Supplemental Material

Metabolomic signature of human aortic valve stenosis

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Supplemental Methods

2.1 Chemicals

All solvents were of LC-MS grade. Ultrapure water, acetonitrile, methanol, 2-propanol, formic acid, ammonium acetate, and ammonium formate were purchased from Thermo Fisher Scientific (Mississauga, ON). Chloroform was purchased from Millipore Sigma (Oakville, ON). Acetic acid and Leucine enkephalin were purchased from Sigma-Aldrich (St.Louis, MO) and the synthetic standard 1-heptadecanoyl-2-hydroxy-sn-glycero-3-phosphate (17:0 lysophosphatidic acid) from Avanti Polar Lipids (Alabaster, AL).

2.2 Procurement of tissues for analysis

AV leaflets were obtained from 106 patients undergoing AV replacement surgery at St. Boniface Hospital (MB, Canada) between June 2014 and July 2015. The study was approved by the ethics committee of both the University of Manitoba and the St. Boniface hospital research ethics boards. Written informed consent was obtained from patients prior to their inclusion in the study. Patients were deemed eligible to participate if they were above 18 years of age and were free of any illness or condition that disallowed their ability to provide consent. Exclusion criteria included end stage renal disease on hemodialysis, suspicion of endocarditis or systemic infection. All valves underwent pathological assessment after removal and if endocarditis was suspected the valves were excluded. The indication for surgery for majority of our population was aortic valve stenosis. For the patients in the mild category the main surgical indication was aortic root surgery and the patients in moderate category was concomitant coronary artery disease. Preoperative transthoracic echocardiography was used to confirm the severity of aortic stenosis

according to the American Society of Echocardiography guidelines on valvular heart disease[1]. To investigate the diagnostic potentials of the differential metabolites and lipids discovered from the tissues, venous blood samples were collected (EDTA-plasma) from a sub-cohort (N=19) of the same population. The patients were on overnight fasting and blood was drawn before the anesthetic infusion prior to surgery. After surgical removal, the AV leaflets were immediately placed in an ice-cold solution containing EDTA/PBS, flash frozen and stored at -80°C until analysis. The average dwell time upon excision to flash-freezing was kept within 30 min. The study was conducted according to the declaration of Helsinki.

2.3 Aortic valve morphology and function

The AV phenotype (i.e. bicuspid vs. tricuspid) was recorded. The Doppler echocardiographic indices of CAVS severity included peak aortic jet velocity (Vmax), peak and mean pressure gradients (MPG) obtained with the use of the modified Bernoulli equation, and the aortic valve area (AVA) calculated by the standard continuity equation.

Left ventricular geometry and function: LV ejection fraction (LVEF) was measured with the use of biplane Simpson method. The relative wall thickness ratio was calculated by dividing the sum of the LV posterior wall and inter-ventricular septal thicknesses by the LV internal dimension. Left ventricular mass was calculated with the corrected formula of the American Society of Echocardiography and was indexed to a 2.7 power of height[2].

The degree of AV calcification was scored according to the criteria proposed by Yousry *et al*[3,4]. A single score value (C-score) from 1 to 5 was assigned for the ultrasound still frames to the whole valve. In short, non-thickened and non-calcified valves were classified as having score '1';

thickened but non-calcified valves as '2'; calcification spot(s) not exceeding one-third of the leaflet area as '3'; calcification spot(s) not exceeding two-thirds of the leaflet area as '4' and calcification spot(s) covering more than two-thirds of the leaflet area as '5'.

2.4 Sample grouping based on CAVS severity

CAVS severity was defined by hemodynamic parameters in accordance with the 2014 AHA/ACC guidelines[5] and accordingly patients were stratified into three disease stages as mild, moderate, and severe. In short, mild AS was defined by a mean pressure gradient (MPG) <20 mm Hg or aortic valve area (AVA) >1.5 cm^2, moderate AS was defined as a MPG between 20 and 40 mm Hg or AVA between 1.0 and 1.5 cm^2, and severe AS by a MPG ≥40 mm Hg or AVA ≤1.0 cm^2. The same subjects were also categorized into three groups based on their echocardiographic calcification score (C-Score) in the 5-grade scoring system[3,4] as low, medium and high. Individual subjects with a score of '1' and '2' formed the 'low' calcification group, individuals with a score of '3' formed the 'medium' group, and individuals with a score of '4' and '5' formed the 'high' calcification group.

2.5 Rate of CAVS progression

The annualized rate of CAVS disease progression was defined as the change in maximum jet velocity (ΔVmax). This was retrospectively analysed in 50 (47%) of the 106 patients in our cohort. The annualized ΔVmax was calculated by deducting the oldest available echocardiography Vmax measurement available from the pre-surgery echocardiography data and dividing it by the time elapsed. The mean time gap between the pre-surgery and oldest available echocardiography reports was 4.4±2.7 yr (range; 0.4-9.8 yr). The following formula was used:

$$\text{Annualized } \Delta\text{Vmax } \left(\text{m/s}/_{\text{yr}}\right) = \frac{\text{Final Vmax} - \text{Oldest Vmax}}{\Delta\text{T}}$$

where 'Final Vmax' is the maximum jet velocity immediately prior to the surgery, 'Oldest Vmax' is the maximum jet velocity from the oldest available echocardiography report and ΔT is the time elapsed (in years) between the latest and oldest echocardiography reports.

2.6 Extraction procedures and sample preparations

The entire explanted valve was cryo-milled until a fine powder was obtained. 100 mg of the frozen powder was weighed and used for metabolite extraction by non-targeted analysis and 100 mg was used for lipid extraction by targeted analysis. The blood samples were collected in EDTA treated tubes and immediately centrifuged at 2500g for 10 minutes at 4^{o}C in a refrigerated centrifuge to harvest plasma. Average time of blood collection to plasma separation and aliquoting were less than 30 min.

For non-targeted metabolomics analysis, the metabolites were extracted using a pre-chilled extraction solution containing 2:2:1 methanol, acetonitrile and water (vol/vol/vol). Approximately 100 mg of frozen tissue powder was suspended in 900 µl of extraction solution, vortexed and sonicated in an ice-cold water bath sonicator for 10 min. The samples were then centrifuged at 3000g at 4°C for 20 min to remove the denatured proteins. The supernatant was dried down in a nitrogen evaporator and stored at -80°C until further analysis. The samples were finally reconstituted in 100 µl of 50% methanol with 0.1% formic acid and transferred into a sampling vial immediately prior to injection. At the same time, a quality control (QC) sample was prepared by pooling equal aliquots (20 µl) from all samples.

For targeted lipidomics analysis, samples were extracted with 2:1 (vol/vol) chloroform and methanol containing 0.01% butylated hydroxytoluene (BHT) and PBS (pH 7.4) using a method previously described by Folch *et al*[6]. Approximately 100 mg of frozen tissue homogenate and 100 µl of plasma were used for extraction. 10 ng of 17:0 lysophosphatidic acid (17: 0 LysoPA) was spiked into each sample as the internal standard for quantitation purposes. The samples were spun at 1900g at 4°C for 5 min. The chloroform extracts were then dried down in a nitrogen evaporator. The dried extracts were finally reconstituted in 100 µl of 60:40 acetonitrile and water in 10 mM ammonium formate and 0.1% formic acid immediately prior to injection.

2.7 Non-targeted metabolomics analysis

The metabolites were first separated using a UPLC® chromatographic system (ACQUITY, WATERS, Milford, USA) in both reverse phase (RP) and hydrophilic interaction liquid chromatography (HILIC) columns to analyze both polar and non-polar compounds. For RP liquid chromatography (RPLC) separation, a C18 (ACQUITY UPLC HSS T3 C18, 100 A0, 1.8 µm, 2.1 mm X 100 mm, Waters) column was used. The oven temperature was maintained at 40°C and the auto-sampler at 4°C. The eluent solutions consisted of aqueous (A) and organic (B) components, where A was ultrapure water and B was acetonitrile. Both A and B contained 0.1% formic acid or 0.1% acetic acid in positive ion mode and negative ion mode respectively. A 22 min linear gradient was performed as follows: 0-1 min, 1% B; 16-20 min, 100 %B; 22 min, 1% B. Samples were ionized in positive and negative ion modes employing the same gradient. HILIC separation was achieved using a BEH (ACQUITY BEH HILIC column, 130 A0, 1.7 µm, 2.1 mm X 150 mm, Waters) column. For HILIC, eluent solutions were 95% acetonitrile in 10 mM ammonium acetate (A) and 50%

acetonitrile in 10 mM ammonium acetate (B). Both A and B contained 0.1% formic acid or 0.1% acetic acid in positive ion mode and negative ion mode respectively. A 20 min linear gradient was performed as follows in both modes as follows: 0 min, 1% B; 5 min, 20% B; 10 min, 50% B; 14-16 min, 95 %B; 17-20 min, 1% B. For both RP and HILIC separations, the sample injection volume was 5 µl and flow rate was set to 400 µl/min.

For mass spectrometry analysis, an ion mobility spectrometry (IMS) in conjunction with the UPLC System coupled to a Quadrupole-Time of Flight (Q-TOF) mass spectrometer (SYNAPT-G2, Waters) was used. Both the systems were operated and controlled by MassLynx4.1 SCN781 workstation (Waters, MA, USA). Mass spectral analysis was conducted in positive and negative ion modes with ESI parameters as follows: capillary voltage, 2.85 KV; sample cone, 40 V; extraction cone, 6 V; IMS gas (N2) flow, 90 ml/min. To perform the mobility separation, the IMS T-Wave™ pulse height was set to 40 V during transmission and the IMS T-Wave™ velocity was set to 650 m/s. The travelling wave height was ramped linearly over 100% of the IMS cycle between 8 V and 20 V.

To ensure the accuracy and active calibration over the length of the sample queue, the *m/z* values of all ions acquired in the Q-TOF/MS were adjusted by LockSpray. The lock mass channel was sampled every 10 s. 2 ng/µl of leucine encephalin (Sigma-Aldrich) was selected as lock mass compound for positive (*m/z* 556.2771) and negative (*m/z* 554.2615) ion mode. The time of flight analyzer (TOF) of the mass spectrometer was calibrated with a solution of 1 mM sodium formate (Sigma-Aldrich). This calibration set the analyzer to detect ions in the range of 50-1200 *m/z*. The data acquisition was done in continuum format. The data were acquired by rapidly alternating

between two functions – Function-1 (low energy) and Function-2 (high energy). In Function-1, only low energy mass spectra (MS) were acquired and in Function-2, mass spectra at elevated collision energy with ion mobility (HDMSE) were acquired. In Function-2, collision energy was set to 4 eV in the Trap region of mass spectrometer and was ramped from 20 eV to 50 eV in the Transfer region of mass spectrometer to attain fragmentation in the HDMSE mode. The scan duration in each function was 0.5 s with an inter scan delay of 0.024 s.

In order to ensure system stabilization, the QC samples were repeatedly injected 10 times for nearly four hours before the formal sampling. To further monitor the reproducibility of the analytical platform, the QC samples were then re-injected after every five samples and at the end of the sample runs in line with the published guidelines[7]. The same volume of blank sample consisting of 50% methanol and 0.1% formic acid were injected in a random manner among all other samples as a means to ensure needle wash and to equilibrate the column, besides avoiding contamination among real samples. It is important to emphasize that global metabolomic extractions are not intended for quantitative extraction of all lipids. While some polar lipids may be fully extracted using the methods described here, others may be partially or minimally extracted. All specific changes in lipid classes measured with these metabolomic-oriented methods are speculatory and must be confirmed with targeted methods as we have done for lysophosphatidic acids (LysoPA).

2.8 Targeted lipidomics analysis

The analysis was carried out on a 4000 QTRAP® triple quadrupole mass spectrometer system (AB Sciex, Massachusetts, USA) as previously described[8]. Briefly, RPLC chromatography was

performed on a C18 (Ascentis Express C18, 2.7 µm, 15 cm × 4.6 mm, Supelco Analytical, Pennsylvania, USA) column. The separation was achieved using a Prominence HPLC system (Shimadzu Corporation, Oregon, USA). The oven temperature was retained at 45°C and the auto-sampler at 4°C. The HPLC system ran a linear gradient elution program consisting of A (acetonitrile/water, 60:40 vol/vol) and B (isopropanol/acetonitrile, 90:10, vol/vol). Both A and B contained 10 mM ammonium formate and 0.1% formic acid. The linear gradient was optimized as follows: 0-1.5 min, 32% B; 4 min, 45% B; 5 min, 52% B; 8 min, 58% B; 11 min, 66% B; 14 min, 70% B; 18 min, 75% B; 21–25 min, 97% B; 25–30 min, 32% B. The sample injection volume was 30 µl and the flow rate was set to 260 µl/min.

Lysophosphatidic acids (LysoPA) were detected in negative ion mode via Multiple Reaction Monitoring (MRM) using the product ion 153.0 *m/z* (Q3 mass) as described by Wijesinghe et al[9]. This ion (153.0 *m/z*) corresponds to the cleaved glycerol phosphate group of LysoPA. The mass spectrometry settings were as follows: curtain gas (psi), 26; collision gas, medium; ion spray voltage (V), -4500; temperature (°C), 500.0; ion source gas 1 (psi), 40.0; ion source gas 2 (psi), 30.0; declustering potential (V), -110, entrance potential (V), -10; collision energy (V), -30; collision cell exit potential (V), -20; and dwell time (ms), 100.

2.9 Data processing

For the non-targeted metabolomics, MassLynx4.1 workstation was used to acquire the data from UPLC-Q-TOF/MS. The raw data were then imported to Progenesis QI v.2.1.5 software (Nonlinear Dynamics, UK) for automatic data processing[10]. Overall, the Progenesis co-detection workflow comprised selecting a reference run, alignment of the runs, peak picking, normalization,

deconvolution, compound identification and statistics. All stages are automated up to the point of compound identification. Retention time (RT)–charge ratio (*m/z*) pairs were used to describe the spectral features. Data processing generated sets of 16,147, 7,056, 9,538 and 4,488 features respectively for RPLC+, RPLC-, HILIC+ and HILIC- ion modes. The repeated injection of QC samples throughout the entire analytical runs was used to ensure the technical reproducibility of UPLC-QTOF/MS. Features were considered reproducible if the coefficient of variation (CV) among QC samples was <30%, as suggested in the guidelines[11,12]. Those features which failed to meet this condition were removed from subsequent data processing. Subsequently, the data set was reduced to 1479, 1483, 980 and 427 features satisfying CV<30% in RPLC+, RPLC-, HILIC+ and HILIC- ion modes, respectively. In the filtered data set, the QC samples are clustered together in the PCA plot representing large homogeneity while experimental samples are clearly separated from the QC samples validating the data quality in non-targeted metabolic profiling (Supplemental Figure.S4). The built-in Metascope search engine of Progenesis was used to identify compounds using the in-house human database. At the end of this workflow, Progenesis QI produced a data matrix containing 583 putatively annotated metabolites and lipids (CV<30%) and their ion abundance (Supplemental Table.S9) which were considered for further statistical analysis. In addition to this ~600 identified metabolites and lipids profiled in the tissue using non-targeted LC-MS, there were approximately 3,700 reproducible unknown peaks, which remain unannotated even after the database search. A summary of the metabolomics workflow is depicted in Figure.1A.

For the targeted lipidomics, data were collected utilizing Analyst® software 1.6 (AB Sciex) from Q-Q-Q/MS. MultiQuant® software 2.1 (AB Sciex) was used to compare peak areas of different

LysoPA species with the internal standard (17:0 LysoPA). Relative amounts of each LysoPA species were then calculated based upon the amount of internal standard added. Results are presented as the amount of LysoPA (ng) detected per mg of AV tissue extracted or the amount of LysoPA (ng) detected per ml of blood plasma. Supplemental Figure S5 provides the sensitivity limits and relative ionization intensities of LysoPA species. Supplemental Table S14 provides the mass spectrometry conditions of quantifiable LysoPA species.

2.10 Tentative identification of metabolites and lipids

At this stage, Progenesis has deconvoluted and quantified the compound ions. Progenesis 'MetaScope' is a built-in search tool used for the identification of compounds. The freely available human metabolite data set was downloaded (v4.0) from HMDB (Human Metabolome Database) database (http://www.hmdb.ca/). It contained 114,100 metabolite entries whose status varied from 'quantified' to 'predicted' in humans. To ensure the confidence in compound identification, the database search was limited to only those compounds from HMDB which are either previously quantified and/or detected in humans. For this purpose, an in-house fragment database was built with the help of Progenesis SDF Studio (Nonlinear Dynamics, UK) software. This in-house human database contained 8,971 entries in SDF (structure-data file) format. Progenesis MetaScope allows us to search for compound identifications based not only on compound properties such as neutral mass, isotopic distribution and retention time, but also on the fragmentation patterns produced by compounds in our experiment. To make definitive identifications, the experimental fragmentation data was compared to theoretical fragmentation patterns of a library of compounds, which are stored in the in-house fragment database. The

theoretical fragmentation patterns are generated by the simulated breaking of bonds in the structures of possible identifications[13] using the 'MetFrag' algorithm in Progenesis. From our list of 72 differential metabolites and lipids, 23 compounds were validated in this way. Compound identifications were searched for with a mass tolerance of ± 10 ppm. Those identifications confirmed with theoretical fragmentation scores were marked using a '*" symbol in the Supplemental Table S7.

2.11 Statistics

Statistical analysis was performed using SPSS v24 (IBM Corporation, Armonk, NY, USA) software. Values are presented as mean ± standard deviation (SD), median (25^{th}, 75^{th} percentiles), or count (percentage) as applicable unless otherwise specified. The Kolmogorov-Smirnov test was used to test the normality assumption of data distribution. The Chi-square test was used for categorical variables, while Kruskal-Wallis test or one-way analysis of variance (ANOVA) was used for continuous variables to assess for statistical significance between sample groups as applicable based on data distribution with Tukey's post-hoc test for multiple pairwise comparisons. Tukey's test is only used if the overall ANOVA test is significant. For comparing two groups, the Chi-square test was used for categorical variables, while Mann-Whitney U test or t-test was used for continuous variables as applicable based on data distribution. Relationships between different continuous variables were calculated using Pearson's (r_p) or Spearman's (r_s) rank correlation coefficient using SPSS v24 or R statistical software v3.5.2. Clustered heatmap analysis and metabolomics pathway analysis (MetPA) were conducted using MetaboAnalyst v4.0 software, an open source R-package for metabolomics data analysis[14] . The Cytoscape software package v3.7.1

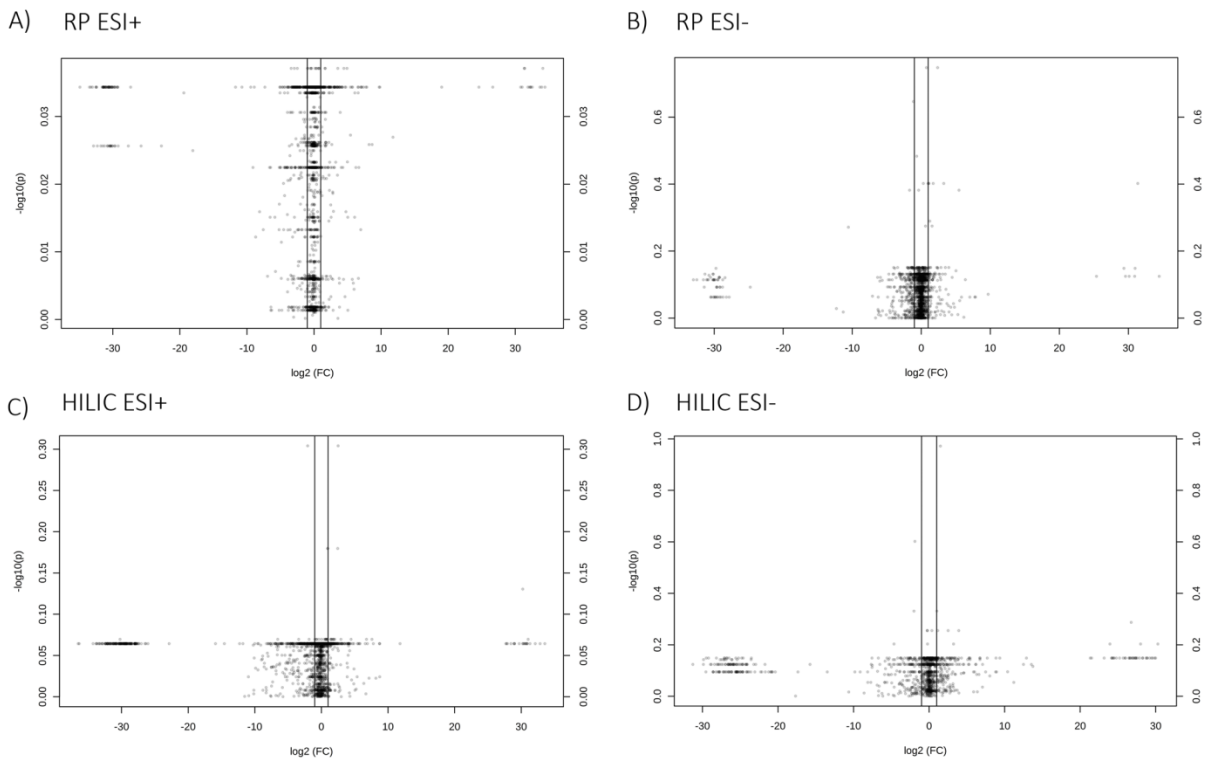
(National Institute of General Medical Sciences, Maryland) was used to plot the correlation network. A 2-sided *p<0.05* was regarded as statistically significant.

2.12 Multiple linear regression analysis

In order to determine the relationship between CAVS severity and the known clinical parameters of CAVS (age, gender, LDL level, HDL level, Triglycerides level, history of smoking, hypertension and presence of a bicuspid valve) along with amount of total LysoPA and individual lysophosphatidic acids, a standard multiple linear regression analysis was performed. Regression analysis was performed using SPSS v24 (IBM Corporation, Armonk, NY, USA) software employing the 'Enter' method of model selection using an alpha level of 0.05 to enter the model and alpha level of 0.10 to remain in the model.

When mean pressure gradient (MPG) was predicted, it was found that total LPA as well as individual lysophosphatidic acids namely 16:0 LysoPA, 18:0 LysoPA, 18:2 LysoPA and 20:4 LysoPA were significant predictors. When aortic valve area (AVA) was predicted, it was found that total LPA as well as individual lysophosphatidic acids namely 16:0 LysoPA, 18:0 LysoPA, 18:2 LysoPA and 22:6 LysoPA were significant predictors. 18:1 LysoPA was not a significant predictor in both models. This shows that that total LPA is an independent predictor of CAVS severity irrespective of other clinical variables. Adjusted regression (Beta) coefficient, standard error and p-value are given in the below table.

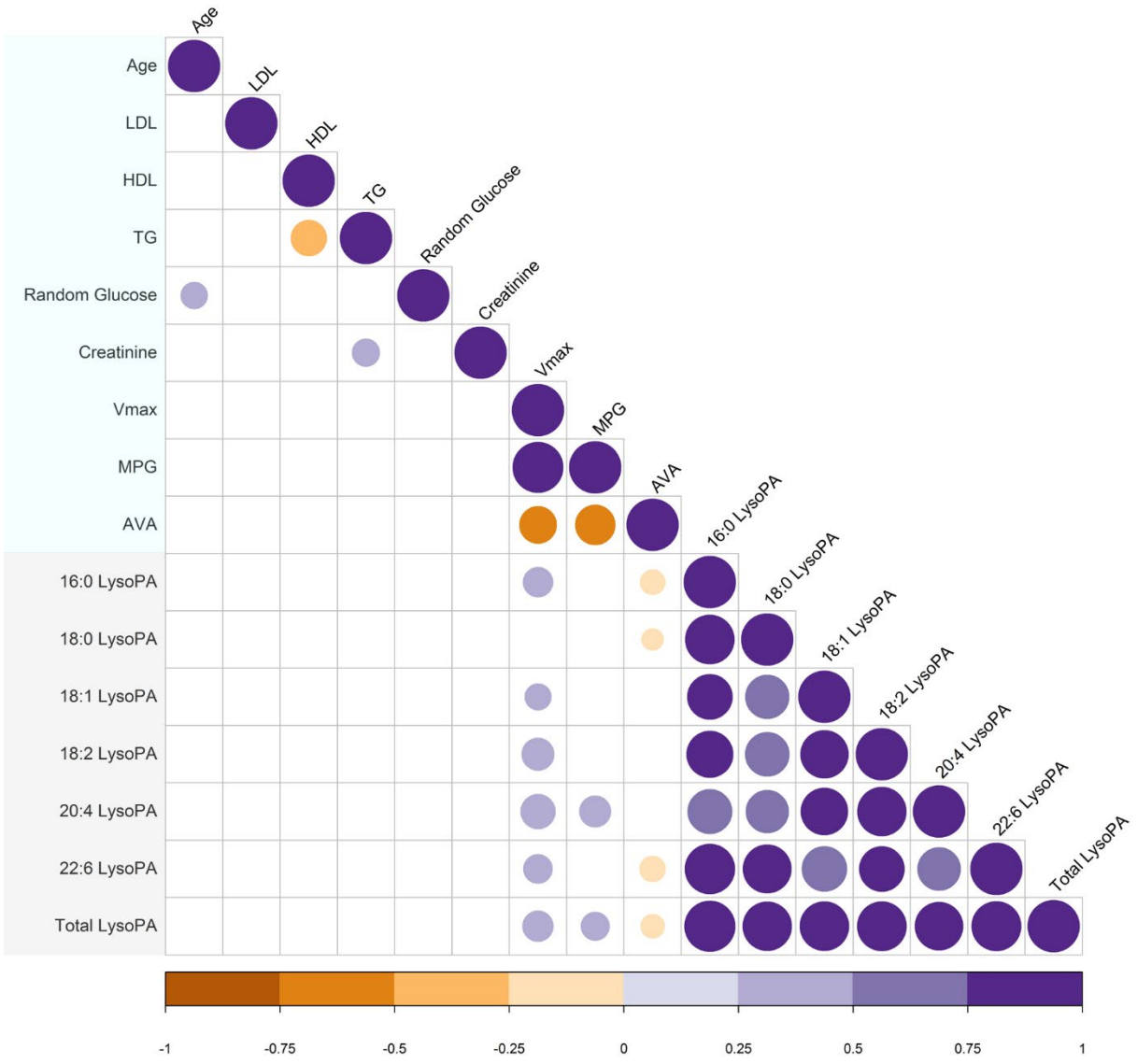
Supplemental Figure S1: Volcano plot comparing bicuspid (N=29) vs. tricuspid (N=73) aortic valve stenosis



The volcano depicts no metabolomics diversity between bicuspid and tricuspid aortic valve stenosis using untargeted metabolic profiling (Fold change threshold: 2, p-value threshold: < 0.05 (FDR adjusted), features with CV<30% in QC samples).

Abbreviations: RP ESI+, Reversed-phase liquid chromatography separation in ESI positive mode; RP ESI-, Reversed-phase liquid chromatography separation in ESI negative mode; HILIC ESI+, Hydrophilic interaction liquid chromatography separation in electrospray ionization (ESI) positive mode; HILIC ESI-, Hydrophilic interaction liquid chromatography separation in ESI negative mode.

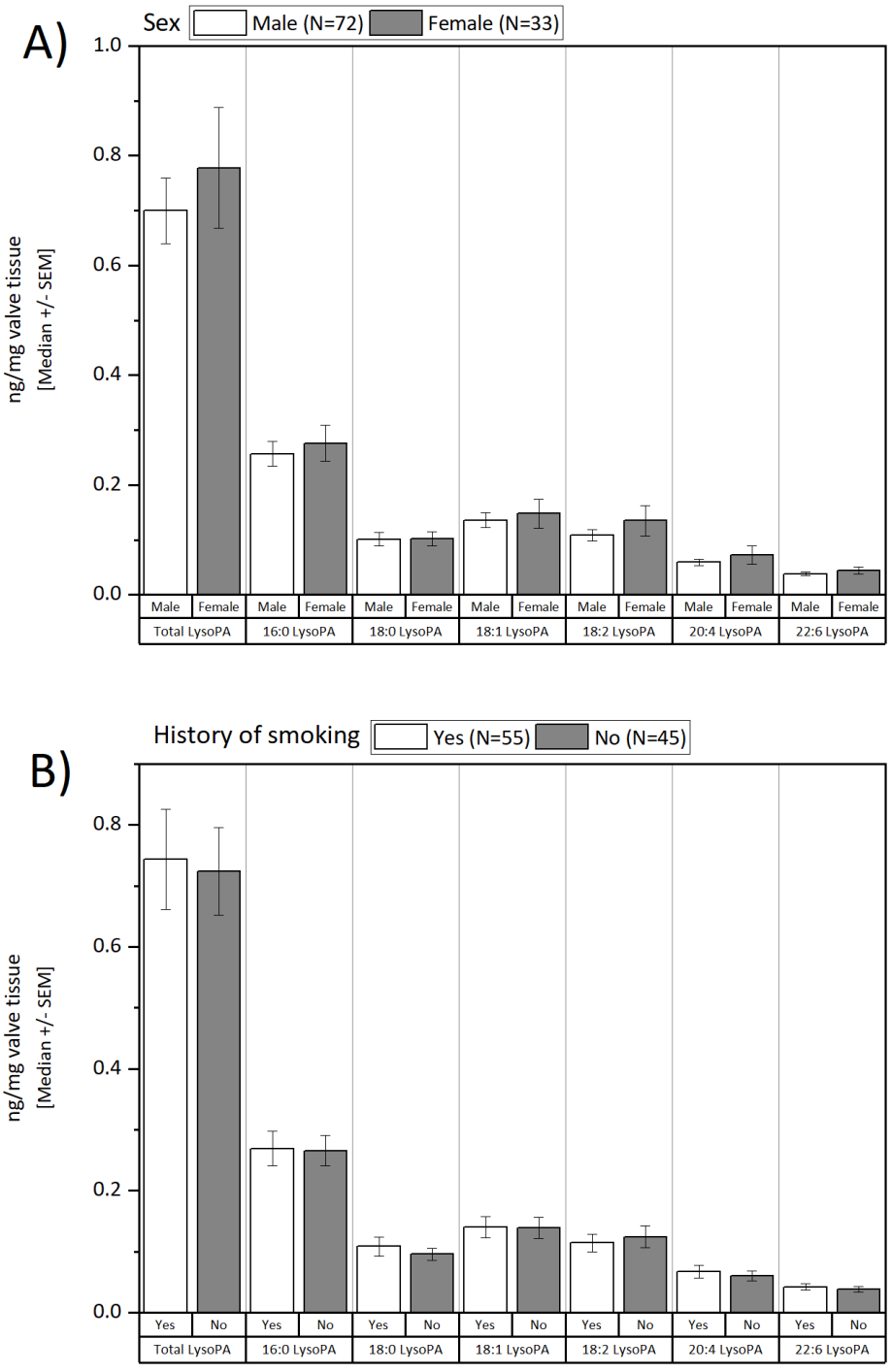
Supplemental Figure S2: Correlation between clinical parameters and lysophosphatidic acids

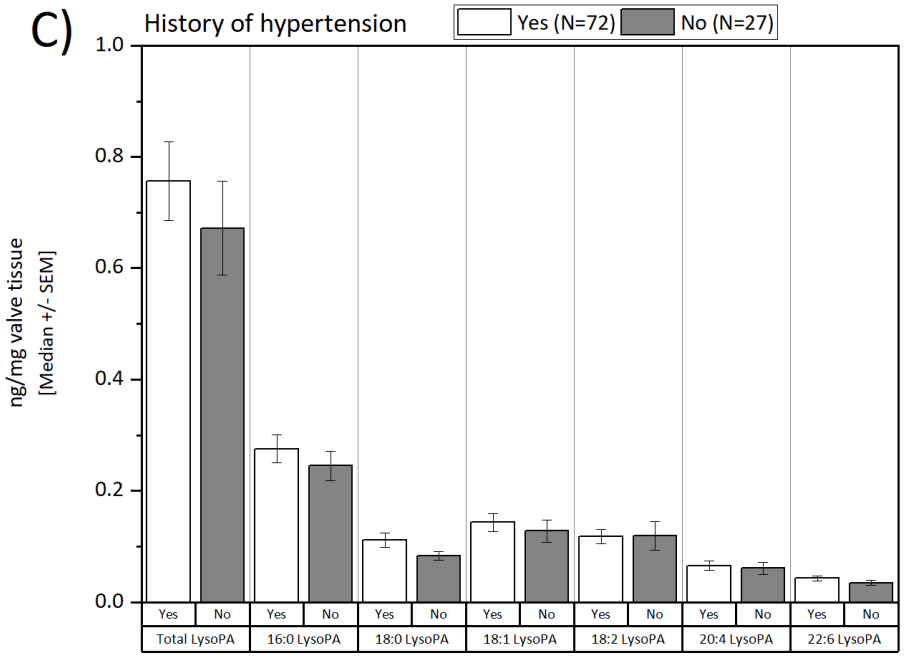


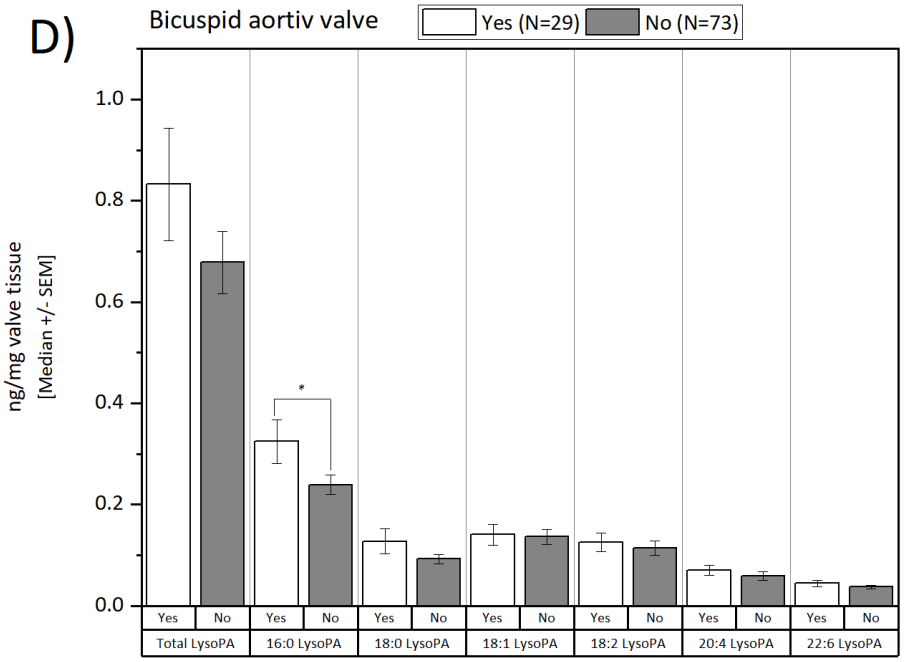
Lysophosphatidic acids (quantified via targeted analysis) were correlated with clinical parameters. Positive correlations are displayed in purple and negative correlations in orange color. Color intensity and the size of the circle are proportional to the correlation coefficients (Spearman correlation, r_s). In this correlogram, correlations with p-value > 0.05 are considered insignificant and are left blank.

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, Triglycerides; Vmax, peak aortic jet velocity; MPG, mean pressure gradient; and AVA, aortic valve area.

Supplemental Figure S3: Important risk factors for CAVS and lysophosphatidic acids



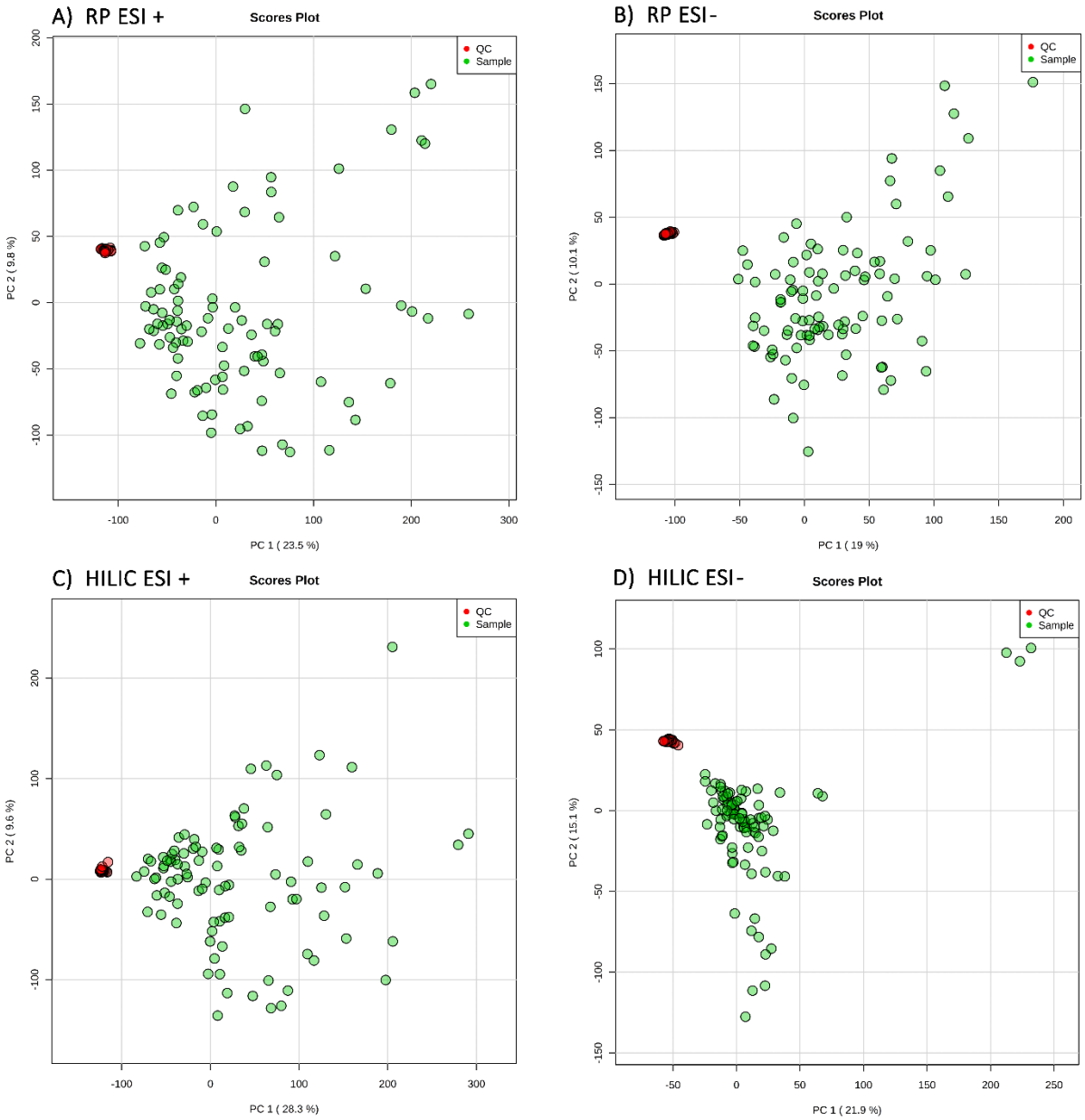




Based on independent t-test, sex difference, smoking habit, prevalence of hypertension and bicuspid aortic valve disease in patients with AS has no significant ($p<0.05$) association with total lysophosphatidic acid amount.

indicates the significance ($p=0.041$*) after Student's t-test and 'N' denotes the number of samples in each category.

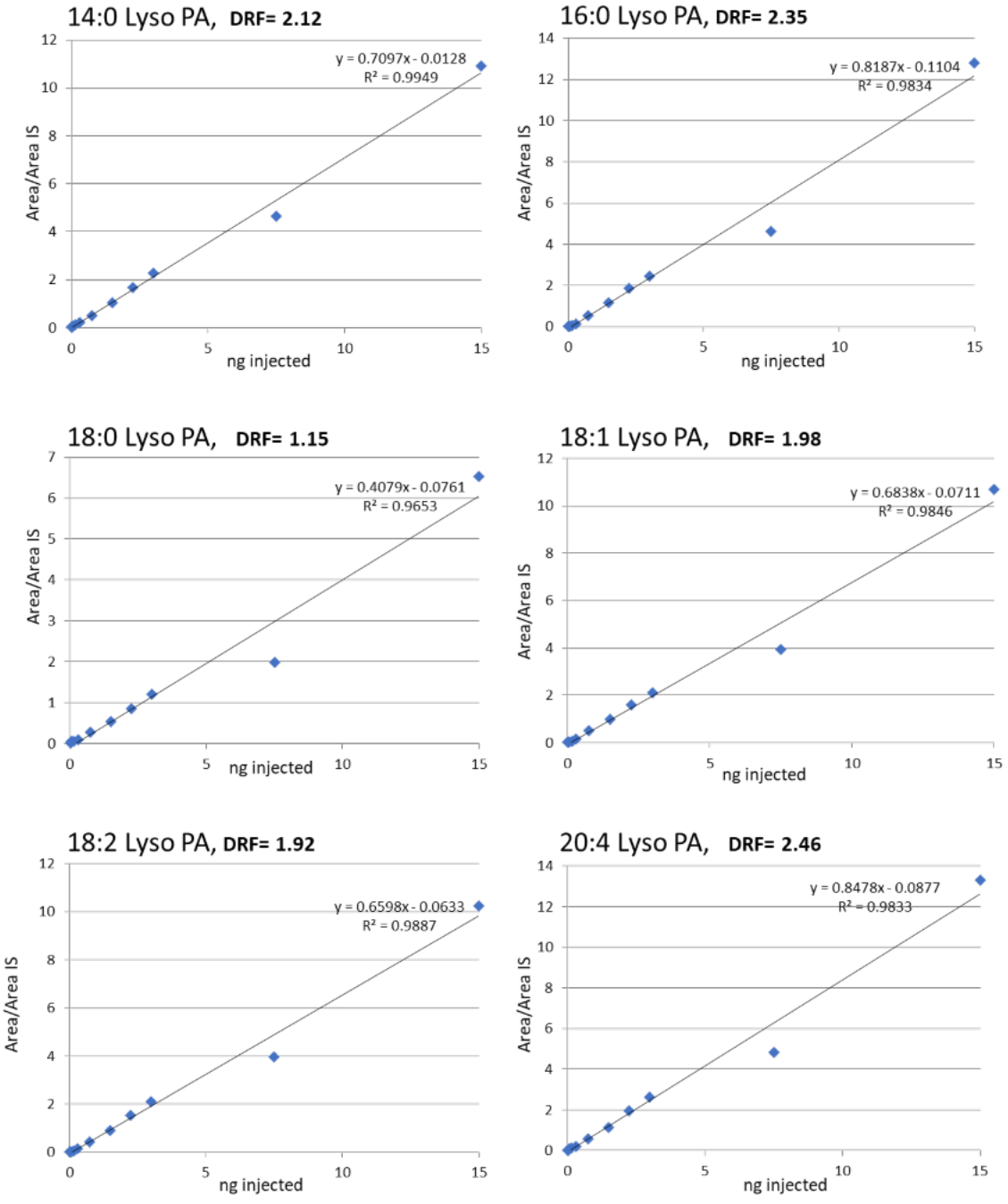
Supplemental Figure S4: Principal component analysis (PCA) score plot



The PCA plot shows that quality control (QC) samples are tightly clustered validating the data quality in untargeted metabolic profiling.

Abbreviations: RP ESI+, Reversed-phase liquid chromatography separation in ESI positive mode; RP ESI-, Reversed-phase liquid chromatography separation in ESI negative mode; HILIC ESI+, Hydrophilic interaction liquid chromatography separation in electrospray ionization (ESI) positive mode; HILIC ESI-, Hydrophilic interaction liquid chromatography separation in ESI negative mode.

Supplemental Figure S5: Dose response factor assessment of commercially available lysophosphatidic acids (LysoPA species) compared to an internal standard (17:0 LysoPA) injected concurrently**.**



To investigate the sensitivity limits and relative ionization intensities of LysoPA species, a standard concentration curve of mixture of 14:0 LysoPA, 16:0 LysoPA, 18:0 LysoPA, 18:1 LysoPA and 20:4 LysoPA was analyzed in relation to 17:0 LysoPA to determine the dose response factor (DRF), and the signal response was measured as the area under the peak. Given the lack of commercially available standard for 22:6 LysoPA, the response factor was based on response factor for 20:4 LysoPA. The relative intensity compared to 17:0 LysoPA was accounted for in our concentration calculations. The method of detection for LPA species shows a linear response in the range from 15 femtogram to 15 nanogram for LPA standards.

Supplemental Table S2: Clinical demographics for subgroup comparison based on aortic valve area (AVA)

Parameter	**Study Population (n=106)**			
Clinical	**Mild**	**Moderate**	**Severe**	***p-value***
	(N=13)	**(N=19)**	**(N=66)**	
Age, yrs	62 (53,77)	71 (63,80)	70 (64,79)	0.189
Male sex, n (%)	9 (69.2)	15 (78.9)	42 (63.6)	0.450
Height, cm	171 (156,179)	171 (168,175)	170 (163,177)	0.877
Weight, kg	78 (51,94)	86 (83,97)	84 (74,100)	0.188
Body surface area, m^2	1.8±0.3	2±0.1	2±0.2	0.092
Body mass index, kg/ m^2	25.3±5.1	30±3.8	30.3±6	0.018
History of hypertension, n (%)	8 (61.5)	16 (84.2)	46 (69.7)	0.644
Smoking History, n (%)	3 (23.1)	9 (47.4)	40 (60.6)	0.199
Current	0	1 (5.2)	9 (13.6)	
Previous	3 (23)	8 (42.1)	29 (43.9)	
Never	7 (53)	7 (36.8)	26 (39.3)	
Medication, n (%)				
Antihypertensive treatment	8 (61.5)	10 (52.6)	42 (63.6)	0.717
ACE inhibitors	5 (38.5)	7 (36.8)	16 (24.2)	0.329
ARBs	1 (7.7)	2 (10.5)	7 (10.6)	0.954
Statins	4 (30.8)	15 (78.9)	36 (54.5)	0.020
Laboratory data				
LDL cholesterol, mmol/l	2.6 (1.3,4.2)	2.2 (1.5,3.0)	2.5 (2.0,3.3)	0.360
HDL cholesterol, mmol/l	1.3 (1.0,1.9)	1.2 (1.0,1.4)	1.3 (1.0,1.6)	0.338
Triglycerides, mmol/l	1.3 (0.9,1.7)	1.3 (1.0,1.7)	1.4 (1.0,1.8)	0.838
Random glucose, mmol/l	6.5 (5.6,8.7)	6.2 (5.3,10.2)	6.4 (5.3,8.0)	0.888
Creatinine, (µmol/l)	80 (65,91)	86 (80,107)	82 (71,103)	0.368
Doppler echocardiographic data				
Bicuspid aortic valve, n (%)	3 (23.1)	6 (31.6)	20 (30.3)	0.816
Aortic valve calcification score, n (%)				
Calcification score = 1	5 (38.4)	1 (5.2)	1 (1.5)	
Calcification score = 2	5 (38.4)	7 (36.8)	13 (19.6)	
Calcification score = 3	2 (15.3)	7 (36.8)	30 (45.4)	
Calcification score = 4	0	2 (10.5)	15 (22.7)	
Calcification score = 5	0	2 (10.5)	5 (7.5)	
Peak aortic jet velocity, m/s	2.5 (1.8,3.6)	4.1 (3.7,4.5)	4.1 (3.8,4.9)	<0.001
Peak pressure gradient, mm Hg	19 (13,46)	64 (50,79)	70 (57,89)	<0.001
Mean pressure gradient, mm Hg	10 (6,23)	38 (30,46)	45 (34,59)	<0.001
Aortic valve area, cm^2	1.8 (1.6,2.4)	1.0 (1.0,1.2)	0.8 (0.7,0.9)	<0.001
Indexed aortic valve area, cm^2/m^2	0.9 (0.7,1.2)	0.5 (0.5,0.6)	0.4 (0.3,0.5)	<0.001
Left ventricular mass index, g/m^2	173 (139,192)	124 (85,149)	114 (101,134)	0.01
Left ventricular ejection fraction	55 (45,60)	60 (60,60)	60 (59,60)	0.058

Values are reported as mean ± standard deviation (SD), median (25th, 75th percentiles), or count (percentage) as applicable. The Chi-square test was used for categorical variables, while Kruskal-Wallis test or one-way analysis of variance (ANOVA) was used for continuous variables to assess for statistical significance between sample groups as applicable based on data distribution. Abbreviations: ACE, angiotensin-converting-enzyme; ARBs, angiotensin II receptor blockers; HDL, high-density lipoprotein; LDL, low-density lipoprotein

Supplemental Table S3: Clinical demographics for subgroup comparison based on aortic valve calcification score (C-Score)

Parameter	**Study Population (n=106)**			
Clinical	**Low**	**Medium**	**High**	***p-value***
	(N=38)	**(N=39)**	**(N=25)**	
Age, yrs	67.5 (60,76)	70 (65,80)	68 (63,78)	0.215
Male sex, n (%)	26 (68.4)	27 (69.2)	18 (72)	0.953
Height, cm	169.2±9.8	170.6±7	171.3±8.1	0.616
Weight, kg	84 (72,95)	86 (78,100)	82 (70,100)	0.415
Body surface area, m^2	2.0 (1.8,2.0)	2.0 (1.9,2.1)	2.0 (1.8,2.1)	0.291
Body mass index, kg/m^2	28.1±5	30.7±5.6	29.2±6.3	0.133
History of hypertension, n (%)	24 (63.2)	26 (66.7)	21 (84)	0.388
Smoking History, n (%)	17 (44.7)	21 (53.8)	15 (60)	0.481
Current	2 (5.2)	4 (10.2)	4 (16)	
Previous	15 (39.4)	17 (43.5)	11 (44)	
Never	19 (50)	16 (41)	9 (36)	
Medication, n (%)				
Antihypertensive treatment	22 (57.9)	25 (64.1)	15 (60)	0.830
ACE inhibitors	16 (42.1)	8 (20.5)	7 (28)	0.117
ARBs	5 (13.2)	4 (10.3)	2 (8)	0.812
Statins	16 (42.1)	24 (61.5)	16 (64)	0.085
Laboratory data				
LDL cholesterol, mmol/l	2.9±1.3	2.4±0.8	2.5±1.1	0.165
HDL cholesterol, mmol/l	1.3 (1.0,1.6)	1.3 (1.1,1.5)	1.3 (1.0,1.6)	0.951
Triglycerides, mmol/l	1.4 (1.0,1.7)	1.4 (0.9,1.7)	1.3 (0.8,1.6)	0.956
Random glucose, mmol/l	6.4 (5.5,8.4)	6.7 (5.3,8.9)	5.7 (5.2,7.2)	0.554
Creatinine, (µmol/l)	80 (62,94)	86 (73,110)	83 (71,99)	0.140
Doppler echocardiographic data				
Bicuspid aortic valve, n (%)	9(23.7)	11(28.2)	9(36)	0.495
Aortic valve calcification score, n (%)				
Calcification score = 1	10 (26.3)	0	0	
Calcification score = 2	28 (73.6)	0	0	
Calcification score = 3	0	39 (100)	0	
Calcification score = 4	0	0	18 (72)	
Calcification score = 5	0	0	7 (28)	

Peak aortic jet velocity, m/s	3.6 (2.6,4.2)	4.1 (3.9,4.5)	4.2 (3.8,5.0)	0.001
Peak pressure gradient, mm Hg	57 (31,70)	67 (56,81)	71 (60,101)	0.001
Mean pressure gradient, mm Hg	30 (14,45)	40 (35,53)	46 (34,60)	0.002
Aortic valve area, cm^2	0.9 (0.8,1.6)	0.8 (0.7,1.0)	0.9 (0.6,1.0)	0.013
Indexed aortic valve area, cm^2/m^2	0.5 (0.4,0.7)	0.4 (0.3,0.5)	0.4 (0.3,0.5)	0.004
Left ventricular mass index, g/m^2	143 (124,172)	111 (97,141)	110 (97,130)	0.004
Left ventricular ejection fraction	60 (60,60)	60 (60,60)	60 (60,60)	0.394

Values are reported as mean ± standard deviation (SD), median (25^{th}, 75^{th} percentiles), or count (percentage) as applicable. The Chi-square test was used for categorical variables, while Kruskal-Wallis test or one-way analysis of variance (ANOVA) was used for continuous variables to assess for statistical significance between sample groups as applicable based on data distribution. Those subjects with a score of '1' and '2' formed the 'low' calcification group, those with a score of '3' formed the 'medium' group and those with a score of '4' and '5' formed the 'high' calcification group. Abbreviations: ACE, angiotensin-converting-enzyme; ARBs, angiotensin II receptor blockers; HDL, high-density lipoprotein; LDL, low-density lipoprotein

Supplemental Table S4: Significant (*p<0.01*) compounds identified by grading AS severity as mild, moderate and severe based on mean pressure gradient (MPG)

Sl.No	Metabolites/Lipids	*m/z*	RT	Anova (p)	q Value	CV%	Max Fold Change	Highest Mean	Lowest Mean
1	5,6-Epoxy-eicosatrienoic acid	319.2275	9.17	8.13E-07	3.82E-04	12.31	2.689391	Moderate	Mild
2	Phenylacetaldehyde	121.0647	1.15	9.52E-07	2.44E-04	7.04	2.864115	Mild	Severe
3	Ketoleucine	95.049	1.14	1.43E-06	3.20E-04	7.91	2.999719	Mild	Severe
4	Linalyl oxide	339.2524	10.72	1.64E-06	7.04E-04	24.82	5.490511	Mild	Severe
5	2,4-Dimethylfuran	119.0487	1.14	3.91E-06	6.09E-04	9.52	2.7907	Mild	Severe
6	Tryptamine	178.1349	4.37	6.50E-06	9.50E-04	23.73	33.09287	Mild	Moderate
7	Uracil	77.0124	1.15	1.18E-05	0.001	25.13	4.048203	Mild	Severe
8	Enterodiol	283.136	7.52	7.37E-05	0.009	12.02	3.907599	Mild	Moderate
9	MG(20:1)	385.333	15.16	8.13E-05	0.006	15.28	2.222748	Moderate	Mild
10	Angiotensin II	1063.569	12.01	8.38E-05	0.007	22.98	4.714409	Severe	Mild
11	Pyridinoline	393.1761	6.68	1.31E-04	0.008	21.9	3.070618	Mild	Moderate
12	LysoPE(18:2)	478.2883	7.78	2.90E-04	0.014	14.52	2.4183	Mild	Severe
13	Deoxypyridinoline	430.2334	4.18	2.99E-04	0.015	25.41	2.784497	Mild	Moderate
14	Indoleacrylic acid	188.0712	5.65	4.99E-04	0.023	16.79	2.799811	Severe	Mild
15	Cholic acid	447.2544	13.82	5.52E-04	0.022	21.44	3.856675	Severe	Mild
16	MG(24:1)	421.3675	12.24	6.53E-04	0.042	14.03	2.041157	Moderate	Mild
17	PC(22:6_p-18:0)	835.6387	12.24	7.01E-04	0.031	24.18	10.38359	Severe	Mild
18	Palmitic amide	256.2631	1.14	7.91E-04	0.033	25.29	3.011334	Moderate	Mild

19	MG(22:5)	387.2899	1.15	0.002	0.057	15.86	2.197309	Mild	Moderate
20	CMPF	263.0885	1.14	0.002	0.065	11.64	2.992936	Mild	Moderate
21	15-Hydroxyeicosatrienoate	321.2421	9.16	0.002	0.082	22.45	2.366902	Moderate	Mild
22	PC(p-18:1_22:1)	843.6963	5.47	0.002	0.047	19.86	2.802354	Severe	Mild
23	5-AMMU	179.0566	3.01	0.004	0.123	20.23	4.743777	Moderate	Mild
24	PC(18:0_22:0)	810.6659	5.33	0.004	0.073	25.23	5.42359	Severe	Mild
25	Tetrahydrocortisone	387.2157	1.14	0.006	0.129	26.39	4.816644	Mild	Severe
26	Uroporphyrin III	831.2429	12.24	0.006	0.131	24.87	7.444387	Severe	Mild
27	N-Methyl-lysine	319.2322	12.11	0.008	0.17	7.34	4.210005	Mild	Moderate
28	PI(16:0_18:0)	877.5197	5.76	0.008	0.102	13.55	5.392857	Severe	Mild

Supplemental Table S5: Significant (*p<0.01*) compounds identified by grading AS severity as mild, moderate and severe based on aortic valve area (AVA).

Sl.No	Metabolites/Lipids	*m/z*	RT	Anova (p)	q Value	CV%	Max Fold Change	Highest Mean	Lowest Mean
1	Phenylacetaldehyde	121.0647	1.15	3.76E-09	4.37E-06	7.04	3.11903	Mild	Moderate
2	Ketoleucine	95.049	1.14	1.19E-07	3.13E-05	7.91	3.313858	Mild	Moderate
3	2,4-Dimethylfuran	119.0487	1.14	1.84E-07	4.49E-05	9.52	2.936738	Mild	Moderate
4	Dodecanoylcarnitine	342.2646	9.73	3.75E-07	3.80E-04	14.28	2.823645	Moderate	Mild
5	5,6-Epoxy-eicosatrienoic acid	319.2275	9.17	2.71E-06	0.001	12.31	6.410601	Severe	Mild
6	15-Hydroxyeicosatrienoate	321.2421	9.16	4.78E-06	0.002	22.45	10.78818	Severe	Mild
7	Palmitic amide	256.2631	1.14	5.57E-06	4.98E-04	25.29	5.675308	Severe	Mild
8	Cholic acid	447.2544	13.82	2.48E-05	0.002	21.44	38.18958	Severe	Mild
9	Deoxypyridinoline	430.2334	4.18	3.12E-05	0.002	25.41	2.822804	Mild	Severe

10	LysoPE(18:2)	478.2883	7.78	3.48E-05	0.002	14.52	2.918989	Mild	Severe
11	Angiotensin II	1063.569	12.01	6.41E-05	0.003	22.98	8.823877	Severe	Mild
12	Linalyl oxide	339.2524	10.72	9.03E-05	0.008	24.82	4.452754	Mild	Severe
13	Pyridinoline	393.1761	6.68	1.26E-04	0.006	21.9	3.347183	Mild	Moderate
14	α-CEHC	277.1431	8.21	1.47E-04	0.01	18.21	2.551602	Mild	Severe
15	PC(p-18:1_22:1)	843.6963	5.47	1.64E-04	0.007	19.86	4.643067	Severe	Mild
16	MG(24:1)	421.3675	12.24	1.92E-04	0.011	14.03	17.15548	Severe	Mild
17	N-Methyl-lysine	319.2322	12.11	2.41E-04	0.012	7.34	5.609939	Mild	Moderate
18	LysoPE(22:6)	526.2909	7.83	2.54E-04	0.009	10.8	2.656826	Mild	Severe
19	Enterodiol	283.136	7.52	3.36E-04	0.014	12.02	3.719567	Mild	Severe
20	LysoPA(16:0)	391.2248	13.14	3.37E-04	0.014	13.48	2.728048	Severe	Mild
21	LysoPC(22:0)	562.4207	11.81	6.00E-04	0.017	17.42	2.517117	Severe	Mild
22	Uracil	77.0124	1.15	6.97E-04	0.015	25.13	4.689703	Mild	Moderate
23	APGPR Enterostatin	477.261	9.34	7.39E-04	0.021	24.05	2.603521	Mild	Severe
24	Tryptamine	178.1349	4.37	7.45E-04	0.016	23.73	36.07912	Mild	Severe
25	MG(20:1)	385.333	15.16	7.54E-04	0.02	20.61	4.684099	Severe	Mild
26	Glycocholic acid	464.3014	5.7	7.79E-04	0.021	26.94	9.442069	Moderate	Mild
27	MG(15:0)	299.2602	1.08	8.44E-04	0.017	19.22	2.025486	Severe	Mild
28	13-Hydroxyoctadecadienoic acid	295.2283	8.57	0.001	0.026	11.66	3.074781	Severe	Mild
29	PE(16:0_18:0)	419.3516	14.03	0.001	0.026	20.94	15.55458	Moderate	Mild
30	9,10-Epoxyoctadecenoic acid	295.2276	8.7	0.001	0.027	14.85	4.550987	Severe	Mild
31	5-AMMU	179.0566	3.01	0.001	0.028	20.23	6.168584	Moderate	Mild
32	Docosatrienoic acid	333.2789	12.97	0.001	0.029	22.54	2.449404	Moderate	Mild
33	2-Phenylethanol	105.07	1.14	0.001	0.02	26.46	2.09323	Mild	Severe
34	2-Methylacetophenone	440.2679	0.84	0.001	0.02	28.45	3.3314	Moderate	Mild
35	PC(22:6_p-18:0)	835.6387	12.24	0.001	0.023	24.18	14.09519	Moderate	Mild
36	Glycoursodeoxycholic acid	448.3071	6.73	0.002	0.032	13.88	4.473352	Moderate	Mild
37	Cholenic acid	355.2638	11.89	0.002	0.034	19.17	2.288637	Moderate	Mild

38	MG(20:3)	417.24	13.52	0.002	0.037	24.97	2.058725	Severe	Mild
39	MG(20:5)	394.295	5.66	0.002	0.03	17.83	2.369179	Moderate	Mild
40	CMPF	263.0885	1.14	0.002	0.029	11.64	2.982952	Mild	Moderate
41	Tetrahydrocortisone	387.2157	1.14	0.002	0.025	26.39	6.167196	Mild	Moderate
42	Uroporphyrin III	831.2429	12.24	0.002	0.03	24.87	7.068897	Moderate	Mild
43	LysoPA(18:2)	399.2274	12.48	0.003	0.048	23.5	3.958626	Severe	Mild
44	Nutriacholic acid	411.2505	11.45	0.003	0.041	15.4	2.61748	Moderate	Mild
45	SM C16:1	680.5661	1.09	0.003	0.037	23.9	9.593328	Severe	Mild
46	Androsterone sulfate	369.1734	6.58	0.004	0.05	22.28	2.97893	Mild	Severe
47	3A,7α-dihydroxycoprostanate	433.3318	12.17	0.004	0.052	11.07	2.252986	Severe	Mild
48	Linoleoyl ethanolamide	324.2893	1.17	0.004	0.049	15.53	2.816207	Severe	Mild
49	Eicosenoic acid	328.3206	1.17	0.004	0.046	23.33	3.794639	Severe	Mild
50	PC(18:0_22:0)	810.6659	5.33	0.005	0.061	25.23	4.977429	Severe	Mild
51	Uric acid	167.0213	0.9	0.005	0.053	7.69	2.567799	Severe	Mild
52	LysoPE(16:0)	474.2614	9.36	0.005	0.056	15.79	2.851689	Mild	Severe
53	MG(22:5)	387.2899	1.15	0.005	0.05	15.86	2.166352	Mild	Moderate
54	LysoPC(20:0)	534.3909	7.44	0.005	0.05	9.86	2.025302	Severe	Mild
55	12(13)Ep-9-KODE	309.2062	8.06	0.006	0.06	17.48	4.598785	Moderate	Mild
56	DG(16:0_18:3)	613.4747	1.01	0.006	0.06	24.91	19.71766	Moderate	Mild
57	Indoleacrylic acid	188.0712	5.65	0.006	0.06	16.79	3.795498	Moderate	Mild
58	LysoPC (O-18:0)	544.3519	9.93	0.007	0.066	25.61	2.809086	Moderate	Mild
59	Calcidiol	383.3333	1.05	0.007	0.063	23.56	2.617499	Severe	Mild
60	LysoPC(24:0)	590.4546	7.24	0.008	0.067	25.1	2.103318	Severe	Mild
61	MG(20:2)	383.3146	17.36	0.009	0.089	15.28	4.055714	Severe	Mild
62	Vitamin D3	367.3377	1.01	0.009	0.074	24.71	2.098275	Severe	Mild

Supplemental Table S6: Significant (*p<0.01*) compounds identified by grading AS severity as mild, moderate and severe based on echocardiography calcification score (C-Score).

Sl.No	Metabolites/Lipids	*m/z*	RT	Anova (p)	q Value	CV%	Max Fold Change	Highest Mean	Lowest Mean
1	LysoPE(22:6)	526.2909	7.83	4.33E-07	9.90E-04	10.8	2.626279	Mild	Severe
2	PC(p-18:1_22:1)	843.6963	5.47	2.71E-06	0.002	19.86	2.818635	Severe	Mild
3	MG(20:4)	361.2742	5.56	9.54E-05	0.028	11.9	2.662581	Mild	Severe
4	MG(22:6)	385.2736	5.52	2.27E-04	0.044	27.77	2.56416	Mild	Severe
5	5-hydroxyoct-5-enoylglycine	180.1023	7.53	2.66E-04	0.046	23.64	8.594037	Moderate	Mild
6	Cholesterol	425.3172	10.13	4.33E-04	0.032	11.55	2.43956	Mild	Severe
7	MG(22:4)	389.3046	5.5	4.92E-04	0.063	26.51	2.587006	Mild	Severe
8	9,10-Epoxyoctadecenoic acid	295.2276	8.7	8.05E-04	0.041	14.85	2.20761	Severe	Mild
9	MG(24:1)	421.3675	12.24	0.002	0.066	14.03	2.388216	Moderate	Mild
10	Angiotensin II	1063.569	12.01	0.002	0.125	22.98	3.386798	Severe	Mild
11	Deoxypyridinoline	413.2037	11.61	0.003	0.093	20.3	3.305308	Severe	Mild
12	LysoPC(18:3)	518.3264	11.43	0.003	0.094	29.79	3.844012	Severe	Mild
13	MG(22:5)	387.2884	5.55	0.003	0.137	19.79	2.815981	Mild	Severe
14	Cortolone-3-glucuronide	541.2644	4.45	0.004	0.099	16.04	2.413344	Moderate	Mild
15	PI(16:0_18:0)	877.5197	5.76	0.005	0.113	13.55	4.274982	Moderate	Mild
16	Tetrahydroaldosterone-3-glucuronide	539.2488	4.56	0.005	0.109	28.58	2.236596	Severe	Mild
17	DG(16:0_18:3)	613.4747	1.01	0.005	0.187	24.91	6.637334	Severe	Mild
18	Serylproline	201.0872	1.05	0.009	0.149	19.16	2.545037	Mild	Severe
19	Uroporphyrin III	831.2429	12.24	0.01	0.249	24.87	2.33145	Severe	Mild

Supplemental Table S7: Comprehensive list of significant (*p<0.01*) compounds identified by grading AS severity as mild, moderate and severe based on mean pressure gradient (MPG), aortic valve area (AVA) and calcification score (C-Score)

Sl.No	Mode[a]	Metabolites/Lipids	*m/z*	RT	Adducts	Formula	Score[b]	Mass Error (ppm)	Isotope similarity[c]
1*	HILIC +	Indoleacrylic acid*	188.0712	5.65	M+H-H2O, M+H	C11H9NO2	50.4	-1.13	94.61
2	HILIC +	Uracil	77.0124	1.15	M+H-2H2O	C4H4N2O2	35.4	-8.82	86.64
3	HILIC +	Tryptamine	178.1349	4.37	M+NH4	C10H12N2	38	6.56	97.47
4	HILIC +	Ketoleucine	95.049	1.14	M+H-2H2O	C6H10O3	38.3	-1.32	93.14
5*	HILIC +	Tetrahydrocortisone*	387.2157	1.14	M+Na	C21H32O5	35.2	4.09	79.65
6	HILIC +	Phenylacetaldehyde	121.0647	1.15	M+H	C8H8O	38.6	-0.69	93.91
7	HILIC +	PC(22:6_p-18:0)	835.6387	12.24	M+NH4	C48H84NO7P	30.5	7.69	61.08
8	HILIC +	Palmitic amide	256.2631	1.14	M+H	C16H33NO	37.6	-1.50	89.96
9	HILIC +	2,4-Dimethylfuran	119.0487	1.14	M+H-H2O, M+Na	C6H8O	38.9	-1.78	96.46
10*	HILIC +	CMPF*	263.0885	1.14	M+Na	C12H16O5	38.7	-1.89	93.96
11	HILIC +	Uroporphyrin III	831.2429	12.24	M+H	C40H38N4O16	31.8	8.82	68.50
12	HILIC +	Angiotensin II	1063.569	12.01	M+NH4	C50H71N13O12	31.7	0.72	59.40
13	HILIC +	MG(22:5)	387.2899	1.15	M+H-H2O	C25H40O4	41.5	1.21	78.71
14*	HILIC +	MG(15:0)*	299.2602	1.08	M+H-H2O	C18H36O4	35.9	6.87	85.62
15*	HILIC +	Linoleoyl ethanolamide*	324.2893	1.17	M+H-H2O, M+H	C20H37NO2	38.5	-1.11	93.92
16	HILIC +	LysoPC(24:0)	590.4546	7.24	M+H-H2O	C32H66NO7P	37	0.40	85.57
17*	HILIC +	LysoPC(20:0)*	534.3909	7.44	M+H-H2O	C28H58NO7P	47	-1.63	82.98
18*	HILIC +	Calcidiol*	383.3333	1.05	M+H-H2O	C27H44O2	39.9	6.19	80.48
19*	HILIC +	Vitamin D3*	367.3377	1.01	M+H-H2O	C27H44O	54	4.63	94.51
20	HILIC +	MG(20:5)	394.295	5.66	M+NH4	C23H36O4	37.7	-0.62	89.08
21	HILIC +	Eicosenoic acid	328.3206	1.17	M+NH4	C20H38O2	37.5	-1.39	89.41

22	HILIC +	2-Phenylethanol	105.07	1.14	M+H-H2O	C8H10O	38.8	1.40	95.84
23	HILIC +	SM C16:1	680.5661	1.09	M+H-2H2O	C40H80N2O6P+	34.4	6.41	79.03
24	HILIC +	2-Methylacetophenone	440.2679	0.84	M+H, M+NH4, M+Na	C20H38O7S	37.3	0.53	87.21
25*	HILIC +	DG(16:0_18:3)*	613.4747	1.01	M+Na	C37H66O5	41	-9.46	81.72
26	HILIC +	MG(20:4)	361.2742	5.56	M+H-H2O	C23H38O4	38.1	1.39	92.33
27	HILIC +	MG(22:6)	385.2736	5.52	M+H-H2O, M+NH4	C25H38O4	37.4	-0.28	87.52
28*	HILIC +	5-hydroxyoct-5-enoylglycine*	180.1023	7.53	M+H-2H2O	C10H17NO4	57.7	1.64	93.17
29	HILIC +	MG(22:4)	389.3046	5.5	M+H-H2O	C25H42O4	37	-1.13	86.52
30	RP -	N-Methyl-lysine	319.2322	12.11	2M-H	C7H16N2O2	36	-9.10	89.85
31	RP -	5,6-Epoxy-eicosatrienoic acid	319.2275	9.17	M-H	C20H32O3	38.4	-1.20	93.36
32*	RP -	5-AMMU*	179.0566	3.01	M-H2O-H	C7H10N4O3	55.9	-4.51	94.47
33	RP -	15-Hydroxyeicosatrienoate	321.2421	9.16	M-H2O-H, M-H	C20H34O3	36	-4.68	85.25
34	RP -	Enterodiol	283.136	7.52	M-H2O-H	C18H22O4	36	6.87	87.73
35	RP -	Linalyl oxide	339.2524	10.72	2M-H	C10H18O2	37.4	-4.85	92.63
36	RP -	MG(24:1)	421.3675	12.24	M-H2O-H	C27H52O4	36.2	-2.75	84.26
37	RP +	PI(16:0_18:0)	877.5197	5.76	M+K	C43H83O13P	32.9	-0.66	65.23
38	RP +	LysoPE(18:2)	478.2883	7.78	M+H, M+Na	C23H44NO7P	35.6	-9.48	88.16
39*	RP +	Cholic acid*	447.2544	13.82	M+K	C24H40O5	34.3	8.98	81.26
40	RP +	Pyridinoline	393.1761	6.68	M+H-2H2O	C18H28N4O8	36.7	-1.82	85.49
41	RP +	PC(18:0_22:0)	810.6659	5.33	M+H-2H2O	C48H96NO8P	30.1	-9.01	60.27
42	RP +	MG(20:1)	385.333	15.16	M+H	C23H44O4	36.8	4.65	89.53
43	RP +	Deoxypyridinoline	430.2334	4.18	M+NH4	C18H28N4O7	36	9.26	89.98
44	RP +	PC(p-18:1_22:1)	843.6963	5.47	M+NH4	C48H92NO7P	32	1.67	62.14
45	RP -	LysoPA(16:0)	391.2248	13.14	M-H2O-H	C19H39O7P	37.5	-1.82	89.45
46*	RP -	Glycocholic acid*	464.3014	5.7	M-H2O-H	C26H43NO6	55.5	-2.59	87.60

47*	RP -	Docosatrienoic acid*	333.2789	12.97	M-H	C22H38O2	40.5	-2.89	87.87
48*	RP -	Glycoursodeoxycholic acid*	448.3071	6.73	M-H	C26H43NO5	58.6	0.53	95.10
49	RP -	Cholenic acid	355.2638	11.89	M-H2O-H	C24H38O3	37	-1.31	86.65
50	RP -	MG(20:3)	417.24	13.52	M+K-2H	C23H40O4	35.9	-3.30	83.41
51	RP -	Nutriacholic acid	411.2505	11.45	M+Na-2H	C24H38O4	37.9	-3.11	93.17
52	RP -	Uric acid	167.0213	0.9	M-H	C5H4N4O3	39.5	1.28	98.88
53*	RP -	LysoPE(16:0)*	474.2614	9.36	M+Na-2H	C21H44NO7P	57.2	2.62	92.07
54*	RP -	12(13)Ep-9-KODE*	309.2062	8.06	M-H	C18H30O4	38.6	-3.05	91.26
55*	RP -	LysoPC(O-18:0)*	544.3519	9.93	M+Cl	C26H56NO6P	51.1	-3.90	81.56
56	RP -	Dodecanoylcarnitine	342.2646	9.73	M-H	C19H37NO4	37.9	-1.09	90.88
57	RP -	α-CEHC	277.1431	8.21	M-H	C16H22O4	37.3	-5.10	92.20
58*	RP -	APGPR Enterostatin*	477.261	9.34	M-H2O-H	C21H36N8O6	39.1	6.13	87.77
59*	RP -	13-Hydroxyoctadecadienoic acid*	295.2283	8.57	M-H2O-H, M-H	C18H32O3	58.7	0.29	95.33
60	RP -	PE(16:0_18:0)	419.3516	14.03	M-H	C27H48O3	36.2	-3.60	85.02
61	RP -	Androsterone sulfate	369.1734	6.58	M-H	C19H30O5S	36.8	-1.93	86.25
62	RP -	3A,7α-dihydroxycoprostanate	433.3318	12.17	M-H	C27H46O4	38	-1.25	91.66
63*	RP -	9,10-Epoxyoctadecenoic acid*	295.2276	8.7	M-H2O-H, M-H	C18H32O3	39.8	-0.53	93.64
64	RP -	Cortolone-3-glucuronide	541.2644	4.45	M-H	C27H42O11	36.9	-1.96	86.87
65	RP -	Tetrahydroaldosterone-3-glucuronide	539.2488	4.56	M-H	C27H40O11	36.9	-1.77	86.87
66*	RP -	Serylproline*	201.0872	1.05	M-H	C8H14N2O4	51.5	-4.27	92.48
67	RP +	LysoPC(22:0)	562.4207	11.81	M+H-H2O	C30H62NO7P	36.2	-4.23	85.84
68	RP +	MG(20:2)	383.3146	17.36	M+H	C23H42O4	37.9	-2.71	92.57
69	RP +	LysoPA(18:2)	399.2274	12.48	M+H-2H2O	C21H39O7P	36	-4.79	85.36
70*	RP +	LysoPE(22:6)*	526.2909	7.83	M+H	C27H44NO7P	37.5	-3.71	90.16

71	RP +	LysoPC(18:3)	518.3264	11.43	M+H	C26H48NO7P	36.7	4.33	88.46
72	RP +	Cholesterol	425.3172	10.13	M+K	C27H46O	36.6	-2.17	85.48

*These compounds are identified by matching the experimental fragmentation data with theoretical fragmentation patterns generated by the simulated breaking of bonds in the structures of possible identifications.

[a]HILIC+ Hydrophilic interaction liquid chromatography separation in electrospray ionization (ESI) positive mode

[a]HILIC- Hydrophilic interaction liquid chromatography separation in ESI negative mode

[a]RP+ Reversed-phase liquid chromatography separation in ESI positive mode

[a]RP- Reversed-phase liquid chromatography separation in ESI negative mode

[b]Scores in Progenesis QI are calculated using the mean of three similarity metrics namely (1) Mass Similarity, (2) Isotope Similarity, and (3) Fragmentation Score.

[c]Isotope similarity compares the intensities of each isotope between observed and theoretical distributions.

Supplemental Table S9: Tentatively identified metabolites/lipids with CV<30% in QC samples *(Sorted by CV %)*					
Sl.No	Compound name	Accepted HMDB ID	RT-*m/z* pair	Chromatography	CV%
1	Panose	HMDB0029937	0.73_504.1702n	RP ESI-	4.42
2	LysoPC(18:0)	HMDB0010384	9.60_523.3658n	RP ESI+	4.91
3	Maltotetraose	HMDB0001296	0.73_666.2218n	RP ESI-	5.23
4	LysoPC(16:0)	HMDB0010382	8.16_495.3325n	RP ESI+	5.35
5	LysoPC(18:1)	HMDB0002815	8.69_521.3441n	RP ESI+	5.39
6	LysoPC(20:4)	HMDB0010396	8.01_543.3353n	RP ESI+	5.42
7	1-Stearoylglycerophosphocholine	HMDB0094688	9.34_525.3761*m/z*	RP ESI+	5.44
8	Stearoylcarnitine	HMDB0000848	9.92_464.3171*m/z*	RP ESI-	5.44
9	LysoPE(20:0)	HMDB0011511	8.96_509.3492n	RP ESI+	5.46
10	LysoPC(20:3)	HMDB0010393	8.42_545.3496n	RP ESI+	5.54
11	N-Palmitoylsphingosine	HMDB0000790	9.58_582.5077*m/z*	RP ESI-	5.56
12	LysoPC(18:2)	HMDB0010386	7.99_519.3336n	RP ESI+	5.58
13	MG(18:0)	HMDB0011131	9.54_341.3044*m/z*	RP ESI+	5.65
14	LysoPC(14:0)	HMDB0010379	7.30_467.2986n	RP ESI+	5.82
15	LysoPC(P-18:0)	HMDB0013122	9.98_507.3665n	RP ESI+	5.94
16	PC(P-18:1_P-18:1)	HMDB0011300	2.24_792.5688*m/z*	HILIC ESI+	6.15
17	LysoPC(P-16:0)	HMDB0010407	8.70_479.3360n	RP ESI+	6.19
18	LysoPC(22:4)	HMDB0010401	8.87_571.3647n	RP ESI+	6.26
19	Docosahexaenoic acid	HMDB0062579	11.04_327.2345*m/z*	RP ESI-	6.28
20	LysoPE(18:1)	HMDB0011475	8.42_478.2946*m/z*	RP ESI-	6.31
21	1,1'-Oxybis[2,4-dibromobenzene]	HMDB0037547	8.65_482.7197*m/z*	RP ESI+	6.52
22	4-(1,1,3,3-Tetramethylbutyl)-phenol	HMDB0013825	1.04_205.1600*m/z*	HILIC ESI-	6.6
23	LysoPE(18:0)	HMDB0011130	9.28_480.3111*m/z*	RP ESI-	6.61
24	LysoPC(20:2)	HMDB0010392	9.01_547.3646n	RP ESI+	6.67
25	Indane	HMDB0059837	1.13_118.0789n	HILIC ESI+	6.71
26	Vitamin K1	HMDB0003555	10.26_450.3534n	RP ESI+	6.75

27	3-hydroxyhexadecanoyl carnitine	HMDB0061642	9.26_450.2985*m/z*	RP ESI-	6.8
28	LysoPC(16:1)	HMDB0010383	7.61_493.3146n	RP ESI+	6.85
29	Eicosatrienoic acid	HMDB0002925	12.11_305.2486*m/z*	RP ESI-	6.86
30	Campesterol	HMDB0002869	10.33_439.3343*m/z*	RP ESI+	6.88
31	LysoPC(20:1)	HMDB0010391	9.85_549.3802n	RP ESI+	6.96
32	LysoPC(20:4)	HMDB0010395	7.31_544.3419*m/z*	HILIC ESI+	6.97
33	Phenylacetaldehyde	HMDB0006236	1.15_121.0647*m/z*	HILIC ESI+	7.04
34	2-Octenal	HMDB0030961	4.94_171.1017*m/z*	RP ESI-	7.05
35	LysoPC(20:5)	HMDB0010397	7.50_542.3232*m/z*	RP ESI+	7.12
36	LysoPE(18:1)	HMDB0011505	8.60_478.2960*m/z*	RP ESI-	7.14
37	LysoPC(22:6)	HMDB0010404	7.99_567.3340n	RP ESI+	7.15
38	5Tetrahydrocortisol	HMDB0000526	0.94_405.2046*m/z*	RP ESI+	7.34
39	N-Methyl-lysine	HMDB0002038	12.11_319.2322*m/z*	RP ESI-	7.34
40	Pentadecanal	HMDB0031078	10.56_226.2278n	RP ESI-	7.35
41	10-Nonadecenoic acid	HMDB0013622	11.33_341.2694*m/z*	RP ESI-	7.38
42	GammTocopherol	HMDB0001492	10.00_455.3309*m/z*	RP ESI+	7.45
43	PE(20:3_P-18:0)	HMDB0009346	0.63_753.5645n	RP ESI+	7.46
44	2-Pentylthiophene	HMDB0040240	6.88_307.1551*m/z*	RP ESI-	7.5
45	Leukotriene D4	HMDB0003080	0.54_533.2092*m/z*	RP ESI-	7.63
46	Uric acid	HMDB0000289	0.90_167.0213*m/z*	RP ESI-	7.69
47	Valyl-Aspartate	HMDB0029123	6.02_269.0544*m/z*	RP ESI-	7.76
48	LysoPC(22:5)	HMDB0010402	8.27_569.3496n	RP ESI+	7.79
49	m-Cresol	HMDB0002048	1.14_91.0541*m/z*	HILIC ESI+	7.81
50	Capric acid	HMDB0000511	7.98_171.1390*m/z*	RP ESI-	7.83
51	LysoPE(16:0)	HMDB0011503	9.24_452.2799*m/z*	RP ESI-	7.83
52	(E)-4-Methyl-2-heptene	HMDB0061910	7.30_157.1234*m/z*	RP ESI-	7.86
53	LysoPC(15:0)	HMDB0010381	7.45_481.3185n	HILIC ESI+	7.89
54	Ketoleucine	HMDB0000695	1.14_95.0490*m/z*	HILIC ESI+	7.91
55	1,3,5-Trimethoxybenzene	HMDB0059963	6.68_335.1494*m/z*	RP ESI-	7.94
56	LysoPE(p-16:0)	HMDB0011152	8.63_437.2935n	RP ESI-	8.07

57	Octyl 4-methoxycinnamic acid	HMDB0061861	10.93_335.1890*m/z*	RP ESI-	8.13
58	Histidinyl-Valine	HMDB0028898	0.73_291.0855*m/z*	RP ESI-	8.17
59	LysoPC(18:1)	HMDB0010385	8.70_521.3476n	RP ESI+	8.22
60	Arabinosylhypoxanthine	HMDB0003040	2.41_267.0742*m/z*	RP ESI-	8.23
61	Isopimaric acid	HMDB0036811	10.52_301.2174*m/z*	RP ESI-	8.23
62	N-Acetylleucine	HMDB0011756	3.42_172.0975*m/z*	RP ESI-	8.45
63	LysoPC(P-18:1)	HMDB0010408	9.01_506.3574*m/z*	RP ESI+	8.47
64	LysoPC(17:0)	HMDB0012108	7.33_509.3514n	HILIC ESI+	8.49
65	Linoleic acid	HMDB0000673	1.08_263.2365*m/z*	HILIC ESI+	8.77
66	PC(14:0_18:4)	HMDB0007877	12.03_708.4905*m/z*	RP ESI+	8.78
67	Hypogeic acid	HMDB0002186	11.04_253.2179*m/z*	RP ESI-	8.89
68	Chenodeoxycholic acid 3-sulfate	HMDB0002586	6.71_507.2234*m/z*	RP ESI-	8.9
69	5b-Cholestane-3a,7a,12a,23S,25-pentol	HMDB0000483	1.11_417.3356*m/z*	HILIC ESI+	8.95
70	Docosapentaenoic acid	HMDB0001976	11.45_329.2495*m/z*	RP ESI-	8.99
71	Heptaethylene glycol	HMDB0061835	11.25_325.1864*m/z*	RP ESI-	9
72	2-Hexyl-3-phenyl-2-propenal	HMDB0031736	7.32_261.1490*m/z*	RP ESI-	9.01
73	11-Dehydro-thromboxane B2	HMDB0004242	11.97_349.2052*m/z*	RP ESI-	9.07
74	Fexofenadine	HMDB0005030	7.83_501.2834n	RP ESI+	9.08
75	Hypoxanthine	HMDB0000157	0.90_135.0307*m/z*	RP ESI-	9.08
76	Oleoylcarnitine	HMDB0005065	5.46_448.3421*m/z*	HILIC ESI+	9.2
77	1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-1-penten-3-one	HMDB0038130	9.41_205.1604*m/z*	RP ESI-	9.22
78	Adenosine triphosphate	HMDB0000538	9.34_525.0331*m/z*	RP ESI+	9.43
79	Phthalic acid	HMDB0002107	1.04_149.0240*m/z*	HILIC ESI+	9.47
80	1,1-Dimethylbiguanide	HMDB0001921	2.56_164.0711*m/z*	RP ESI-	9.49
81	6-Phenylundecane	HMDB0061857	10.61_277.2175*m/z*	RP ESI-	9.5
82	2,4-Dimethylfuran	HMDB0032965	1.14_96.0573n	HILIC ESI+	9.52
83	11b-PGE2	HMDB0060041	11.45_397.2263*m/z*	RP ESI-	9.61
84	2-Aminoheptanoate	HMDB0094649	10.39_184.0748*m/z*	HILIC ESI+	9.67
85	Glycogen	HMDB0000757	7.77_689.2099*m/z*	HILIC ESI+	9.7

86	4-Hydroxy-5-(dihydroxyphenyl)-valeric acid-O-methyl-O-sulphate	HMDB0059977	0.59_336.0547n	RP ESI-	9.79
87	LysoPC(22:1)	HMDB0010399	11.17_578.4179*m/z*	RP ESI+	9.81
88	LysoPC(20:0)	HMDB0010390	7.44_534.3909*m/z*	HILIC ESI+	9.86
89	Oleic acid	HMDB0000207	1.17_282.2582n	HILIC ESI+	9.87
90	PC(o-16:1_14:1)	HMDB0013410	10.06_688.5220*m/z*	RP ESI+	9.88
91	Xylose	HMDB0000098	0.68_195.0506*m/z*	RP ESI-	10.1
92	Isoleucyl-Isoleucine	HMDB0028910	0.79_265.1513*m/z*	HILIC ESI-	10.12
93	Dodecanoic acid	HMDB0000638	9.34_199.1712*m/z*	RP ESI-	10.15
94	2-Hydroxymyristic acid	HMDB0002261	9.73_225.1850*m/z*	RP ESI-	10.19
95	Hexylbenzene	HMDB0061815	1.35_180.1746*m/z*	HILIC ESI+	10.19
96	Guanosine	HMDB0000133	2.41_282.0842*m/z*	RP ESI-	10.24
97	Iso-Valeraldehyde	HMDB0006478	10.39_104.1072*m/z*	HILIC ESI+	10.28
98	betDamascenone	HMDB0013804	8.35_235.1331*m/z*	RP ESI-	10.3
99	Trans-urocanate	HMDB0062562	0.54_174.9918*m/z*	RP ESI-	10.31
100	AlphLinolenic acid	HMDB0001388	1.08_279.2310*m/z*	HILIC ESI+	10.34
101	Butanone	HMDB0000474	6.63_143.1070*m/z*	RP ESI-	10.36
102	gammGlutamylmethionine	HMDB0034367	0.68_315.0410*m/z*	RP ESI-	10.42
103	Taurocholic acid	HMDB0000036	5.37_514.2843*m/z*	RP ESI-	10.5
104	Myristic acid	HMDB0000806	10.74_227.2032*m/z*	RP ESI-	10.58
105	Heptadecanoic acid	HMDB0002259	1.10_269.2487*m/z*	HILIC ESI-	10.71
106	2-Hexenal	HMDB0031496	7.24_195.1391*m/z*	RP ESI-	10.78
107	Oenanthic ether	HMDB0000798	7.96_315.2543*m/z*	RP ESI-	10.78
108	LysoPE(22:6)	HMDB0011526	7.83_526.2909*m/z*	RP ESI+	10.8
109	Undecanoic acid	HMDB0000947	8.65_185.1539*m/z*	RP ESI-	10.92
110	3-Phenyl-1-propanol	HMDB0033962	1.17_159.0774*m/z*	HILIC ESI+	11.04
111	3A,7α-dihydroxycoprostanate	HMDB0000359	12.17_433.3318*m/z*	RP ESI-	11.07
112	DG(14:0_16:1)	HMDB0007012	17.82_577.4229*m/z*	RP ESI+	11.11
113	Biliverdin	HMDB0001008	6.42_583.2553*m/z*	RP ESI+	11.12
114	Ricinoleic acid	HMDB0034297	9.02_297.2443*m/z*	RP ESI-	11.13

115	trans-Hexadec-2-enoyl carnitine	HMDB0006317	5.61_398.3271*m/z*	HILIC ESI+	11.19
116	Mono-(2-ethyl-5-carboxypentyl) phthalate	HMDB0094647	5.87_289.1076*m/z*	RP ESI-	11.23
117	TG(22:5_18:3_22:5)	HMDB0010548	2.42_999.7436*m/z*	HILIC ESI+	11.28
118	Glycocholic acid	HMDB0000138	5.70_464.3014*m/z*	RP ESI-	11.29
119	PC(18:3_14:0)	HMDB0008163	8.13_772.5138*m/z*	RP ESI-	11.29
120	PC(14:1_18:2)	HMDB0007907	8.36_772.5132*m/z*	RP ESI-	11.33
121	Leu-Leu-Leu	HMDB0094648	1.56_340.2568*m/z*	HILIC ESI+	11.36
122	Glycylproline	HMDB0000721	0.56_217.0821*m/z*	RP ESI-	11.4
123	DG(20:5_20:2)	HMDB0007573	14.83_666.5287n	RP ESI+	11.46
124	MG(18:1)	HMDB0011536	5.62_339.2892*m/z*	HILIC ESI+	11.46
125	Dimethyl sulfone	HMDB0004983	0.76_187.0091*m/z*	HILIC ESI-	11.49
126	Tetranor 12-HETE	HMDB0060055	6.78_265.1797*m/z*	RP ESI-	11.54
127	1-Phenyl-2-hexanone	HMDB0094661	6.14_221.1174*m/z*	RP ESI-	11.55
128	Cholesterol	HMDB0000067	10.13_425.3172*m/z*	RP ESI+	11.55
129	DG(14:0_16:0)	HMDB0007011	18.81_579.4386*m/z*	RP ESI+	11.57
130	Trigonelline	HMDB0000875	1.19_138.0552*m/z*	HILIC ESI+	11.62
131	CMPF	HMDB0061112	1.14_263.0885*m/z*	HILIC ESI+	11.64
132	13-Hydroxyoctadecadienoic acid	HMDB0004667	8.57_296.2352n	RP ESI-	11.66
133	4-Hydroxynonenal	HMDB0004362	8.36_311.2217*m/z*	RP ESI-	11.68
134	Solanidine	HMDB0003236	7.19_398.3412*m/z*	RP ESI+	11.68
135	17-Hydroxyandrostane-3-glucuronide	HMDB0010359	7.86_449.2539*m/z*	RP ESI-	11.71
136	6-Methyl-3,5-heptadien-2-one	HMDB0031582	8.75_247.1699*m/z*	RP ESI-	11.72
137	Phenylethylamine	HMDB0012275	6.38_158.0377*m/z*	RP ESI-	11.78
138	16-Hydroxypregnenolone	HMDB0000315	0.83_332.2338n	HILIC ESI-	11.9
139	MG(20:4)	HMDB0004666	5.56_361.2742*m/z*	HILIC ESI+	11.9
140	MG(14:0)	HMDB0011530	12.46_302.2443n	RP ESI-	11.94
141	Palmitoylcarnitine	HMDB0000222	5.60_400.3457*m/z*	HILIC ESI+	12
142	Enterodiol	HMDB0005056	7.52_283.1360*m/z*	RP ESI-	12.02
143	Kynurenic acid	HMDB0000715	7.17_377.0764*m/z*	RP ESI-	12.02
144	lysoPC(28:0)	HMDB0029206	0.65_664.5337*m/z*	RP ESI+	12.03

145	LysoPC(22:2)	HMDB0010400	9.18_598.3874*m/z*	RP ESI+	12.04
146	PE(22:1_22:2)	HMDB0000520	5.44_454.3896*m/z*	HILIC ESI+	12.04
147	CE(15:1)	HMDB0060056	12.12_647.5189*m/z*	RP ESI+	12.09
148	Pyridoxine 5'-phosphate	HMDB0001319	1.17_285.9887*m/z*	RP ESI-	12.16
149	GammButyrolactone	HMDB0000549	10.39_124.9999*m/z*	HILIC ESI+	12.19
150	SM(d18:0_22:3)	HMDB0013468	2.34_819.5717*m/z*	HILIC ESI+	12.26
151	5,6-Epoxy-eicosatrienoic acid	HMDB0002190	9.17_319.2275*m/z*	RP ESI-	12.31
152	gammGlutamylthreonine	HMDB0029159	1.05_248.0999n	RP ESI-	12.33
153	Docosadienoic acid	HMDB0061714	1.09_336.3020n	HILIC ESI-	12.34
154	Atorvastatin	HMDB0005006	11.54_581.2428*m/z*	RP ESI+	12.37
155	1,3-Diacetylpropane	HMDB0029165	3.96_127.0752*m/z*	RP ESI-	12.42
156	Methyl dihydrojasmonate	HMDB0031740	6.51_207.1407*m/z*	RP ESI-	12.52
157	2,4-Di-tert-butylphenol	HMDB0013816	7.45_249.1509*m/z*	RP ESI-	12.79
158	N-Octyl phenyl ketone	HMDB0094674	7.59_263.1661*m/z*	RP ESI-	12.82
159	3-Hexanone	HMDB0000753	0.91_199.1689*m/z*	HILIC ESI-	12.94
160	LysoPC(24:1)	HMDB0010406	12.71_605.4434n	RP ESI+	12.94
161	Prolyl-Tryptophan	HMDB0029028	1.16_319.1777*m/z*	HILIC ESI+	12.94
162	Cyclotetradecane	HMDB0033567	11.43_241.2173*m/z*	RP ESI-	12.99
163	p-Cresol sulfate	HMDB0011635	3.91_187.0078*m/z*	RP ESI-	12.99
164	all-trans-Retinoic acid	HMDB0001852	9.81_299.2024*m/z*	RP ESI-	13.06
165	Diltiazem	HMDB0014487	5.09_415.1690*m/z*	RP ESI+	13.11
166	Saccharopine	HMDB0000279	6.69_551.2624*m/z*	HILIC ESI-	13.12
167	(E)-3-decen-1-ol	HMDB0013810	14.96_311.2960*m/z*	RP ESI-	13.14
168	Indoxyl sulfate	HMDB0000682	0.78_212.0031*m/z*	HILIC ESI-	13.2
169	Coprocholic acid	HMDB0000601	1.06_450.3337n	HILIC ESI+	13.27
170	9E-Heptadecenoic acid	HMDB0031046	11.70_267.2326*m/z*	RP ESI-	13.29
171	Isoursodeoxycholic acid	HMDB0000686	7.88_392.2925n	RP ESI-	13.3
172	LysoPA(16:0)	HMDB0007849	13.14_391.2248*m/z*	RP ESI-	13.48
173	Deoxyuridine	HMDB0000012	1.05_273.0734*m/z*	RP ESI-	13.49
174	PI(16:0_18:0)	HMDB0009781	5.76_877.5197*m/z*	RP ESI+	13.55

175	25,26-dihydroxyvitamin D	HMDB0001420	1.10_415.3218*m/z*	HILIC ESI-	13.57
176	18-Oxocortisol	HMDB0000332	0.54_375.1826*m/z*	RP ESI-	13.63
177	Tryptophan	HMDB0000929	2.92_203.0822*m/z*	RP ESI-	13.69
178	Dihydrotestosterone	HMDB0002961	0.85_290.2268n	HILIC ESI-	13.7
179	13-OxoODE	HMDB0004668	8.92_293.2115*m/z*	RP ESI-	13.72
180	Ubiquinone-1	HMDB0002012	7.02_231.1032*m/z*	RP ESI-	13.72
181	DG(22:5_16:1)	HMDB0007708	19.27_663.5005*m/z*	RP ESI+	13.73
182	Glycoursodeoxycholic acid	HMDB0000708	6.73_448.3071*m/z*	RP ESI-	13.88
183	2-Butylfuran	HMDB0040272	8.40_247.1689*m/z*	RP ESI-	13.91
184	Serylvaline	HMDB0029052	0.58_204.1095n	RP ESI-	13.91
185	Tauroursodeoxycholic acid	HMDB0000874	6.07_498.2894*m/z*	RP ESI-	13.95
186	Phloretin	HMDB0003306	4.83_273.0795*m/z*	RP ESI-	13.97
187	Dehydroepiandrosterone sulfate	HMDB0001032	5.72_367.1590*m/z*	RP ESI-	13.98
188	DG(14:0_20:3)	HMDB0007023	20.01_613.4817*m/z*	RP ESI+	13.98
189	MG(24:1)	HMDB0011559	12.24_421.3675*m/z*	RP ESI-	14.03
190	Borneol	HMDB0034976	6.80_199.1332*m/z*	RP ESI-	14.13
191	PC(14:0_20:4)	HMDB0007883	8.67_798.5286*m/z*	RP ESI-	14.16
192	Scopolamine	HMDB0003573	1.13_286.1446*m/z*	HILIC ESI+	14.18
193	SM(d18:0_14:1(OH))	HMDB0013462	20.39_689.5191*m/z*	RP ESI+	14.18
194	DG(16:0_20:4)	HMDB0007113	20.16_599.5048*m/z*	RP ESI+	14.21
195	Cortol	HMDB0003180	7.07_386.2900*m/z*	HILIC ESI+	14.24
196	19,20-DiHDPA	HMDB0010214	0.82_397.2148*m/z*	HILIC ESI-	14.25
197	Dodecanoylcarnitine	HMDB0002250	9.73_342.2646*m/z*	RP ESI-	14.28
198	Matairesinol	HMDB0035789	0.61_357.1320*m/z*	RP ESI-	14.33
199	Myristoleic acid	HMDB0002000	7.34_247.1692*m/z*	RP ESI-	14.35
200	DG(14:0_18:2)	HMDB0007016	19.60_587.4640*m/z*	RP ESI+	14.37
201	1-Stearoylglycerophosphoglycerol	HMDB0061697	10.59_533.2872*m/z*	RP ESI-	14.39
202	Mono-(2-ethyl-5-oxohexyl) phthalate	HMDB0094645	7.02_291.1231*m/z*	RP ESI-	14.46
203	LysoPE(18:2)	HMDB0011507	7.78_477.2810n	RP ESI+	14.52
204	Adrenic acid	HMDB0002226	12.21_331.2659*m/z*	RP ESI-	14.53

205	Pantothenol	HMDB0004231	10.48_242.0807*m/z*	HILIC ESI-	14.58
206	Traumatic acid	HMDB0000933	1.05_228.1365n	HILIC ESI+	14.59
207	Undecanal	HMDB0030941	16.64_339.3266*m/z*	RP ESI-	14.6
208	Capryloylglycine	HMDB0000832	1.14_236.1054*m/z*	HILIC ESI-	14.62
209	LysoPE(20:2)	HMDB0011513	7.33_523.3479*m/z*	RP ESI+	14.63
210	Hexadecanedioic acid	HMDB0000672	6.56_267.1962*m/z*	RP ESI-	14.65
211	1-Tridecene	HMDB0030930	1.12_227.2019*m/z*	HILIC ESI-	14.66
212	6-Hydroxyhexanoic acid	HMDB0012843	4.88_131.0702*m/z*	RP ESI-	14.68
213	Phytol	HMDB0002019	11.89_341.3053*m/z*	RP ESI-	14.76
214	1-Phenylheptane	HMDB0061825	1.14_221.1541*m/z*	HILIC ESI-	14.82
215	1-Phenyl-1-propanone	HMDB0032623	1.13_134.0728n	HILIC ESI+	14.85
216	9,10-Epoxyoctadecenoic acid	HMDB0004701	8.70_296.2350n	RP ESI-	14.85
217	Dimethylbenzimidazole	HMDB0003701	8.38_291.1589*m/z*	RP ESI-	14.99
218	p-Xylene	HMDB0059924	1.34_124.1121*m/z*	HILIC ESI+	15.03
219	AlphLactose	HMDB0000186	0.71_377.0848*m/z*	RP ESI-	15.04
220	Pentadecanoic acid	HMDB0000826	1.11_241.2168*m/z*	HILIC ESI-	15.04
221	Desmosine	HMDB0000572	9.14_571.2880*m/z*	RP ESI-	15.06
222	Hexaethylene glycol	HMDB0061822	1.17_283.1757*m/z*	HILIC ESI+	15.21
223	Undecanedioic acid	HMDB0000888	5.38_216.1364n	RP ESI-	15.26
224	MG(20:1)	HMDB0011543	15.16_385.3330*m/z*	RP ESI+	15.28
225	2-Methoxyestrone	HMDB0000010	5.95_300.1724n	RP ESI-	15.31
226	Octanoylcarnitine	HMDB0000791	5.70_268.1932*m/z*	RP ESI-	15.31
227	DG(22:6_22:6)	HMDB0007788	8.63_677.4905*m/z*	RP ESI+	15.35
228	Nutriacholic acid	HMDB0000467	11.45_411.2505*m/z*	RP ESI-	15.4
229	Azelaic acid	HMDB0000784	4.42_187.0976*m/z*	RP ESI-	15.46
230	Histidine	HMDB0000177	7.24_154.0624*m/z*	RP ESI-	15.46
231	alphTerpineol acetate	HMDB0032051	16.69_241.1433*m/z*	RP ESI-	15.51
232	Linoleoyl ethanolamide	HMDB0012252	1.17_323.2821n	HILIC ESI+	15.53
233	SM(d18:0_16:1(OH))	HMDB0013463	12.85_699.5490*m/z*	RP ESI+	15.54
234	2-Nonadecanone	HMDB0061862	12.11_327.2901*m/z*	RP ESI-	15.57

235	Histidinol	HMDB0003431	9.34_281.1724*m/z*	RP ESI-	15.58
236	Raffinose	HMDB0003213	6.98_527.1582*m/z*	HILIC ESI+	15.58
237	Tetrahydrocorticosterone	HMDB0000268	5.77_368.2797*m/z*	HILIC ESI+	15.59
238	4-Hydroxybenzoic acid	HMDB0000500	1.04_121.0282*m/z*	HILIC ESI+	15.6
239	Phosphate	HMDB0001429	10.39_98.9834*m/z*	HILIC ESI+	15.66
240	3-Oxotetradecanoic acid	HMDB0010730	7.88_241.1798*m/z*	RP ESI-	15.73
241	LysoPE(16:0)	HMDB0011473	9.36_474.2614*m/z*	RP ESI-	15.79
242	3-Hydroxycapric acid	HMDB0002203	7.67_169.1224*m/z*	RP ESI-	15.82
243	Pyrocatechol sulfate	HMDB0059724	20.80_188.9845*m/z*	RP ESI-	15.84
244	MG(22:5)	HMDB0011555	1.15_387.2899*m/z*	HILIC ESI+	15.86
245	6-Keto-prostaglandin F1a	HMDB0002886	13.35_351.2210*m/z*	RP ESI-	15.91
246	Methylpyrazine	HMDB0033112	4.17_187.0981*m/z*	RP ESI-	15.92
247	Picolinoylglycine	HMDB0059766	9.00_217.0023*m/z*	HILIC ESI-	15.92
248	Cuminaldehyde	HMDB0002214	6.83_169.0645*m/z*	RP ESI-	15.99
249	Cortolone-3-glucuronide	HMDB0010320	4.45_541.2644*m/z*	RP ESI-	16.04
250	xi-10-Hydroxyoctadecanoic acid	HMDB0037396	12.36_281.2510*m/z*	RP ESI-	16.13
251	P,P-Dioctyldiphenylamine	HMDB0061926	7.55_416.3271*m/z*	RP ESI+	16.29
252	Oxychlordane	HMDB0059571	0.63_384.7667*m/z*	RP ESI+	16.32
253	3-Hydroxydodecanoic acid	HMDB0000387	4.88_253.1220*m/z*	RP ESI-	16.39
254	Tetradecanoylcarnitine	HMDB0005066	5.73_372.3120*m/z*	HILIC ESI+	16.4
255	PC(20:4_16:0)	HMDB0008462	5.11_782.5737*m/z*	HILIC ESI+	16.54
256	Styrene	HMDB0034240	5.67_149.0607*m/z*	RP ESI-	16.55
257	Anandamide	HMDB0004080	1.17_347.2820n	HILIC ESI+	16.57
258	5-Tetradecenoic acid	HMDB0000499	1.17_227.2007*m/z*	HILIC ESI+	16.62
259	5-Hydroxylysine	HMDB0000450	8.40_162.1007n	RP ESI-	16.65
260	Dodecanedioic acid	HMDB0000623	7.30_211.1328*m/z*	RP ESI-	16.68
261	PC(22:4_14:0)	HMDB0008623	8.38_818.5181*m/z*	RP ESI-	16.71
262	1-Dodecene	HMDB0059874	9.83_213.1850*m/z*	RP ESI-	16.72
263	2-Heptenal	HMDB0033827	9.11_223.1691*m/z*	RP ESI-	16.75
264	gammCEHC	HMDB0001931	7.56_245.1173*m/z*	RP ESI-	16.75

265	Indoleacrylic acid	HMDB0000734	5.65_187.0631n	HILIC ESI+	16.79
266	Pregnanetriol	HMDB0006070	5.66_337.2732*m/z*	HILIC ESI+	16.83
267	(-)-Neoisomenthol	HMDB0035764	1.09_311.2955*m/z*	HILIC ESI-	16.87
268	3,5-Bis(1,1-dimethylethyl)-4-hydroxy-benzoic acid ethyl ester	HMDB0061935	19.65_309.1705*m/z*	HILIC ESI-	16.95
269	Glycyl-Histidine	HMDB0028843	1.04_212.0912n	HILIC ESI+	16.97
270	3'-Hydroxy-e,e-caroten-3-one	HMDB0002020	18.00_549.4108*m/z*	RP ESI+	17.07
271	4-Hydroxy-5-(dihydroxyphenyl)-valeric acid-O-sulphate	HMDB0059978	11.39_271.0246*m/z*	HILIC ESI+	17.07
272	3-Hexenyl salicylic acid	HMDB0061823	7.11_220.1090n	RP ESI-	17.09
273	MG(16:0)	HMDB0011533	5.71_313.2733*m/z*	HILIC ESI+	17.19
274	N6-Carbamoyl-L-threonyladenosine	HMDB0041623	18.75_411.1492n	RP ESI-	17.19
275	Norepinephrine	HMDB0000216	0.81_150.0570*m/z*	HILIC ESI-	17.19
276	Myo-inositol hexakisphosphate	HMDB0003502	0.59_659.8632n	RP ESI+	17.23
277	Benzeneacetamide-4-O-sulphate	HMDB0059994	2.75_230.0127*m/z*	RP ESI-	17.29
278	Pregnenolone sulfate	HMDB0000774	0.81_395.1898*m/z*	HILIC ESI-	17.31
279	trans-Jasmone	HMDB0031454	5.31_209.1175*m/z*	RP ESI-	17.33
280	Trihexosylceramide (d18:1_18:0)	HMDB0004880	1.68_1050.6845*m/z*	HILIC ESI-	17.33
281	Tridecanoic acid	HMDB0000910	7.32_235.1695*m/z*	RP ESI-	17.34
282	LysoPC(22:0)	HMDB0010398	11.81_562.4207*m/z*	RP ESI+	17.42
283	PC(14:1_16:1)	HMDB0007903	9.80_719.5386*m/z*	RP ESI+	17.42
284	Tetraethylene glycol	HMDB0094708	6.75_387.2199*m/z*	HILIC ESI-	17.44
285	Ibuprofen	HMDB0001925	7.99_251.1286*m/z*	RP ESI-	17.45
286	12(13)Ep-9-KODE	HMDB0013623	8.06_309.2062*m/z*	RP ESI-	17.48
287	Oxoglutaric acid	HMDB0000208	0.90_191.0197*m/z*	RP ESI-	17.51
288	PC(22:5_14:0)	HMDB0008689	5.12_779.5493n	HILIC ESI+	17.53
289	PG(16:0_22:4)	HMDB0010581	5.41_816.5722*m/z*	HILIC ESI+	17.55
290	Heptanoic acid	HMDB0000666	16.25_129.0910*m/z*	RP ESI-	17.56
291	4-Hydroxyphenytoin	HMDB0041905	2.99_267.0765*m/z*	HILIC ESI-	17.57
292	Lidocaine	HMDB0014426	12.82_467.3381*m/z*	RP ESI-	17.7

293	N-Acetylglutamine	HMDB0006029	1.05_187.0717*m/z*	RP ESI-	17.7
294	Ethylhexyl salicylate	HMDB0061839	8.33_231.1379*m/z*	RP ESI-	17.72
295	CE(15:0)	HMDB0060057	12.58_649.5311*m/z*	RP ESI+	17.78
296	Agmatine	HMDB0001432	4.71_165.0910*m/z*	RP ESI-	17.81
297	MG(20:5)	HMDB0011550	5.66_394.2950*m/z*	HILIC ESI+	17.83
298	LysoPA(18:0)	HMDB0007850	11.06_483.2720*m/z*	RP ESI-	17.86
299	LysoPE(15:0)	HMDB0011502	3.34_460.2405*m/z*	RP ESI-	17.87
300	CE(16:2)	HMDB0060058	11.72_659.5164*m/z*	RP ESI+	18
301	9,12,13-TriHOME	HMDB0004708	5.60_329.2325*m/z*	RP ESI-	18.01
302	Squalene	HMDB0000256	1.08_449.3570*m/z*	HILIC ESI+	18.01
303	2-Tetradecanone	HMDB0030924	3.68_230.2489*m/z*	HILIC ESI+	18.06
304	Perfluorooctanesulfonic acid	HMDB0059586	0.71_498.9298*m/z*	HILIC ESI-	18.14
305	xi-gammUndecalactone	HMDB0038311	5.31_165.1276*m/z*	RP ESI-	18.15
306	Eugenol	HMDB0005809	1.10_147.0799*m/z*	HILIC ESI+	18.16
307	α-CEHC	HMDB0001518	8.21_277.1431*m/z*	RP ESI-	18.21
308	Erucic acid	HMDB0002068	1.09_338.3185n	HILIC ESI-	18.22
309	N-Acetylhistamine	HMDB0013253	8.46_305.1750*m/z*	RP ESI-	18.22
310	2,4-Dimethyladipic acid	HMDB0059727	4.28_173.0808*m/z*	RP ESI-	18.29
311	2,3-Dinor-TXB2	HMDB0002904	7.73_323.1853*m/z*	RP ESI-	18.33
312	PC(16:1_20:0)	HMDB0008010	10.55_716.4586*m/z*	RP ESI+	18.34
313	PS(18:0_20:3)	HMDB0012382	5.27_796.5468*m/z*	HILIC ESI+	18.49
314	4-Hydroxy-5-(3'-hydroxyphenyl)-valeric acid-3'-O-sulphate	HMDB0059975	11.13_311.0216*m/z*	HILIC ESI-	18.52
315	TG(20:0_18:0_22:4)	HMDB0046248	7.40_991.8446*m/z*	HILIC ESI+	18.64
316	PC(18:1_20:4)	HMDB0008081	5.01_830.5667*m/z*	HILIC ESI+	18.72
317	DG(18:4_18:1)	HMDB0007334	18.55_637.4833*m/z*	RP ESI+	18.78
318	DG(22:6_16:0)	HMDB0007765	19.24_679.4761*m/z*	RP ESI+	18.78
319	Naproxen	HMDB0001923	6.32_267.0419*m/z*	RP ESI-	18.79
320	DG(20:5_16:1)	HMDB0007563	10.84_651.4443*m/z*	RP ESI+	18.8
321	CE(22:5)	HMDB0010375	2.25_737.5675*m/z*	HILIC ESI+	18.95

322	1-Stearoylglycerophosphoinositol	HMDB0061696	9.71_621.3038*m/z*	RP ESI-	18.97
323	Oleoyl glycine	HMDB0013631	7.05_374.2441*m/z*	RP ESI-	18.98
324	4-Hydroxydebrisoquine	HMDB0006468	6.80_236.1045*m/z*	RP ESI-	19.06
325	4alphCarboxy-5alphcholest8-en-3betol	HMDB0012166	16.61_430.3406n	RP ESI+	19.09
326	Aconitic acid	HMDB0000072	1.04_174.0165n	HILIC ESI+	19.1
327	Urocanic acid	HMDB0000301	20.77_174.9903*m/z*	RP ESI-	19.13
328	Serylproline	HMDB0029047	1.05_201.0872*m/z*	RP ESI-	19.16
329	Cholenic acid	HMDB0000308	11.89_355.2638*m/z*	RP ESI-	19.17
330	MG(15:0)	HMDB0011532	1.08_299.2602*m/z*	HILIC ESI+	19.22
331	TG(22:6_22:6_22:6)	HMDB0010562	8.60_1021.7208*m/z*	RP ESI-	19.22
332	LysoPE(20:2)	HMDB0011483	7.96_504.3105*m/z*	RP ESI-	19.23
333	Phenol sulphate	HMDB0060015	3.24_172.9915*m/z*	RP ESI-	19.28
334	2-Heptanone	HMDB0003671	2.85_227.2014*m/z*	HILIC ESI-	19.3
335	Pentadecane	HMDB0059886	7.37_212.2519n	RP ESI-	19.38
336	PE(18:1_P-18:0)	HMDB0009049	2.41_712.5674*m/z*	HILIC ESI+	19.4
337	Triethanolamine	HMDB0032538	1.57_130.0867*m/z*	RP ESI-	19.47
338	Methyl stearate	HMDB0034154	1.09_297.2791*m/z*	HILIC ESI-	19.48
339	SM(d18:0_14:0)	HMDB0012085	20.78_677.5569*m/z*	RP ESI+	19.49
340	DG(18:3_18:2)	HMDB0007306	1.01_653.4487*m/z*	HILIC ESI+	19.59
341	PE(16:0_20:4)	HMDB0008937	12.01_704.4953*m/z*	RP ESI+	19.68
342	3,4-Methylene suberic acid	HMDB0059768	5.50_197.0808*m/z*	RP ESI-	19.72
343	PC(18:2_18:0)	HMDB0008135	5.16_786.6035*m/z*	HILIC ESI+	19.72
344	DG(18:4_20:2)	HMDB0007341	19.08_640.5087n	RP ESI+	19.73
345	PC(14:1_P-18:1)	HMDB0007930	8.60_758.5325*m/z*	RP ESI-	19.79
346	4-Hydroxyproline	HMDB0000725	11.37_131.0586n	HILIC ESI+	19.84
347	PC(p-18:1_22:1)	HMDB0011290	5.47_843.6963*m/z*	RP ESI+	19.86
348	Phenylglyoxal	HMDB0061916	0.92_152.0707*m/z*	HILIC ESI+	19.86
349	Trimethylsilyl l-Alanine	HMDB0094699	7.20_184.0755*m/z*	HILIC ESI+	19.86
350	5-Aminopentanoic acid	HMDB0003355	7.53_118.0867*m/z*	HILIC ESI+	19.92
351	Alphdimorphecolic acid	HMDB0004670	1.11_296.2349n	HILIC ESI-	20.04

352	Lysyl-Tyrosine	HMDB0028963	1.18_327.2018*m/z*	HILIC ESI+	20.13
353	Homoarginine	HMDB0000670	4.59_223.0960*m/z*	RP ESI-	20.15
354	PC(P-18:1_18:4)	HMDB0011280	0.63_802.5116*m/z*	RP ESI+	20.2
355	5-AMMU	HMDB0004400	3.01_179.0566*m/z*	RP ESI-	20.23
356	5-(3',5'-Dihydroxyphenyl)-gammvalerolactone-O-sulphate-O-methyl	HMDB0060031	0.58_302.0445n	RP ESI-	20.29
357	p-Menth-1-en-4-ol	HMDB0035833	12.63_307.2645*m/z*	RP ESI-	20.32
358	Eicosadienoic acid	HMDB0005060	1.10_329.2489*m/z*	HILIC ESI-	20.33
359	LysoPE(18:3)	HMDB0011478	8.16_498.2560*m/z*	RP ESI+	20.33
360	5-Tetradecenoylcarnitine	HMDB0002014	5.71_370.2955*m/z*	HILIC ESI+	20.34
361	Carnitine	HMDB0000062	7.73_144.1032*m/z*	HILIC ESI+	20.41
362	2-Ethyl-2-hexenal	HMDB0061945	1.04_149.0929*m/z*	HILIC ESI+	20.51
363	4-Aminohippuric acid	HMDB0001867	6.72_387.1322*m/z*	HILIC ESI-	20.52
364	PC(16:0_18:1)	HMDB0007971	5.21_760.5872*m/z*	HILIC ESI+	20.6
365	MG(20:2)	HMDB0011544	17.36_383.3146*m/z*	RP ESI+	20.61
366	SM(d16:1_24:1)	HMDB0011694	9.61_806.6273*m/z*	RP ESI-	20.71
367	PS(16:0_18:2)	HMDB0012358	8.38_804.5027*m/z*	RP ESI-	20.82
368	Histidylproline diketopiperazine	HMDB0002053	1.38_231.1225*m/z*	HILIC ESI+	20.83
369	Carotene-3,3'-dione	HMDB0002193	13.98_564.3941n	RP ESI+	20.86
370	DG(15:0_20:1)	HMDB0007079	19.50_589.5168*m/z*	HILIC ESI-	21.03
371	Phosphoribosyl pyrophosphate	HMDB0000280	6.18_390.9575*m/z*	HILIC ESI+	21.03
372	3,4-Methyleneazelaic acid	HMDB0059744	1.36_213.1120*m/z*	HILIC ESI+	21.04
373	Histidinyl-Proline	HMDB0028893	6.97_275.1102*m/z*	HILIC ESI+	21.14
374	2-Biphenylol	HMDB0032582	6.38_169.0659*m/z*	RP ESI-	21.19
375	Di-2-propenyl sulfide	HMDB0036491	17.03_227.0917*m/z*	RP ESI-	21.19
376	PS(14:0_18:2)	HMDB0012336	5.50_732.4795*m/z*	HILIC ESI+	21.2
377	Oxypurinol	HMDB0000786	3.74_133.0153*m/z*	HILIC ESI-	21.21
378	3-Oxocholic acid	HMDB0000502	11.81_387.2509*m/z*	RP ESI-	21.22
379	Hydroxytyrosol	HMDB0005784	16.39_307.1179*m/z*	RP ESI-	21.22
380	SM(d18:0_20:2)	HMDB0013465	0.61_772.6347*m/z*	RP ESI+	21.3

381	CE(18:2)	HMDB0000610	13.35_687.5487*m/z*	RP ESI+	21.33
382	PC(18:0_22:6)	HMDB0008057	5.01_856.5799*m/z*	HILIC ESI+	21.39
383	2-Nonenal	HMDB0031269	6.16_185.1173*m/z*	RP ESI-	21.42
384	Cholic acid	HMDB0000619	13.82_447.2544*m/z*	RP ESI+	21.44
385	PC(20:4_18:0)	HMDB0008431	5.07_810.6040*m/z*	HILIC ESI+	21.6
386	Benzoic acid	HMDB0001870	3.81_121.0288*m/z*	RP ESI-	21.68
387	lysoPC(26:1)	HMDB0029220	8.35_678.4700*m/z*	RP ESI-	21.74
388	Phenyllactic acid	HMDB0000563	2.58_166.0622n	RP ESI-	21.75
389	Methyl propenyl ketone	HMDB0001184	5.01_167.1063*m/z*	RP ESI-	21.82
390	Isoleucyl-Tryptophan	HMDB0028918	0.87_298.1581*m/z*	HILIC ESI-	21.83
391	Naphthalene	HMDB0029751	3.59_128.0637n	RP ESI-	21.88
392	Pyridinoline	HMDB0000851	6.68_393.1761*m/z*	RP ESI+	21.9
393	Glutaminylglutamine	HMDB0028795	8.83_292.1593*m/z*	HILIC ESI+	21.92
394	Farnesol	HMDB0004305	5.53_240.2322*m/z*	HILIC ESI+	21.95
395	3-Methyl-2-butenal	HMDB0012157	9.08_102.0912*m/z*	HILIC ESI+	21.98
396	3-Hydroxysuberic acid	HMDB0000325	1.05_190.0851n	HILIC ESI+	22.02
397	3-Hydroxy-4,5-dimethyl-2(5H)-furanone	HMDB0031306	0.73_109.0287*m/z*	HILIC ESI-	22.06
398	Prostaglandin E3	HMDB0002664	5.05_350.2085n	RP ESI-	22.06
399	Indole	HMDB0000738	2.94_116.0498*m/z*	RP ESI-	22.11
400	5-Dodecenoic acid	HMDB0000529	7.13_243.1594*m/z*	RP ESI-	22.26
401	Kyotorphin	HMDB0005768	9.03_338.1835*m/z*	HILIC ESI+	22.26
402	Androsterone sulfate	HMDB0002759	6.58_369.1734*m/z*	RP ESI-	22.28
403	Octadecanedioic acid	HMDB0000782	12.11_314.2449n	RP ESI-	22.3
404	Diethanolamine	HMDB0004437	7.00_209.1521*m/z*	RP ESI-	22.34
405	Cer(d18:1_14:0)	HMDB0011773	16.94_509.4777n	RP ESI+	22.36
406	15-Hydroxyeicosatrienoate	HMDB0005045	9.16_322.2493n	RP ESI-	22.45
407	Sebacic acid	HMDB0000792	16.89_223.0957*m/z*	RP ESI-	22.48
408	PS(16:1_18:1)	HMDB0012368	8.13_804.5027*m/z*	RP ESI-	22.52
409	Docosatrienoic acid	HMDB0002823	12.97_333.2789*m/z*	RP ESI-	22.54
410	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-	HMDB0032913	7.37_193.1602*m/z*	RP ESI-	22.59

	butanone				
411	Lysyl-Valine	HMDB0028964	13.49_266.1493*m/z*	RP ESI-	22.59
412	5-Aminoimidazole-4-carboxamide	HMDB0003192	3.70_251.1031*m/z*	RP ESI-	22.62
413	PE(16:1_P-16:0)	HMDB0008982	9.65_691.5439*m/z*	RP ESI+	22.65
414	Reticuline	HMDB0003601	1.12_312.1597*m/z*	HILIC ESI+	22.68
415	9-HPODE	HMDB0006940	8.06_293.2134*m/z*	RP ESI-	22.72
416	4-phenylbutanic acid-O-sulphate	HMDB0059983	6.18_227.0348*m/z*	HILIC ESI+	22.77
417	2-Oleoylglycerophosphocholine	HMDB0061701	14.79_505.3483*m/z*	RP ESI+	22.8
418	CE(10:0)	HMDB0003603	7.99_523.4827*m/z*	RP ESI+	22.81
419	15-Deoxy-d-12,14-PGJ2	HMDB0005079	0.87_316.2032n	HILIC ESI-	22.94
420	Angiotensin II	HMDB0001035	12.01_1063.5691*m/z*	HILIC ESI+	22.98
421	Cotinine	HMDB0001046	7.15_351.1804*m/z*	RP ESI-	23.01
422	2-Methylbutyrylglycine	HMDB0000339	7.53_160.0969*m/z*	HILIC ESI+	23.13
423	Stearoylethanolamide	HMDB0013078	1.12_310.3099*m/z*	HILIC ESI+	23.17
424	Diaminopimelic acid	HMDB0001370	1.05_171.0761*m/z*	RP ESI-	23.23
425	Eicosenoic acid	HMDB0002231	1.17_328.3206*m/z*	HILIC ESI+	23.33
426	LysoPA(p-16:0)	HMDB0011154	10.37_431.1937*m/z*	RP ESI-	23.34
427	Valyl-Serine	HMDB0029136	0.59_225.0846*m/z*	RP ESI-	23.38
428	AlphTocotrienol	HMDB0006327	9.80_463.2992*m/z*	RP ESI+	23.4
429	Retinoyl b-glucuronide	HMDB0003141	20.79_457.2249*m/z*	RP ESI-	23.41
430	6A-hydroxy-dhea 3-sulfate	HMDB0062544	4.73_383.1522*m/z*	RP ESI-	23.42
431	Tridecanal	HMDB0030928	7.82_243.1952*m/z*	RP ESI-	23.42
432	LysoPA(18:2)	HMDB0007856	12.48_399.2274*m/z*	RP ESI+	23.5
433	Calcidiol	HMDB0003550	1.05_383.3333*m/z*	HILIC ESI+	23.56
434	Thyroxine sulfate	HMDB0002728	12.71_839.6394*m/z*	RP ESI+	23.59
435	CE(16:1)	HMDB0000658	2.39_605.5620*m/z*	HILIC ESI+	23.61
436	5-hydroxyoct-5-enoylglycine	HMDB0094763	7.53_180.1023*m/z*	HILIC ESI+	23.64
437	Menadione	HMDB0001892	3.61_207.0233*m/z*	HILIC ESI-	23.68
438	PC(14:1_24:0)	HMDB0007926	9.49_854.6102*m/z*	RP ESI+	23.69
439	PC(14:0_P-18:1)	HMDB0007897	5.16_715.5504n	HILIC ESI+	23.7

440	Tryptamine	HMDB0000303	4.37_178.1349*m/z*	HILIC ESI+	23.73
441	4-Methylcatechol	HMDB0000873	16.40_247.0960*m/z*	RP ESI-	23.77
442	Benzaldehyde	HMDB0006115	0.73_151.0399*m/z*	HILIC ESI-	23.77
443	Tetradecanedioic acid	HMDB0000872	7.71_257.1751*m/z*	RP ESI-	23.8
444	11-beta-Hydroxyandrosterone-3-glucuronide	HMDB0010351	1.27_483.2565*m/z*	HILIC ESI+	23.87
445	SM C16:1	HMDB0029216	1.09_680.5661*m/z*	HILIC ESI+	23.9
446	APGPR Enterostatin	HMDB0006117	9.34_477.2610*m/z*	RP ESI-	24.05
447	3-Buten-2-one 1-(2,3,6-trimethyl phenyl)	HMDB0059677	7.57_233.1168*m/z*	RP ESI-	24.07
448	(3R,6'R)-3-Hydroxy-3',4'-didehydro-beta,gammcarotene	HMDB0112260	15.54_533.4166*m/z*	RP ESI+	24.11
449	Lithocholyltaurine	HMDB0000722	10.17_504.2719*m/z*	RP ESI-	24.14
450	Nonadecanoic acid	HMDB0000772	0.43_297.2792*m/z*	HILIC ESI-	24.14
451	PC(22:6_p-18:0)	HMDB0008752	12.24_835.6387*m/z*	HILIC ESI+	24.18
452	Trihydroxycoprostanoic acid	HMDB0002163	1.11_464.3469n	HILIC ESI-	24.2
453	PC(20:4_P-16:0)	HMDB0008488	5.04_766.5774*m/z*	HILIC ESI+	24.25
454	Cinnamaldehyde	HMDB0003441	1.13_132.0575n	HILIC ESI+	24.27
455	Cer(d18:1_18:1)	HMDB0004948	19.74_563.5262n	RP ESI+	24.46
456	5-Heptadecyl-1,3-benzenediol	HMDB0038530	18.67_393.2977*m/z*	RP ESI-	24.47
457	Creatinine	HMDB0000562	4.88_225.1124*m/z*	RP ESI-	24.47
458	m-Chlorobenzoic acid	HMDB0001544	9.87_157.0058*m/z*	HILIC ESI+	24.57
459	Daidzein	HMDB0003312	6.87_291.0070*m/z*	HILIC ESI-	24.64
460	Stigmastanol	HMDB0000494	1.07_455.3668*m/z*	HILIC ESI+	24.65
461	Sulfolithocholylglycine	HMDB0002639	6.58_512.2678*m/z*	RP ESI-	24.65
462	PE(16:0_18:0)	HMDB0003990	14.03_419.3516*m/z*	RP ESI-	24.66
463	Pyridoxine	HMDB0000239	1.18_152.0699*m/z*	HILIC ESI+	24.71
464	Vitamin D3	HMDB0000876	1.01_367.3377*m/z*	HILIC ESI+	24.71
465	3,4-Methylenesebacic acid	HMDB0059729	6.21_207.1008*m/z*	RP ESI-	24.76
466	PC(20:3_18:0)	HMDB0008399	5.06_794.6077*m/z*	HILIC ESI+	24.76
467	1-Methoxy-4-propylbenzene	HMDB0032626	1.14_173.0928*m/z*	HILIC ESI+	24.81
468	Luteolin	HMDB0005800	11.13_267.0296*m/z*	HILIC ESI-	24.81

469	Linalyl oxide	HMDB0035907	10.72_339.2524*m/z*	RP ESI-	24.82
470	5-(3',4'-Dihydroxyphenyl)-gammvalerolactone	HMDB0029185	5.20_415.1384*m/z*	RP ESI-	24.84
471	DG(22:2_18:4)	HMDB0007657	13.37_668.5348n	RP ESI+	24.84
472	Uroporphyrin III	HMDB0000916	12.24_831.2429*m/z*	HILIC ESI+	24.87
473	DG(16:0_18:3)	HMDB0007104	1.01_613.4747*m/z*	HILIC ESI+	24.91
474	2-Methoxy-4-vinylphenol	HMDB0013744	1.04_151.0755*m/z*	HILIC ESI+	24.95
475	MG(20:3)	HMDB0011546	13.52_417.2400*m/z*	RP ESI-	24.97
476	Niacinamide	HMDB0001406	1.68_123.0556*m/z*	HILIC ESI+	25.07
477	LysoPC(24:0)	HMDB0010405	7.24_590.4546*m/z*	HILIC ESI+	25.1
478	Uracil	HMDB0000300	1.15_77.0124*m/z*	HILIC ESI+	25.13
479	SM(d18:1_14:0)	HMDB0012097	14.06_674.5416n	RP ESI+	25.15
480	1-Methylnicotinamide	HMDB0000699	11.39_160.0612*m/z*	HILIC ESI+	25.18
481	PC(18:0_22:0)	HMDB0008051	5.33_810.6659*m/z*	RP ESI+	25.23
482	Fructose 6-phosphate	HMDB0000124	6.77_305.0263*m/z*	HILIC ESI-	25.29
483	Palmitic amide	HMDB0012273	1.14_256.2631*m/z*	HILIC ESI+	25.29
484	3'-O-Methyl-(-)-epicatechin-5-O-sulphate	HMDB0029176	6.77_387.0306*m/z*	HILIC ESI-	25.3
485	9-Hexadecenoylcarnitine	HMDB0013207	7.59_478.2936*m/z*	RP ESI-	25.41
486	Deoxypyridinoline	HMDB0000569	4.18_430.2334*m/z*	RP ESI+	25.41
487	dCMP	HMDB0001202	0.75_352.0578*m/z*	HILIC ESI-	25.53
488	2-Acetyl-4,5-dihydrothiazole	HMDB0033561	4.81_150.0007*m/z*	RP ESI-	25.54
489	LysoPC(O-18:0)	HMDB0011149	9.93_544.3519*m/z*	RP ESI-	25.61
490	Threo-beta-phenylserine	HMDB0002184	0.95_180.0667*m/z*	RP ESI-	25.63
491	PC(o-18:1_18:2)	HMDB0013429	5.15_770.6006*m/z*	HILIC ESI+	25.65
492	Guanine	HMDB0000132	3.75_150.0420*m/z*	HILIC ESI-	25.66
493	Trimethylsilyl nonanoic acid	HMDB0094668	0.96_275.1682*m/z*	HILIC ESI-	25.66
494	17-betEstradiol-3-glucuronide	HMDB0006224	20.79_429.1938*m/z*	RP ESI-	25.71
495	Glycyl-Methionine	HMDB0028847	6.61_227.0463*m/z*	RP ESI-	25.74
496	Cer(d18:0_14:0)	HMDB0011759	5.93_529.5269*m/z*	HILIC ESI+	25.76
497	PE(14:0_18:0)	HMDB0008826	12.09_730.4779*m/z*	RP ESI+	25.79

498	PC(14:0_P-16:0)	HMDB0007895	20.78_707.5736*m/z*	RP ESI+	25.81
499	Stearic acid	HMDB0000827	10.24_321.2209*m/z*	RP ESI-	25.89
500	PS(18:2_14:1)	HMDB0012397	10.16_730.4688*m/z*	RP ESI+	25.9
501	5-Methoxyindoleacetate	HMDB0004096	5.95_188.0709*m/z*	HILIC ESI+	25.92
502	2-Deoxytetronic acid	HMDB0000337	6.79_141.0160*m/z*	HILIC ESI-	25.93
503	Suberylglycine	HMDB0000953	4.52_252.0865*m/z*	RP ESI-	25.93
504	Phenylacetylglutamine	HMDB0006344	3.41_263.1030*m/z*	RP ESI-	25.94
505	TG(16:0_16:0_18:1)	HMDB0005360	2.37_855.7384*m/z*	HILIC ESI+	25.95
506	3beta,7alpha-Dihydroxy-5-cholestenoate	HMDB0012454	1.13_415.3233*m/z*	HILIC ESI+	25.99
507	Umbelliferone	HMDB0029865	1.09_163.0393*m/z*	HILIC ESI+	26.04
508	Histidinyl-Glycine	HMDB0028885	1.32_195.0877*m/z*	HILIC ESI+	26.07
509	Sinapic acid	HMDB0032616	6.38_447.1331*m/z*	RP ESI-	26.08
510	DG(18:1_16:1)	HMDB0007186	1.00_592.5037n	HILIC ESI+	26.11
511	Terephthalic acid	HMDB0002428	19.69_149.0232*m/z*	HILIC ESI+	26.11
512	Tyramine-O-sulfate	HMDB0006409	4.59_198.0226*m/z*	RP ESI-	26.12
513	5-alphDihydrotestosterone glucuronide	HMDB0006203	5.74_465.2487*m/z*	RP ESI-	26.13
514	5,6-Dihydrouridine	HMDB0000497	6.02_245.0799*m/z*	RP ESI-	26.16
515	Cyclo(proline-leucine)	HMDB0034276	9.03_249.0979*m/z*	HILIC ESI+	26.21
516	gammGlutamylcysteine	HMDB0001049	9.90_215.0472*m/z*	HILIC ESI+	26.21
517	PC(14:0_20:3)	HMDB0007881	5.13_755.5402n	HILIC ESI+	26.23
518	Tetrahydrocortisone	HMDB0000903	1.14_387.2157*m/z*	HILIC ESI+	26.39
519	TG(14:0_18:0_20:5)	HMDB0042957	2.39_821.7354*m/z*	HILIC ESI+	26.41
520	2-Phenylethanol	HMDB0033944	1.14_105.0700*m/z*	HILIC ESI+	26.46
521	Queuine	HMDB0001495	0.89_553.2297*m/z*	HILIC ESI-	26.47
522	MG(22:4)	HMDB0011554	5.50_389.3046*m/z*	HILIC ESI+	26.51
523	Mono-(2-ethyl-5-hydroxyhexyl) phthalate	HMDB0094679	5.62_293.1389*m/z*	RP ESI-	26.6
524	PS(18:2_14:0)	HMDB0012396	11.33_714.4756*m/z*	RP ESI+	26.71
525	Isoleucyl-Serine	HMDB0028916	7.37_435.2490*m/z*	RP ESI-	26.79
526	Tridecanol	HMDB0013316	7.66_221.1893*m/z*	RP ESI-	26.83
527	Quinidine	HMDB0015044	4.04_324.1828n	HILIC ESI+	26.85

528	Acetyl citrate	HMDB0059808	11.13_269.0089*m/z*	HILIC ESI-	26.87
529	20-Carboxy-leukotriene B4	HMDB0006059	1.35_367.2085*m/z*	HILIC ESI+	26.88
530	PC(P-18:1_16:1)	HMDB0011306	5.05_764.5582*m/z*	HILIC ESI+	26.94
531	Ergocalciferol	HMDB0000900	13.82_379.3399*m/z*	RP ESI+	27
532	PS(18:2)_18:0)	HMDB0012400	5.31_788.5440*m/z*	HILIC ESI+	27.03
533	2-Furoylglycine	HMDB0000439	11.48_187.0712*m/z*	HILIC ESI+	27.15
534	PC(18:0_22:5)	HMDB0008055	5.01_858.5938*m/z*	HILIC ESI+	27.16
535	Tyrosyl-Phenylalanine	HMDB0029112	8.03_328.1430n	RP ESI-	27.18
536	12,13-DHOME	HMDB0004705	11.04_335.2194*m/z*	RP ESI-	27.24
537	DG(16:0_22:6)	HMDB0007121	8.60_675.4705*m/z*	RP ESI-	27.24
538	PC(18:0_14:1)	HMDB0008032	5.26_754.5354*m/z*	HILIC ESI+	27.27
539	DG(18:3_18:2)	HMDB0007277	18.44_615.4999*m/z*	RP ESI+	27.32
540	N1-Methyl-2-pyridone-5-carboxamide	HMDB0004193	3.59_152.0580n	RP ESI-	27.33
541	PC(o-16:0_18:0)	HMDB0013405	1.05_770.5970*m/z*	HILIC ESI+	27.34
542	PS(18:0_18:0)	HMDB0012378	5.30_774.5640*m/z*	HILIC ESI+	27.46
543	2-Hydroxyhexadecanoic acid	HMDB0031057	9.11_272.2345n	RP ESI-	27.49
544	Lactosylceramide (d18:1_22:0)	HMDB0011594	2.16_980.6804*m/z*	HILIC ESI-	27.66
545	MG(22:6)	HMDB0011557	5.52_402.2769n	HILIC ESI+	27.77
546	2-Hydroxyadipic acid	HMDB0000321	9.89_185.0415*m/z*	HILIC ESI+	27.8
547	5-Phenylvaleric acid	HMDB0002043	18.76_161.0962*m/z*	HILIC ESI+	27.85
548	14,15-DiHETrE	HMDB0002265	5.82_356.2791*m/z*	HILIC ESI+	27.89
549	PI(16:0_20:2)	HMDB0009786	13.37_880.5890*m/z*	RP ESI+	27.93
550	Phytoene	HMDB0002181	11.40_581.4543*m/z*	RP ESI-	27.96
551	5-Tricosyl-1,3-benzenediol	HMDB0038524	10.74_477.3913*m/z*	RP ESI-	28.01
552	Cystinylglycine	HMDB0000709	11.53_315.0802*m/z*	HILIC ESI+	28.02
553	3,4-Methylenepimelic acid	HMDB0059730	20.80_221.0237*m/z*	RP ESI-	28.06
554	PC(22:6_18:0)	HMDB0008727	5.05_834.6019*m/z*	HILIC ESI+	28.1
555	Glycoursodeoxycholic acid 3-sulfate	HMDB0002409	5.89_529.2691n	RP ESI-	28.11
556	Cholesterol sulfate	HMDB0000653	15.04_465.3043*m/z*	RP ESI-	28.26
557	Carnosine	HMDB0000033	18.48_261.0761*m/z*	RP ESI-	28.29

558	N-Oleoylethanolamine	HMDB0002088	4.90_308.2946*m/z*	HILIC ESI+	28.3
559	Urobilin	HMDB0004161	8.21_569.2725*m/z*	RP ESI-	28.37
560	Phenol	HMDB0000228	0.75_93.0351*m/z*	HILIC ESI-	28.38
561	2-Methylacetophenone	HMDB0032386	0.84_422.2340n	HILIC ESI+	28.45
562	PG(18:0_22:6)	HMDB0010614	5.33_840.5728*m/z*	HILIC ESI+	28.53
563	Tetrahydroaldosterone-3-glucuronide	HMDB0010357	4.56_539.2488*m/z*	RP ESI-	28.58
564	AlphCryptoxanthin	HMDB0002268	18.70_535.4307*m/z*	RP ESI+	28.59
565	2,6-Di-tert-butyl-4-methylphenol	HMDB0033826	7.00_220.1810n	RP ESI-	28.71
566	2-Undecanol	HMDB0030942	1.14_172.1843n	HILIC ESI-	28.91
567	Sucralose	HMDB0031554	0.58_441.0164*m/z*	RP ESI-	29
568	Ergosterol	HMDB0000878	11.48_435.3044*m/z*	RP ESI+	29.03
569	Hexadecyl Benzoic acid	HMDB0094685	5.72_367.2616*m/z*	RP ESI-	29.24
570	PC(14:1_18:1)	HMDB0007905	5.26_730.5396*m/z*	HILIC ESI+	29.28
571	PG(18:1_18:0)	HMDB0010617	18.58_777.5701*m/z*	RP ESI+	29.34
572	Octaethylene glycol	HMDB0094680	1.18_371.2274*m/z*	HILIC ESI+	29.35
573	3-Hepteneoylglycine	HMDB0094729	7.64_185.1053n	HILIC ESI+	29.38
574	3-Hydroxydodecanedioic acid	HMDB0000413	6.48_267.1202*m/z*	RP ESI-	29.47
575	13-HPODE	HMDB0003871	6.66_333.2059*m/z*	RP ESI-	29.54
576	9,12-Hexadecadienoylcarnitine	HMDB0013334	1.05_413.3351*m/z*	HILIC ESI+	29.62
577	Sphingosine 1-phosphate	HMDB0000277	7.24_424.2457*m/z*	RP ESI-	29.65
578	Dihyroxy-1H-indole glucuronide I	HMDB0059997	0.91_325.0812n	RP ESI-	29.77
579	Inosine	HMDB0000195	2.91_291.0700*m/z*	HILIC ESI+	29.79
580	LysoPC(18:3)	HMDB0010387	11.43_518.3264*m/z*	RP ESI+	29.79
581	PG(16:0_16:0)	HMDB0010570	13.47_740.5389*m/z*	RP ESI+	29.8
582	Sumiki's acid	HMDB0002432	9.87_160.0603*m/z*	HILIC ESI+	29.86
583	3-Sulfodeoxycholic acid	HMDB0002504	8.16_459.2420*m/z*	RP ESI+	30

Supplemental Table S10-A: Adjusted Beta Coefficients*
Dependent Variable: Mean pressure gradient

		Unstandardized Coefficients		Standardized Coefficients	t-value	p-value
		Beta	**Std. Error**	**Beta**		
1	Total LPA	8.523	3.825	0.253	2.229	*0.029*
2	16:0 Lyso PA	24.066	11.035	0.25	2.181	*0.033*
3	18:0 Lyso PA	44.401	21.281	0.243	2.086	*0.041*
4	18:1 Lyso PA	24.776	18.426	0.155	1.345	*NS*
5	18:2 Lyso PA	44.592	20.264	0.248	2.201	*0.031*
6	20:4 Lyso PA	64.529	29.975	0.243	2.153	*0.035*
7	22:6 Lyso PA	107.243	62.43	0.197	1.718	*NS*

* All models are adjusting for age, gender, LDL, HDL, TG, history of hypertension, history of smoking and presence of bicuspid aortic valve. Abbreviations: Std. Error, Standard Error

Supplemental Table S10-B: Adjusted Beta Coefficients*
Dependent Variable: Aortic valve area

		Unstandardized Coefficients		Standardized Coefficients	t-value	p-value
		Beta	**Std. Error**	**Beta**		
1	Total LPA	-0.214	0.083	-0.292	-2.571	*0.013*
2	16:0 Lyso PA	-0.71	0.233	-0.343	-3.05	*0.003*
3	18:0 Lyso PA	-1.009	0.461	-0.257	-2.188	*0.032*
4	18:1 Lyso PA	-0.741	0.416	-0.209	-1.782	*NS*
5	18:2 Lyso PA	-0.948	0.463	-0.236	-2.048	*0.045*
6	20:4 Lyso PA	-0.999	0.667	-0.174	-1.499	*NS*
7	22:6 Lyso PA	-3.445	1.336	-0.291	-2.578	*0.012*

* All models are adjusting for age, gender, LDL, HDL, TG, history of hypertension, history of smoking and presence of bicuspid aortic valve. Abbreviations: Std. Error, Standard Error

Supplemental Table S11:Clinical demographics for subgroup comparison based on annualized change in maximum jet velocity (ΔVmax)

Parameter	**Study Population (n=50)**		
Clinical	**Slow progressors (N=25)**	**Rapid progressors (N=25)**	***p-value***
Annualized ΔVmax ((m/s)/yr)	0.18 (0.14,0.22)	0.5 (0.41,0.85)	<0.001
Age, yrs	66 ± 14	69 ± 10	0.552
Male sex, n	17(68)	20 (80)	0.333
Height, cm	171 ± 7	172 ±7	0.452
Weight, kg	78 (72,96)	86 (81,101)	0.095
Body surface area, m2	1.9 ± 0.2	2.0 ± 0.16	0.345
Body mass index, kg/m2	29 ± 5.6	29 ± 3.5	0.788
History of hypertension, n (%)	13 (59)	19 (79)	0.139
Smoking History, n (%)	14 (58)	11 (46)	0.386
Current	4 (17)	2 (8)	
Previous	10 (42)	9 (38)	
Never	10 (42)	13 (54)	
Medication, n (%)			
Antihypertensive treatment	14 (58)	15 (63)	0.768
ACE inhibitors	5 (21)	8 (33)	0.330
ARBs	3 (13)	3 (14)	1.000
Statins	14 (58)	17 (71)	0.365
Laboratory data			
LDL cholesterol, mmol/l	3.0 (2.4,3.7)	2.0 (1.4,2.5)	0.004
HDL cholesterol, mmol/l	1.4 (1.1,1.5)	1.2 (1.0,1.5)	0.233
Triglycerides, mmol/l	1.0 (0.8,1.6)	1.2 (0.9,1.6)	0.568
Random glucose, mmol/l	6.2 (5.5,8.5)	6.5 (5.3,8.9)	0.793
Creatinine, (µmol/l)	83.4 ± 20.8	86.8 ± 17.8	0.535
Doppler echocardiographic data			
Bicuspid aortic valve, n (%)	6 (24)	6 (24)	0.935
Aortic valve calcification score, n (%)			
Calcification score = 1	2 (8)	1 (4)	
Calcification score = 2	4 (16)	6 (24)	
Calcification score = 3	12 (48)	13 (52)	
Calcification score = 4	5 (20)	3 (12)	
Calcification score = 5	2 (8)	2 (8)	
Peak aortic jet velocity, m/s	4.1 (3.6,4.4)	3.9 (3.8,4.6)	0.711
Peak pressure gradient, mm Hg	65 (53,78)	61 (57,88)	0.674
Mean pressure gradient, mm Hg	40 (28,49)	39 (34,54)	0.624
Aortic valve area, cm2	0.9 (0.7,1.0)	1.0 (0.9,1.1)	0.164
Indexed aortic valve area, cm2/m2	0.4 (0.3,0.6)	0.5 (0.4,0.5)	0.307
Left ventricular mass index, g/m2	134 (98,146)	111 (88,137)	0.285
Left ventricular ejection fraction	60 (60,60)	60 (60,60)	0.646

Values are reported as mean ± standard deviation (SD), median (25^{th}, 75^{th} percentiles), or count (percentage) as applicable. The Chi-square test was used for categorical variables, while Student's t-test or Mann-Whitney U test was used for continuous variables to assess for statistical significance across sample groups as applicable based on data distribution. Abbreviations: ΔVmax, annualized change in maximum jet velocity; ACE, angiotensin-converting-enzyme; ARBs, angiotensin II receptor blockers; HDL, high-density lipoprotein; LDL, low-density lipoprotein

Supplemental Table S12: Clinical demographics for 19 patients used for plasma LysoPA lipidomics	
Parameter	**Plasma study population (n=19)**
Clinical	
Age, yrs	70.3 (66,78)
Male sex, n (%)	12 (63)
Height, cm	170 (162,173)
Weight, kg	91 (78,96)
Body surface area, m^2	2.04 (1.8,2.1)
Body mass index, kg/m^2	30.5 (27.1,33.9)
History of hypertension, n (%)	12 (63)
Smoking History, n (%)	6 (32)
Current	3 (16)
Previous	1 (5)
Never	10 (52)
Medication, n (%)	
Antihypertensive treatment	9 (47)
ACE inhibitors	5 (26)
ARBs	2 (11)
Statins	13 (68)
Laboratory data	
LDL cholesterol, mmol/l	2.6 (2.0,3.0)
HDL cholesterol, mmol/l	1.1 (1.0,1.6)
Triglycerides, mmol/l	1.2 (1.0,1.5)
Random glucose, mmol/l	5.7 (5.2,7.1)
Creatinine, (µmol/l)	85 (72,104)
Doppler echocardiographic data	
Bicuspid aortic valve, n (%)	2 (11)
Aortic valve calcification score, n (%)	
Calcification score = 1	1 (5)
Calcification score = 2	2 (11)
Calcification score = 3	9 (47)
Calcification score = 4	3 (16)
Calcification score = 5	2 (11)
Peak aortic jet velocity, m/s	4.4 (3.4,5.1)
Peak pressure gradient, mm Hg	77 (45,103)
Mean pressure gradient, mm Hg	46 (32,65)
Aortic valve area, cm^2	0.81 (0.64,1.11)
Indexed aortic valve area, cm^2/m^2	0.46 (0.33,0.57)
Left ventricular mass index, g/m^2	128 (109,159)

Left ventricular ejection fraction	60 (55,60)
Values are reported as median (25th, 75th percentiles) or count (percentage) as applicable. Abbreviations: ACE, angiotensin-converting-enzyme; ARBs, angiotensin II receptor blockers; HDL, high-density lipoprotein; LDL, low-density lipoprotein	

Supplemental Table S13: Pearson correlation (r_p)

	Correlations	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	P-16:0 LPA														
2	P-18:0 LPA	.87**													
3	P-18:1 LPA	.92**	.74**												
4	P-18:2 LPA	.71**	.48*	.88**											
5	P-20:4 LPA	.72**	.47*	.79**	.79**										
6	P-22:6 LPA	.94**	.81**	.89**	.76**	.81**									
7	P-Total LPA	.98**	.84**	.97**	.82**	.79**	.96**								
8	T-16:0 LPA	.4	.17	.45	.35	.49*	.43	.41							
9	T-18:0 LPA	.28	.09	.38	.33	.42	.31	.32	.94**						
10	T-18:1 LPA	.09	-.17	.24	.44	.36	.14	.17	.58**	.66**					
11	T-18:2 LPA	.4	.14	.49*	.47*	.70**	.46*	.45	.82**	.78**	.66**				
12	T-20:4 LPA	.27	.07	.36	.33	.70**	.33	.32	.70**	.68**	.61**	.86**			
13	T-22:6 LPA	.44	.33	.37	.23	.23	.42	.4	.83**	.77**	.33	.53*	.27		
14	T-Total LPA	.34	.08	.44	.43	.56*	.39	.39	.94**	.93**	.79**	.91**	.82**	.69**	

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Abbreviations: P –Plasma, T-Tissue

Supplemental Table S14: MS-MS transitions and experimental conditions of Lysophosphatidic acids (LysoPA)

Sl.No	Analyte ID	Q1 mass (Da)	Q3 mass (Da)	Internal standard	CE (volts)	DP (volts)
1	LysoPA 14:0	381.2	153.0	LysoPA 17:0	-25.0	-100.0
2	LysoPA 16:0	409.2	153.0	LysoPA 17:0	-30.0	-110.0
3	LysoPA 18:0	437.2	153.0	LysoPA 17:0	-30.0	-110.0
4	LysoPA 18:1	435.2	153.0	LysoPA 17:0	-30.0	-110.0
5	LysoPA 18:2	433.2	153.0	LysoPA 17:0	-30.0	-100.0
6	LysoPA 20:4	457.2	153.0	LysoPA 17:0	-30.0	-110.0
7	LysoPA 22:6	481.3	153.0	LysoPA 17:0	-30.0	-100.0

Abbreviations: CE-Collision Energy; DP-Declustering Potential

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