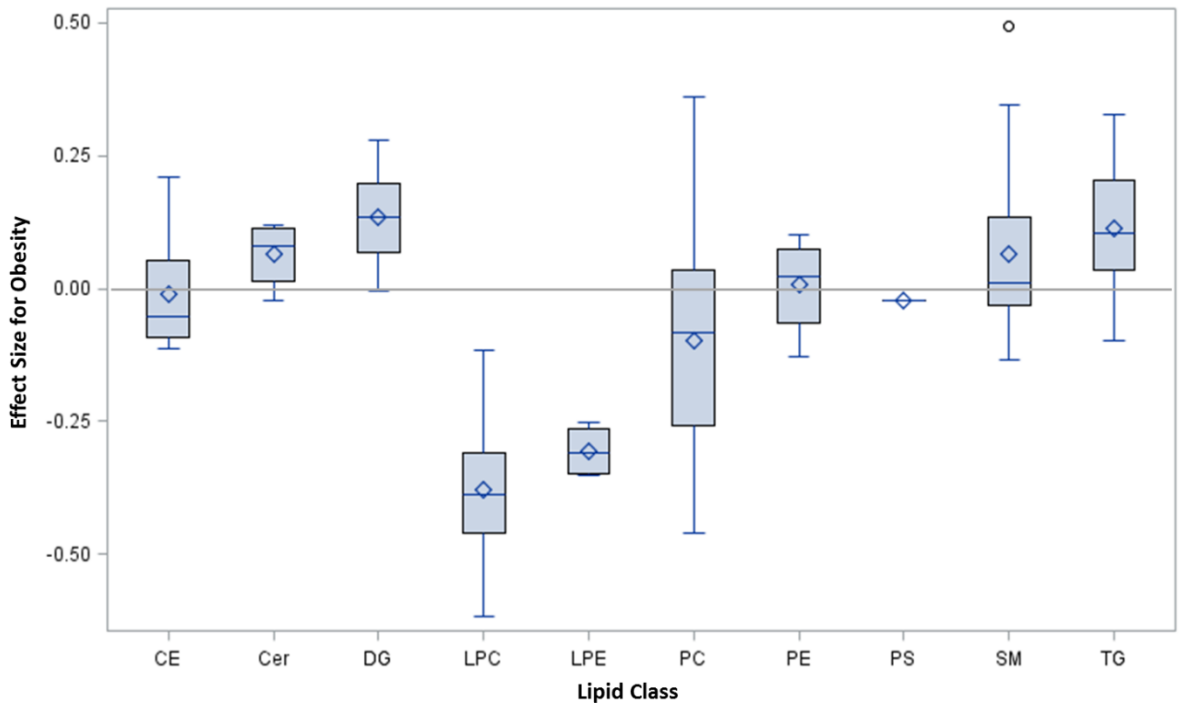
Abbreviations: LPC=lysophosphatidylcholine; SM=sphingomyelin.

Supplementary Table 6. Internal Lipid Standards Used in Lipidomic Analysis of PESA Samples

Compound	Reference
1,3(d5)-dihexadecanoyl-glycerol	110537, Avanti Polar Lipids
1,3(d5)-dihexadecanoyl-2-octadecanoyl-glycerol	110543, Avanti Polar Lipids
1-hexadecanoyl(d31)-2-(9Z-octadecenoyl)-sn-glycero-3-phosphate	110920, Avanti Polar Lipids
1-hexadecanoyl(d31)-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine	110918, Avanti Polar Lipids
1-hexadecanoyl(d31)-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine	110921, Avanti Polar Lipids
1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1'-rac-glycerol-1',1',2',3',3'-d5)	110899, Avanti Polar Lipids
1-hexadecanoyl(d31)-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-myo-inositol	110923, Avanti Polar Lipids
1-hexadecanoyl(d31)-2-(9Z-octadecenoyl)-sn-glycero-3-[phospho-L-serine]	110922, Avanti Polar Lipids
26:0-d4 Lyso PC	860389, Avanti Polar Lipids
18:1 Chol (D7) ester	111015, Avanti Polar Lipids
cholest-5-en-3ß-ol(d7)	LM-4100, Avanti Polar Lipids
D-erythro-sphingosine-d7	860657, Avanti Polar Lipids
D-erythro-sphingosine-d7-1-phosphate	860659, Avanti Polar Lipids
N-palmitoyl-d31-D-erythro-sphingosine	868516, Avanti Polar Lipids
N-palmitoyl-d31-D-erythro-sphingosylphosphorylcholine	868584, Avanti Polar Lipids
Octadecanoic acid-2,2-d2	19905-58-9, Sigma Aldrich

Supplementary Figures

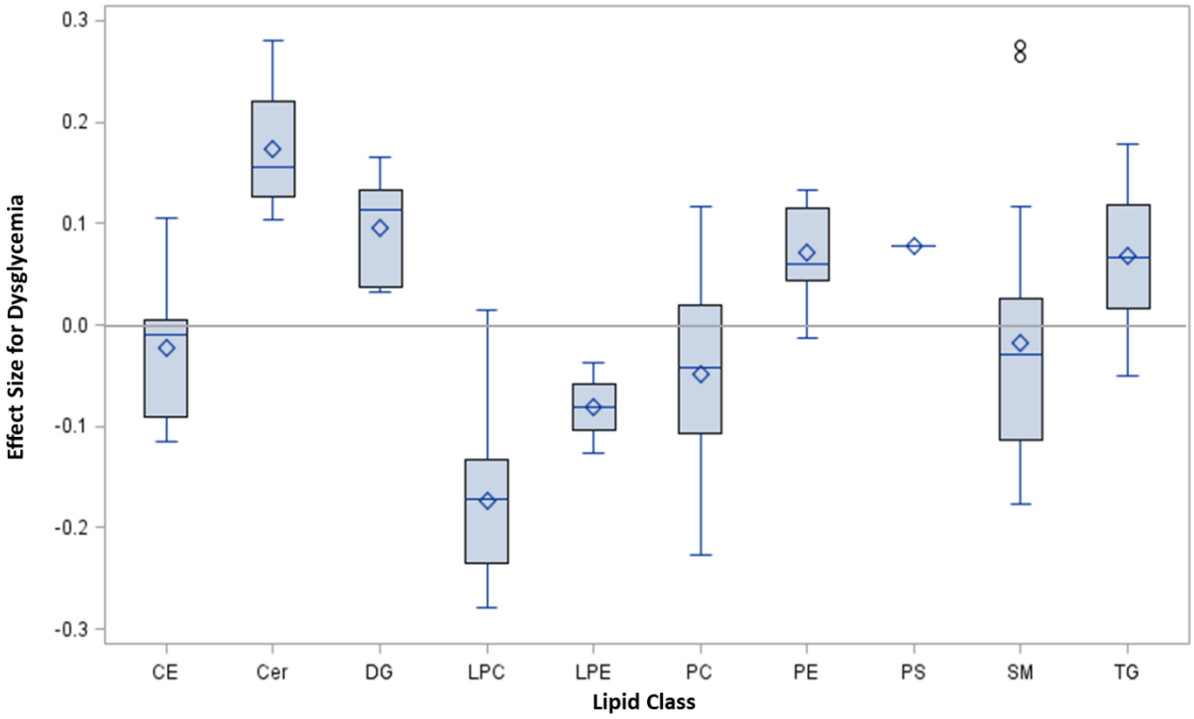
Supplementary Figure 1. Correlation Matrix of Lipid Markers



Supplementary Figure 2. Association of Lipid Classes with Obesity in FHS

Lipid species significantly associated with obesity were DAGs, LPCs, LPEs, PCs, SMs, or TAGs.

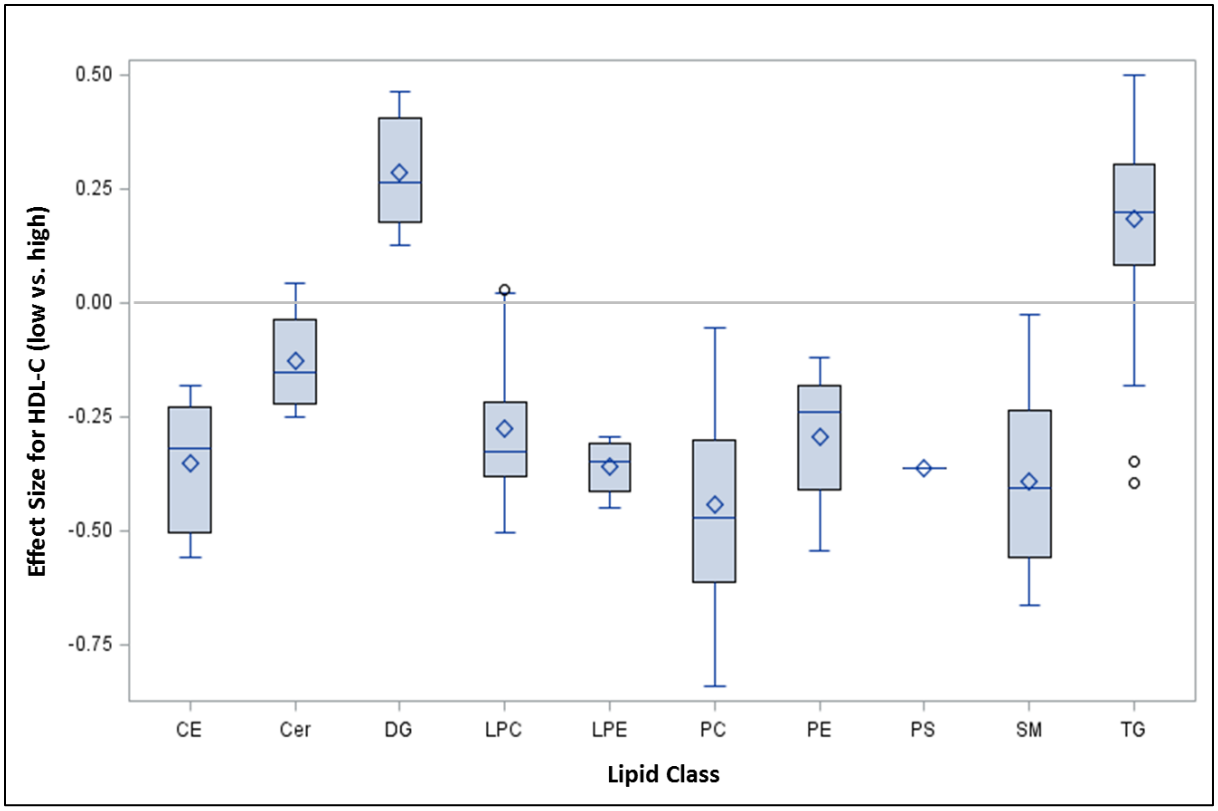
Abbreviations: CE=cholesteryl ester; Cer=ceramide; DAG=diacylglycerol; LPC=lysophosphatidylcholine; LPE=lysophosphatidylethanolamine; PC=phosphatidylcholine; PE=phosphatidylethanolamine; PS=phosphatidylserine; SM=sphingomyelin; TAG=triacylglycerol.



Supplementary Figure 3. Association of Lipid Classes with Dysglycemia in FHS

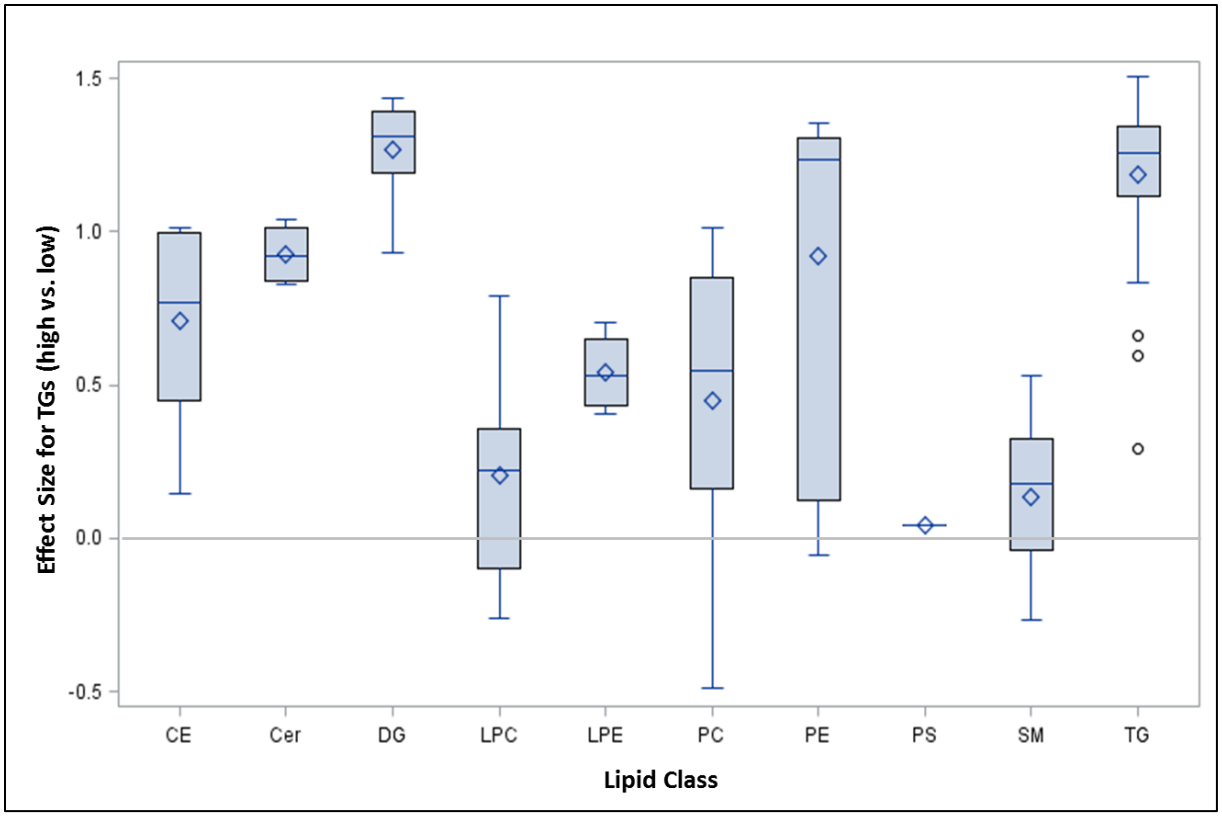
Lipid species significantly associated with dysglycemia were Cers, LPCs, or SMs.

Abbreviations: CE=cholesteryl ester; Cer=ceramide; DAG=diacylglycerol; LPC=lysophosphatidylcholine; LPE=lysophosphatidylethanolamine; PC=phosphatidylcholine; PE=phosphatidylethanolamine; PS=phosphatidylserine; SM=sphingomyelin; TAG=triacylglycerol.



Supplementary Figure 4. Associations of Lipid Classes with Low vs. High HDL-C

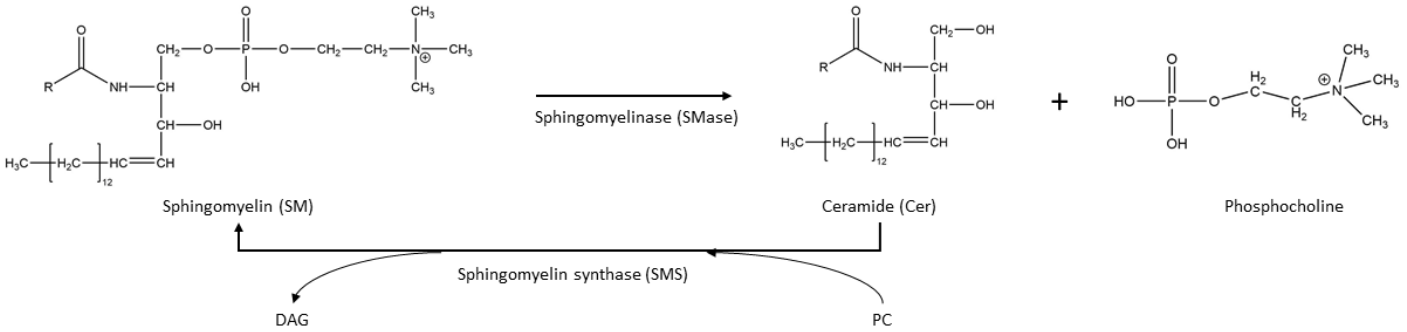
Abbreviations: CE=cholesteryl ester; Cer=ceramide; DAG=diacylglycerol; LPC=lysophosphatidylcholine; LPE=lysophosphatidylethanolamine; PC=phosphatidylcholine; PE=phosphatidylethanolamine; PS=phosphatidylserine; SM=sphingomyelin; TAG=triacylglycerol.



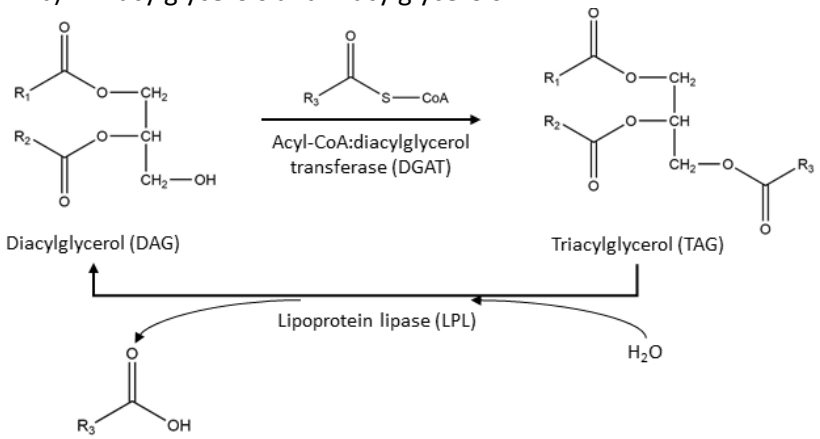
Supplementary Figure 5. Associations of Lipid Classes with High vs. Low TAG

Abbreviations: CE=cholesteryl ester; Cer=ceramide; DAG=diacylglycerol; LPC=lysophosphatidylcholine; LPE=lysophosphatidylethanolamine; PC=phosphatidylcholine; PE=phosphatidylethanolamine; PS=phosphatidylserine; SM=sphingomyelin; TAG=triacylglycerol.

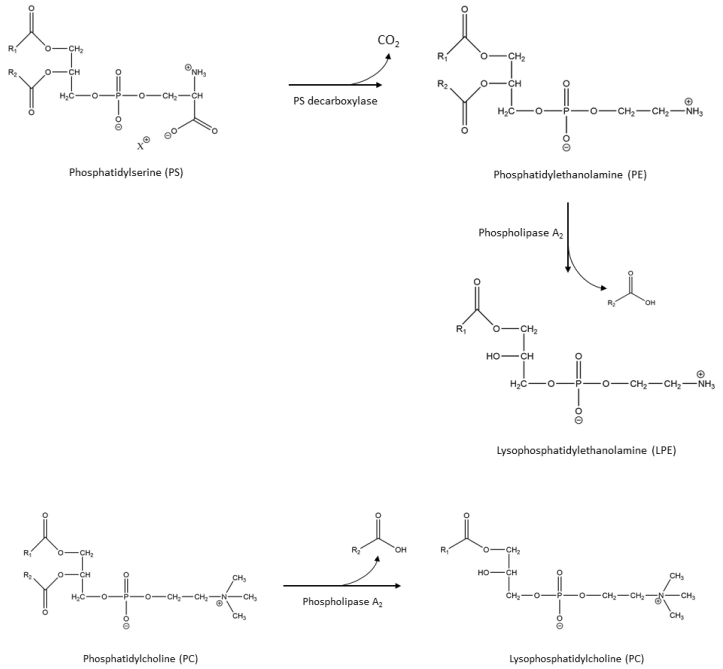
a) Sphingolipids: Sphingomyelins and Ceramides



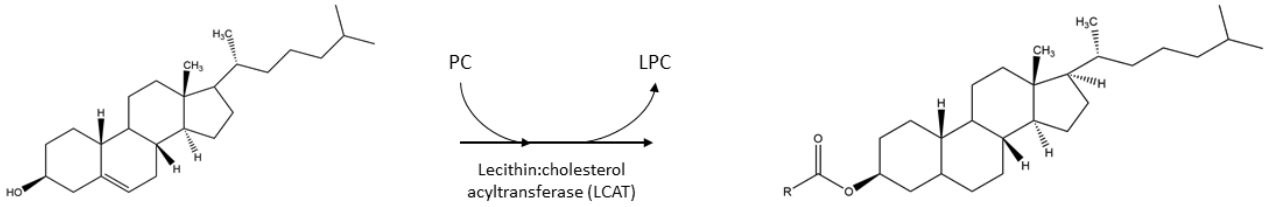
b) Triacylglycerols and Diacylglycerols



c) Phospholipids: Phosphatidylserine, Phosphatidylethanolamine, Lysophosphatidylethanolamine (Top); Phosphatidylcholine, Lysophosphatidylcholine (Bottom)



d) Cholesterol (left) and Cholesterol Ester (right)



Supplementary Figure 6: Chemical Structures and Interconvertibility of Metabolite Species

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