Data S1. Materials and methods

Sample preparation and lipid extraction. Lipids were extracted from liver samples using a modified version of Folch method. Briefly, liver tissues (50.0±2.0 mg) were homogenized in 0.5 ml cold double distilled water and 1.2 ml dichloromethane:methanol (2:1 v/v). The mixture was mixed using vortex for 5 min followed by centrifugation at 11,000 x g at 4˚C for 15 min to enable separation of layers. The dichloromethane fraction was carefully decanted and evaporated under nitrogen stream to dryness and was stored at -20˚C until further experimentation.

Ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) analysis. The dichloromethane fractions (lipid extracts) were suspended in isopropanol-acetonitrile-water (2:1:1 v/v/v). The lipid extracts were subjected to MS/MS using an UPLC system (UltiMate 3000; Thermo Fisher Scientific, Inc.) coupled to an ESI-quadrupole/Orbitrap mass spectrometer (Q Exactive™; Thermo Fisher Scientific, Inc.) in both positive and negative ionization modes. Lipid extract (5 μl) was added to an Agela-Halo C18 column (2.1x100 mm x2.7 μm; Advanced Materials Technology, Inc.) at 45˚C and the autosampler at 4˚C. The binary gradient system comprised 10 mM ammonium acetate in acetonitrile:water (40:60, v/v; solvent A) and 10 mM ammonium acetate in acetonitrile:isopropanol (10:90, v/v; solvent B). The gradient profile was 15-40% B over 5 min, 40-70% B from 5-5.5 min, 70-75% B from 5.5-7.5 min, 75-78% B from 7.5-15 min and 78-85% B from 15-24 min. The mobile phase was reverted back to 15% B and equilibrated for 3 min for subsequent runs. The flow rate was maintained at 300 μl/min for 27 min.

Data was collected in the full scan mode in positive and negative electrospray ionisation with the following parameters: Sheath gas flow rate, 45 (arbitrary units); sheath gas pressure, 35 psi; nitrogen gas consumption, 8 l/min; auxiliary gas flow rate, 10 (arbitrary units); sweep gas flow rate, 0 (arbitrary units); spray voltage, 3.5 kV in positive mode and 3.0 kV in negative mode; mass collecting range, m/z 150-2000; resolution, 70,000; and (S)-lens radio frequency level, 55. Capillary temperature and auxiliary gas temperature were maintained at 320˚C. MS/MS analysis was performed on potential biomarker ions and the collision energy was automatically optimized.

To ensure that significant differences of serum metabolites in LC-MS resulted from the inherent differences between groups rather than from instrumental drift, the instrument stability and analytical repeatability were evaluated by analyzing quality control (QC) samples during the analytical run. The instrument repeatability and method repeatability were validated by analysing one QC sample in six continuous times and six replicates of QC samples, separately. Ten ion peaks (248.23454, 378.26383, 429.22728, 544.33933, 644.53092, 704.45111, 722.41715, 809.77014, 886.54955, 1302.94654) from the positive ion mode and ten ion peaks (171.08348, 207.06549, 304.23624, 479.35554, 591.44109, 669.33079, 839.58870, 900.56900, 1175.77652, 1520.06893) from the negative ion mode were extracted for method validation. In positive ion mode, the instrument repeatability and method repeatability, relative standard deviation of the peak intensities and retention times in positive ion mode were estimated to be 0.36-2.19 and 0.68-4.04%, respectively, and in negative ion mode were estimated to be 0.83-2.15 and 2.32-5.38%, respectively (Table SI). The deviation variation of all QC samples was further accessed via principal component analysis for method validation. The results showed that 12 QC samples in the positive ion mode and six in negative ion mode both fell within the 2 standard deviation region and 95% confidence interval (Fig. S3). QC samples were also further subjected to principal component analysis and partial least squares discriminant analysis with the experimental samples (Fig. S4). The score plots showed that most QC samples were clustered closely. These data indicated that the analytical platform provided the excellent precision and repeatability required for a large-scale metabolomics study.

MS/MS analysis. Standard references were used to monitor whether the experimental samples are lipid compounds and were used to find fragments of lipid mass spectrometry. For sphingolipids, as shown in Fig. S5a-1 and a-2, the m/z=239.059 is fatty acid (FA) chain (C18:1). For phospholipids, as shown in Fig. S5b-1 and b-2, m/z=716.524 is phosphatidylcholine (PC) (17:0/14:1) and m/z=239.05886 is lysoPC (14:1), which was broken down from PC (17:0/14:1). As shown in Fig. S5c-1 and c-2, m/z=782.534 is the standard of phosphatidylglycerol (PG) (17:0/20:4), m/z=800.545 is $[PG+NH4^{+}-2H]^{-}$, m/z=722.506 is the product ion $[M-CH_4O_2-H]^{-}$, and m/z=303.232 and 283.264 are $[lysoPG1-H]^{-}$ and $[lysoPG2-H]^{-}$, respectively, which are broken by ester bonds.

In addition, lysoPC (18:0) species were identified phosphocholine (-284.331) from protonated pseudomolecular ions of their MS/MS spectra (Fig. S5d-1 and d-2), whereas sphingomyelin (SM) (d18:0/12:0) species were identified by the loss of two fatty acyl chains from protonated pseudomolecular ions of their MS/MS spectra (Fig. S5e-1 and e-2).

For the analysis of the lipid profile structure in the samples, MS/MS data were collected and analyzed. MS and MS/MS data for these reference standards and QC samples were obtained by collision-induced dissociation (CID). The principle of CID is that molecules collide with neutral particles (helium, nitrogen or argon) to produce ion fragments; by collecting the data analysis of these ion fragments, the molecular structure of some or all of the ions can be determined (2). Both positive mode and negative mode fragmentation of these species yielded a wealth of structural information. In each case, head group fragmentation, lysoPC formation and FA fragments aided in the lipid identification process (Table SIII) (1). For example, lysoPC (18:1) species were identified: $[M-H]^{-}$ (m/z=521.350); $[M-H_2O-H]^{-}$ (m/z=503.341); and $[FA\ C18:1]^{-}$ (m/z=281.248). SM (d18:0/22:0) fragmentation in negative mode was identified: $[FA\ C18:0]^{-}$, m/z=283.264; and $[FA\ C22:0]^{-}$, m/z=339.200. In addition, a previous study found fragment ions of glycerophospholipids (1); in the present study, fragmentation of lactosylceramide (LacCer) (d18:1/25:0) and LacCer (d18:1/20:0) in positive mode exclusively yielded

a [HOP(O)OHOCH$_2$CH$_2$NMe3-H$_2$O]$^+$ ion (m/z=184.074), [OHCH2CH2NMe3]$^+$ (m/z=86.097), [PhCholine-H2O]$^+$ (m/z=166.159) and [choline]$^+$ (104.108). In addition, acyl chain information on triglyceride (TG) species, such as TG (56:11) and TG (58:13), were obtained via the loss of fatty acyl chains from pseudomolecular ions (Table SIII).

References

1. Milne S, Ivanova P, Forrester J and Alex Brown H: Lipidomics: An analysis of cellular lipids by ESI-MS. Methods 39: 92-103, 2006.
2. Wolfender J-L, Marti G and Ferreira Queiroz E: Advances in techniques for profiling crude extracts and for the rapid identification of Natural products: Dereplication, Quality Control and Metabolomics. Curr Org Chem 14: 1808-1832, 2010.

Figure S1. Total ion chromatograms from UPLCESI-MS/MS analysis. Chromatograms of (A) negative control, (B) spontaneously hypertensive and (C) oleanolic acid groups in positive ion mode obtained from UPLCESI-MS/MS analysis. UPLC-ESI-MS/MS, ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry.

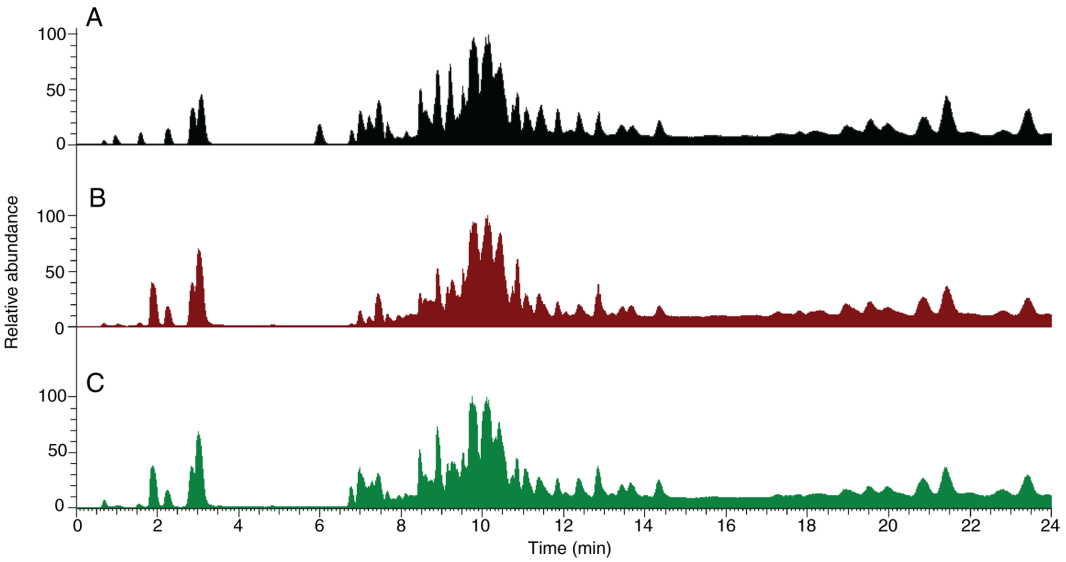

Figure S2. Total ion chromatograms from UPLCESI-MS/MS analysis. Chromatograms of (A) negative control, (B) spontaneously hypertensive and (C) oleanolic acid groups in negative ion mode obtained from UPLCESI-MS/MS analysis. UPLC-ESI-MS/MS, ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry.

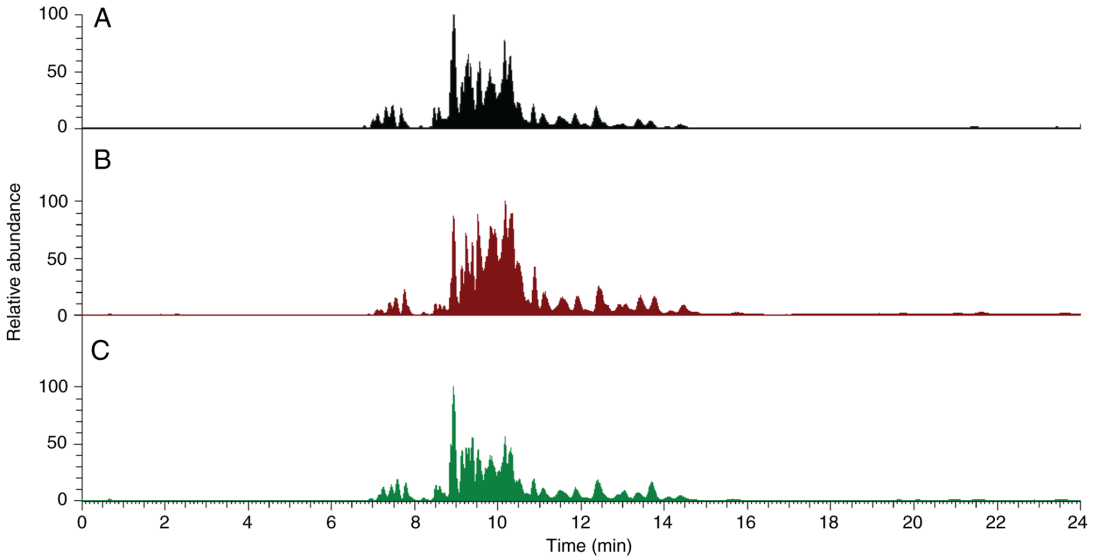

Figure S3. Principal component analysis line score plots of different injections of QC samples. X-axis represents the run order of QC samples; y-axis represents standard deviation or Hotelling's T2 range. Standard deviation in (A) ESI+ and (B) ESI- mode; Hotelling's T2 range in (C) ESI+ and (D) ESI- mode. ESI, electrospray ionization; QC, quality control.

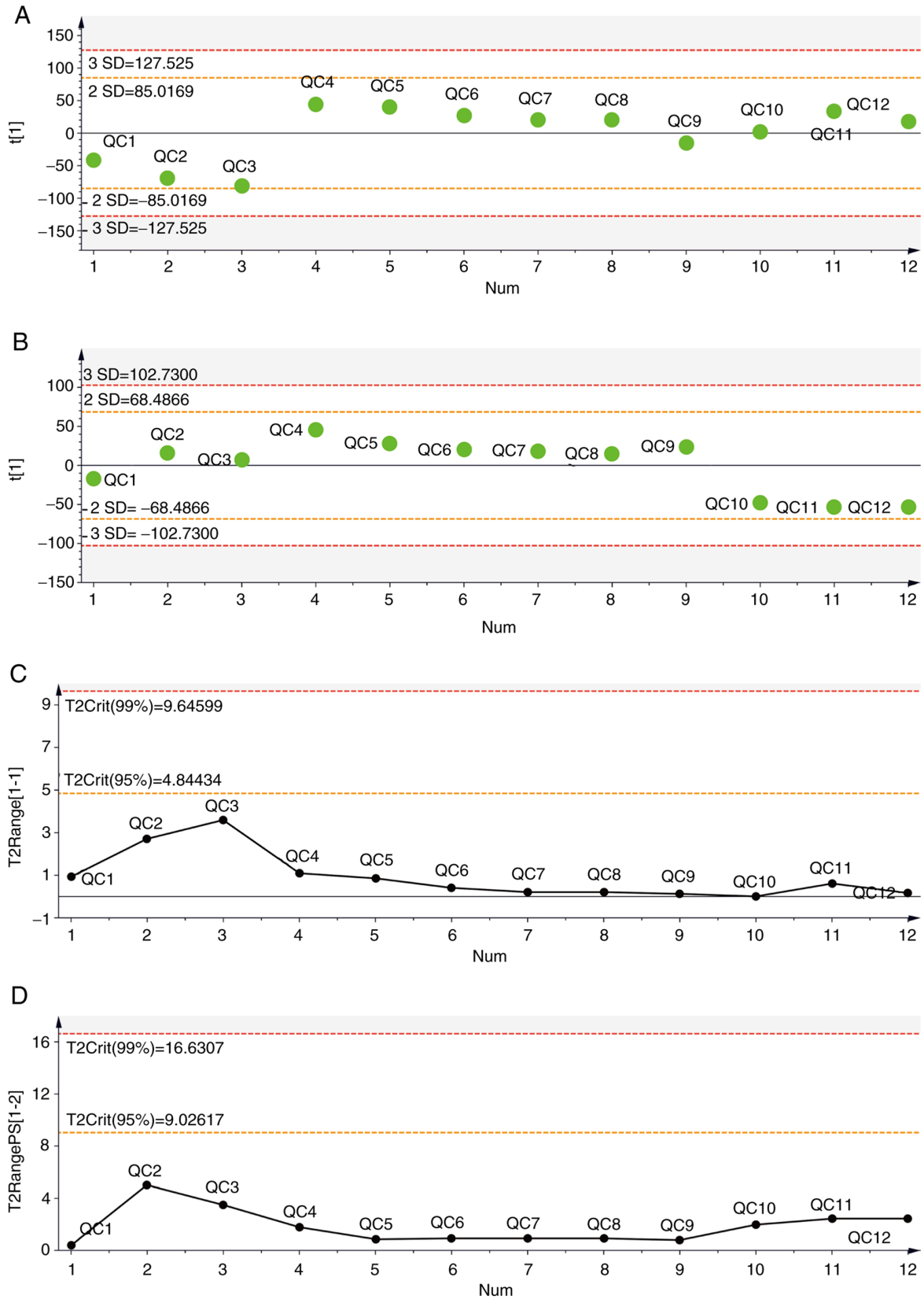

Figure S4. PCA and PLS-DA score plots of lipids in rats derived from ultra-performance liquid chromatographyelectrospray ionization-tandem mass spectrometry profiling. (A) PCA score plots in positive mode of the NC, SH and OA groups and QC. (B) PLS-DA score plots in positive mode of the NC, SH and OA groups and QC. (C) PCA score plots in negative mode of the NC, SH and OA groups and QC. (D) PLS-DA score plots in negative mode of the NC, SH and OA groups and QC. NC, negative control; SH, spontaneously hypertensive; OA, oleanolic acid; QC, quality control; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis.

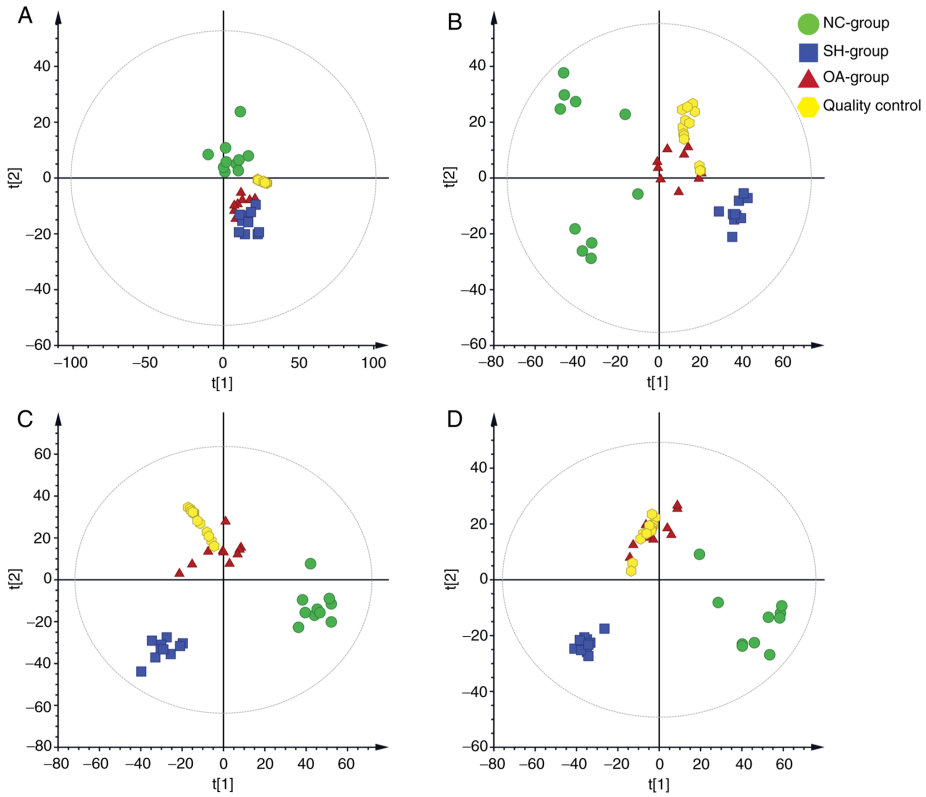

Figure S5. Mass spectrometry analysis of potential biomarkers. (a) Ceramide (d18:1/18:0) m/z 726.588 negative ion mode in (1) internal standard and (2) sample. (b) Phosphatidylcholine (17:0/14:1) m/z 716.524 negative ion mode in (1) internal standard and (2) sample. (c) Phosphatidylglycerol (17:0/20:4) m/z 800.54480 negative ion mode in (1) internal standard and (2) sample. (d) Lysophosphatiylcholine (18:0) m/z 508.377 positive ion mode in (1) internal standard and (2) sample. (e) Sphingomyelin (d18:0/12:0) m/z 649.527 positive ion mode in (1) internal standard and (2) sample. (f) Phosphatidylethanolamine internal standard m/z 591.44891 in (1) positive ion mode and m/z 589.43528 in (2) negative ion mode.

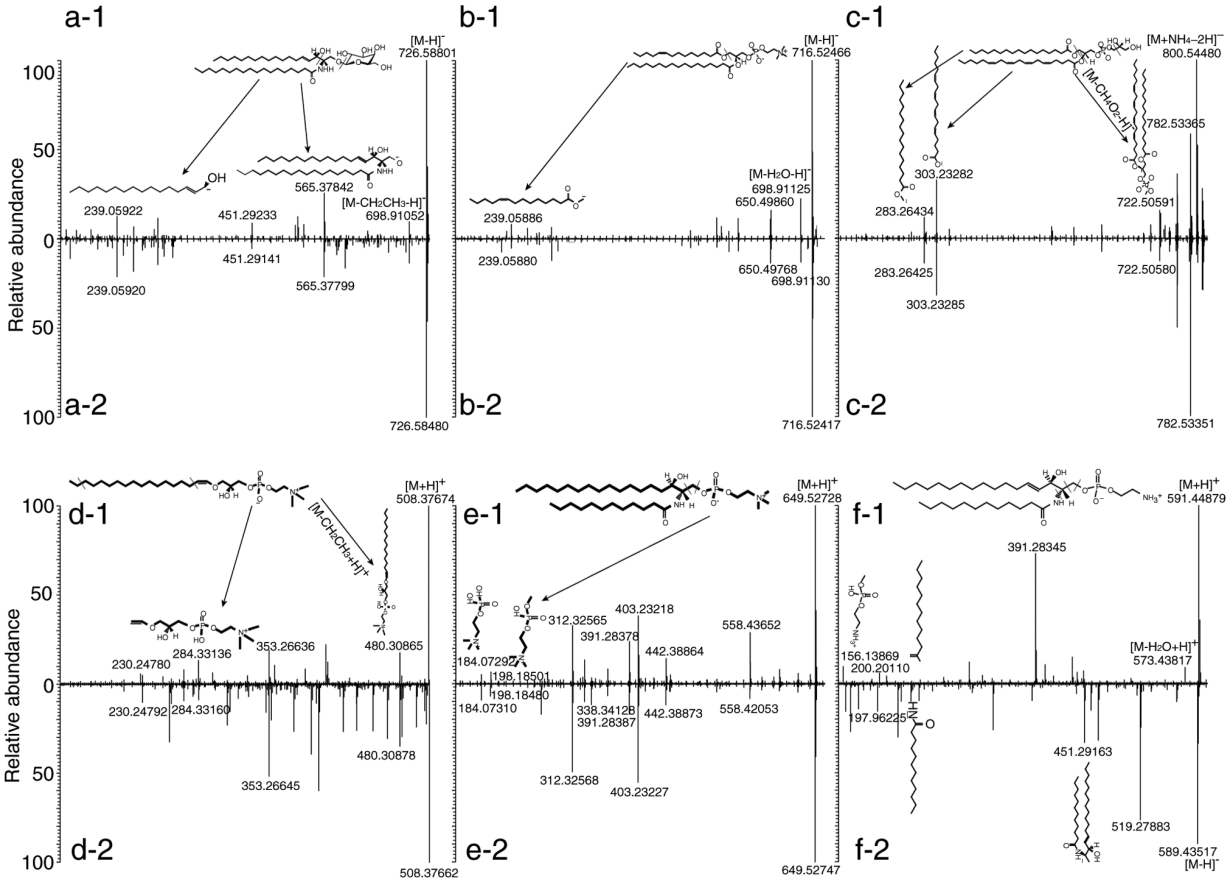

Table SI. RSD values of 10 ion signals in positive and negative ion modes.

A, Positive ion mode

m/z	t_R/min	RSD1, %	RSD2, %
248.23454	1.56	0.36	0.68
378.26383	4.82	0.44	1.04
429.22728	4.23	0.57	1.56
544.33933	6.98	0.53	1.52
644.53092	11.44	0.64	1.05
704.45111	8.94	0.72	1.88
722.41715	9.11	2.19	4.04
809.77014	12.38	1.96	3.92
886.54955	9.05	1.97	2.46
1302.94654	10.44	1.95	3.51

B, Negative ion mode

m/z	t_R/min	RSD1, %	RSD2, %
171.08348	0.78	0.83	4.44
207.06549	10.85	1.41	5.12
304.23624	8.95	1.02	2.83
479.35554	9.15	1.42	4.69
591.44109	9.26	2.15	2.82
669.33079	9.03	1.93	5.00
839.58870	9.98	1.81	4.34
900.56900	9.61	2.02	4.02
1175.77652	9.68	2.14	2.32
1520.06893	9.85	2.00	5.38

RSD, relative standard deviation; t_R, retention time.

Table SII. Identification of lipids from negative control and spontaneously hypertensive rats using ultra-performance liquid chromatography-electrospray ionization-MS/MS and MS^2 analysis.

Number	Formula	Metabolite	MS fragments	MS^2 fragment 1	MS^2 fragment 2	MS^2 fragment 3	MS^2 fragment 4
1	$C_{34}H_{67}NO_3$	GluCer (d18:1/25:0)	$[M-H]^-$ m/z=825.70577	$[M-H_2O-H]^-$ m/z=803.53656	$[FA\ C18:1]^-$ 281.24801	$[FA\ C25:0]^-$ 381.19515	N/A
2	$C_{39}H_{68}O_5$	DG (36:4)	$[M+H]^+$ m/z=616.50624	$[FA1+H]^+$ m/z=442.39006	$[FA2+H]^+$ m/z=403.23347	FA^+ 73.39056	N/A
3	$C_{50}H_{95}NO_{13}$	LacCer (d18:1/20:0)	$[M+H]^+$ m/z=917.68544	$[X]^+$ m/z=184.07364	$[Y-H_2O]^+$ m/z=86.09721	$[PhCholine-H_2O]^+$ m/z=166.15924	$[Y]^+$(choline) m/z=104.10764
4	$C_{55}H_{105}NO_{13}$	LacCer (d18:1/25:0)	$[M+H]^+$ m/z=987.75350	$[X]^+$ m/z=184.07382	$[Y-H_2O]^+$ m/z=86.09727	$[PhCholine-H_2O]^+$ m/z=166.15962	$[Y]^+$(choline) m/z=104.10747
5	$C_{26}H_{52}NO_7P$	LysoPC (18:1)	$[M-H]^-$ m/z=521.34950	$[M-H_2O-H]^-$ m/z=503.34071	$[FA\ C18:1]^-$ m/z=281.24839	$[GA–H_2O-H]^-$ m/z=152.95615	N/A
6	$C_{43}H_{85}O_8P$	PA (40:0)	$[M-H]^-$ m/z=759.5954	[FA C20:0] m/z=311.16874	N/A	N/A	N/A
7	$C_{40}H_{76}NO_8P$	PC (32:2)	$[M-H]^-$ m/z=729.53166	$[M-H_2O-H]^-$ m/z=711.54077	N/A	N/A	N/A
8	$C_{42}H_{80}NO_8P$	PC (34:2)	$[M-H]^-$ m/z=757.56290	$[FA\ C16:2]^-$ m/z=255.23252	N/A	N/A	N/A
9	$C_{42}H_{76}NO_8P$	PC (34:4)	$[M-H]^-$ m/z=753.53070	$[FA\ C18:0]^-$ m/z=283.26406	N/A	N/A	N/A
10	$C_{37}H_{74}NO_8P$	PE (32:0)	$[M-H]^-$ m/z=691.51380	$[M-H_2O-H]$ m/z=673.51978	N/A	N/A	N/A
11	$C_{45}H_{93}N_2O_6P$	SM (d18:0/22:0)	$[M-H]^-$ m/z=788.67284	$[FA\ C18:0]^-$ m/z=283.26406	$[FA\ C22:0]^-$ m/z=339.19961	N/A	N/A
12	$C_{59}H_{92}O_6$	TG (56:11)	$[M+H]^+$ m/z=896.68605	$[FA1+H]^+$ m/z=425.21518	$[FA2+H]^+$ m/z=403.23325	FA^+ m/z=73.38606	N/A
13	$C_{61}H_{92}O_6$	TG (58:13)	$[M+H]^+$ m/z=920.68623	$[FA1+H]^+$ m/z=427.22147	$[FA2+H]^+$ m/z=403.23334	FA^+ m/z=73.46214	N/A
14	$C_{60}H_{111}NO_{18}$	TriCer	$[M-H]^-$ m/z=1133.7749	N/A	N/A	N/A	N/A

LacCer, lactosylceramide; TriCer, trihexosylceramide; GluCer, glucosylceramide; SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; lysoPC, lysophosphatidylcholine; DG, diglyceride; TG, triglyceride; FA, fatty acid; GA, glycerophosphate; X, $C_5H_{15}NO_4P$; Y, $C_5H_{14}NO$.

Table SIII. Pathway impact analysis using Metabolomics Pathway Analysis for differential lipid species.

Number[a]	Pathway	Metabolites	Hits	P-value	-log(P)	Holm P	FDR	Impact
1	Sphingolipid metabolism	21	5	<0.001	15.799	<0.001	<0.001	0.424
2	Glycerophospholipid metabolism	36	4	<0.001	9.432	0.007	0.003	0.356
3	Glycerolipid metabolism	16	2	0.005	5.206	0.44982	0.154	0.012
4	Linoleic acid metabolism	5	1	0.036	3.325	1.000	0.755	0.000
5	α-Linolenic acid metabolism	13	1	0.091	2.396	1.000	1.000	0.000
6	Glycosylphosphatidylinositol-anchor biosynthesis	14	1	0.098	2.325	1.000	1.000	0.004
7	Phosphatidylinositol signaling system	28	1	0.187	1.678	1.000	1.000	0.002
8	Arachidonic acid metabolism	36	1	0.234	1.453	1.000	1.000	0.000

[a]Number corresponds to the numbers in Fig. 7A. FDR, false discovery rate.