Electronic Supplementary Material (ESI) for Molecular Omics.

Supporting Information

Serum Metabolic Profile Of Septic Shock Patients Based Upon Co-Morbidities and Other Underlying Conditions

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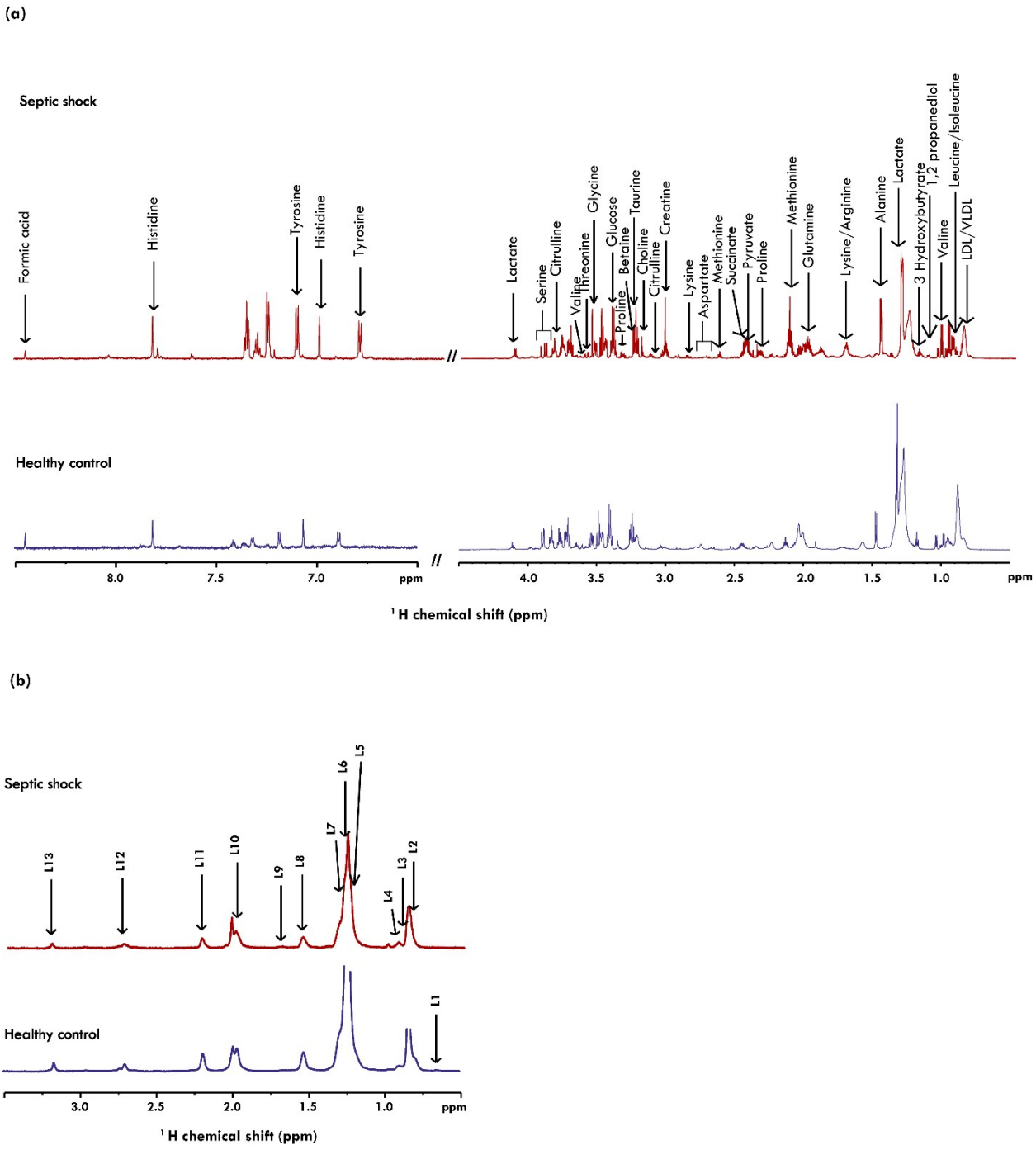

Figure S1: (a) A representative 800-MHz ^{1}H CPMG NMR spectra of serum obtained from septic shock patients and healthy control. (b) A representative diffusion edited 1H NMR spectra of serum obtained from septic shock patients and healthy control. L1-L12: Fatty acyl chain protons of various lipid types (triglycerides, phospholipids, cholesteryl esters) all as part of lipoproteins (HDL, LDL, VLDL). L1: CH_3 of bile acids; L2: CH_3 of HDL; L3: CH_3 of LDL; L4: CH_3 of VLDL; L5: $(CH_2)n$ OF HDL; L6: CH_2 OF LDL; L7: CH_2 OF VLDL; L8: $CH_2CH_2CH_2CO$; L9: CH_2CH_2CO; L10: NAG; L11: CH_2CO; L12: =CHCH2CH=; L13: Choline head group protons from phospholipids, mainly in lipoproteins. L14: $N(CH_3)_3$.

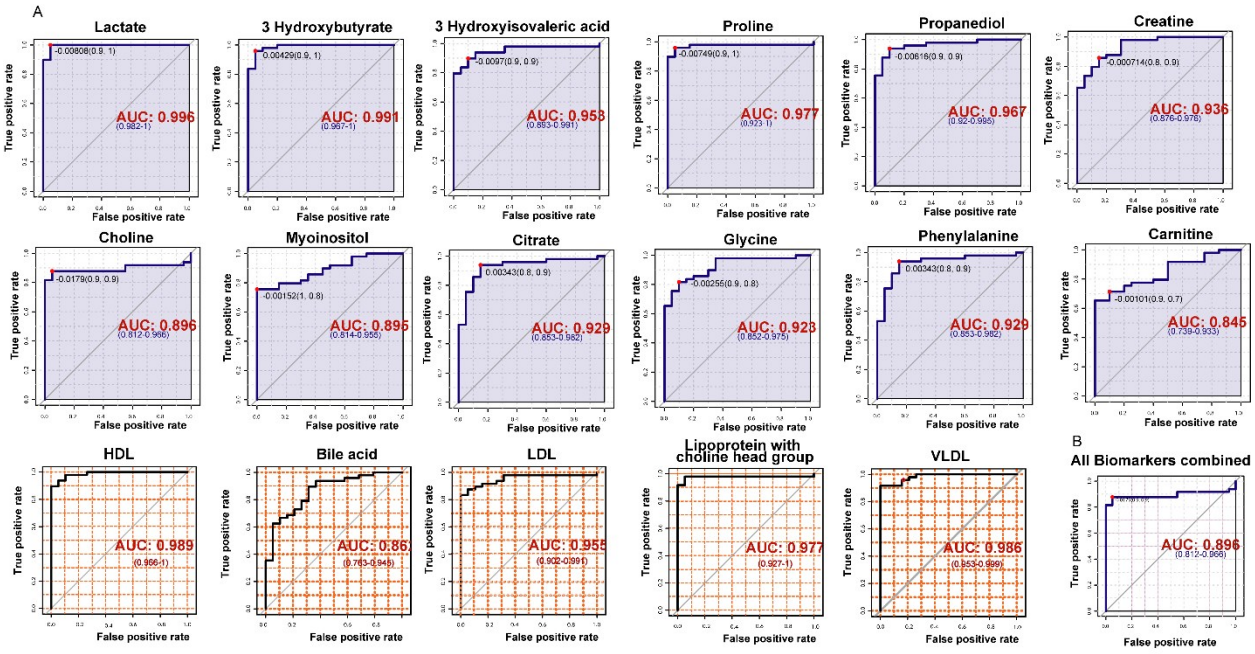

Figure S2: (A)Potential biomarker candidate metabolites identified after ROC curve analysis performed with all key discriminatory small molecular weight metabolites and lipid molecules, responsible for the separation between septic shock and healthy control groups tabulated in **SI Table 1**. (B) Shows the ROC curve of all potential biomarkers combined.

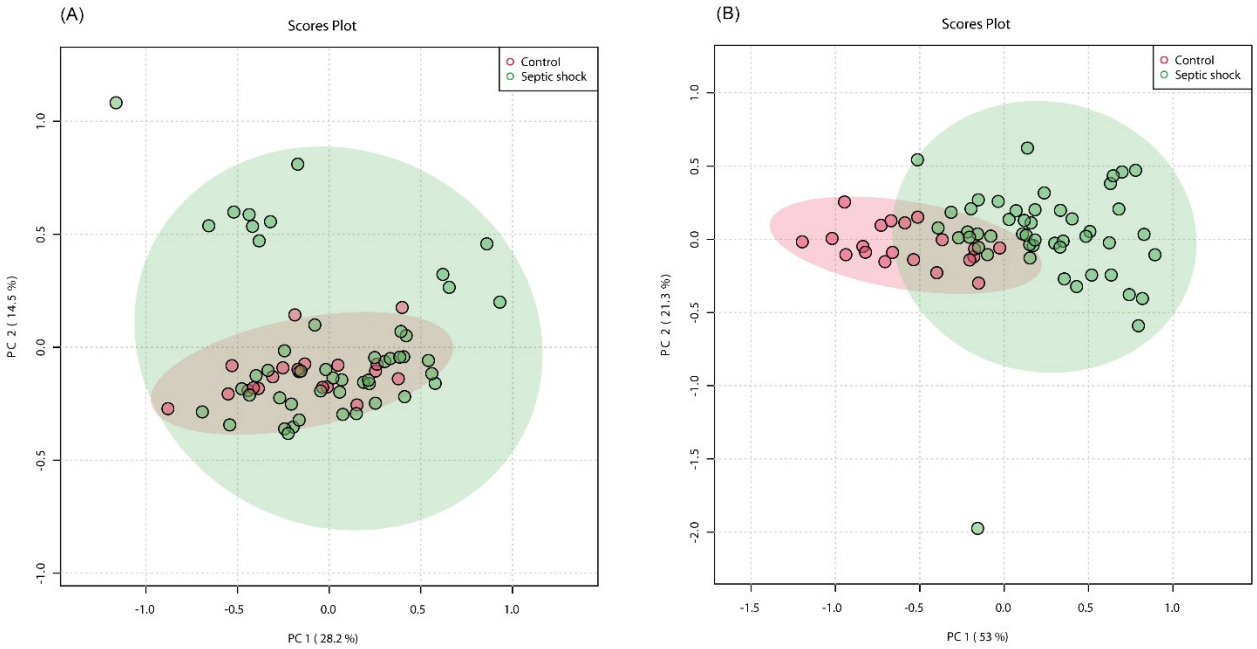

Figure S3: PCA score plots derived from (A)1D CPMG (B) 1D diffusion edited ^{1}H NMR spectra of serum samples between patients with septic shock and healthy control.

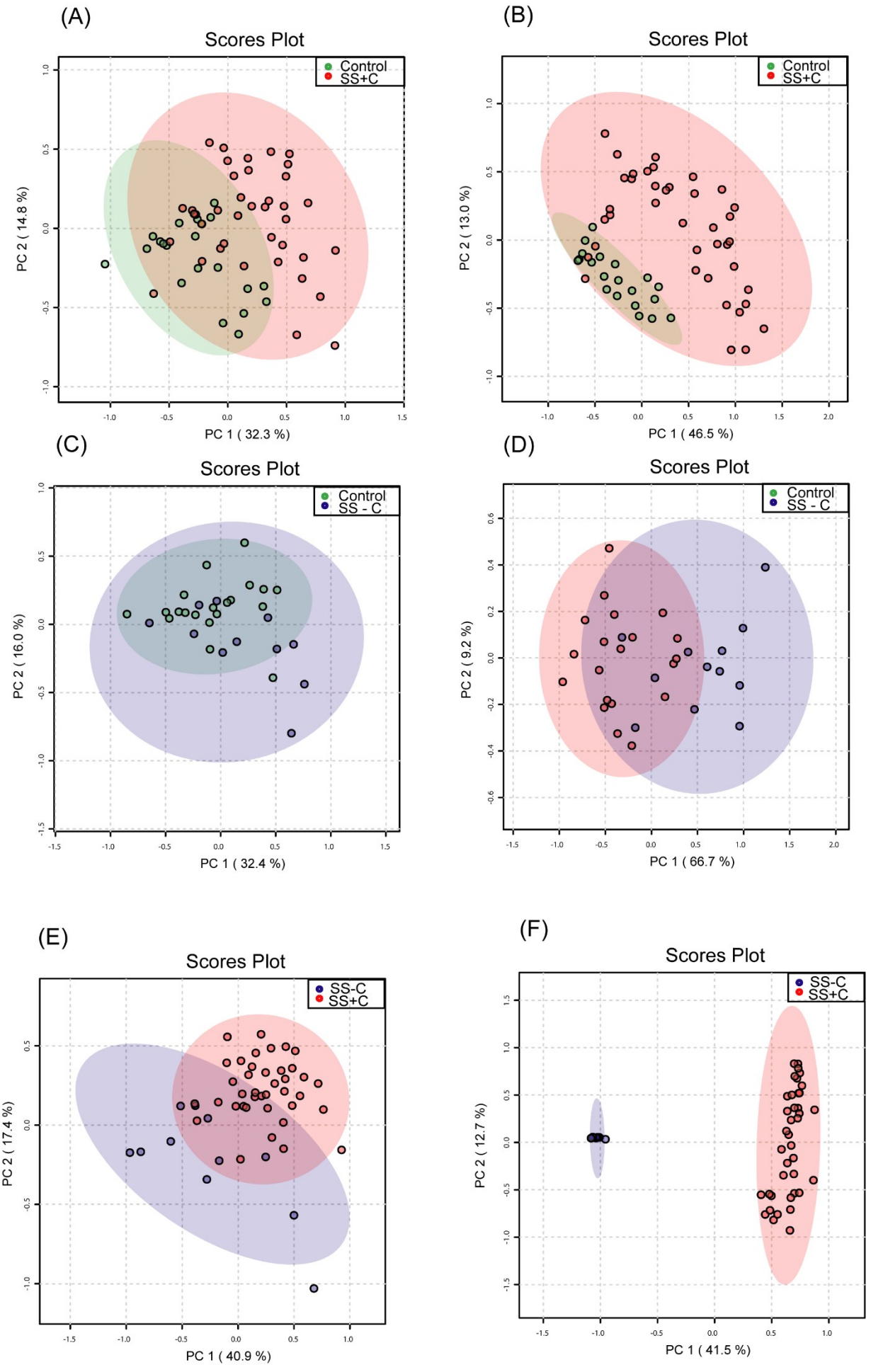
(A)
Scores Plot
Control
SS+C
PC 2 (14.8 %)
PC 1 (32.3 %)
(B)
Scores Plot
Control
SS+C
PC 2 (13.0 %)
PC 1 (46.5 %)
(C)
Scores Plot
Control
SS - C
PC 2 (16.0 %)
PC 1 (32.4 %)
(D)
Scores Plot
Control
SS - C
PC 2 (9.2 %)
PC 1 (66.7 %)
(E)
Scores Plot
SS-C
SS+C
PC 2 (17.4 %)
PC 1 (40.9 %)
(F)
Scores Plot
SS-C
SS+C
PC 2 (12.7 %)
PC 1 (41.5 %)

Figure S4: (A)(B)PCA score plots derived from 1D CPMG and 1D diffusion edited ^{1}H NMR spectra of serum samples between SS+C vs C respectively.(C)(D) PCA score plots derived from 1D CPMG and 1D diffusion edited ^{1}H NMR spectra of serum samples between SS-C vs C respectively.(E)(F) PCA score plots derived from 1D CPMG and 1D diffusion edited ^{1}H NMR spectra of serum samples between SS-C vs SS+C respectively. SS+C:septic shock with comorbidities, SS-C : septic shock without comorbidities,C : control

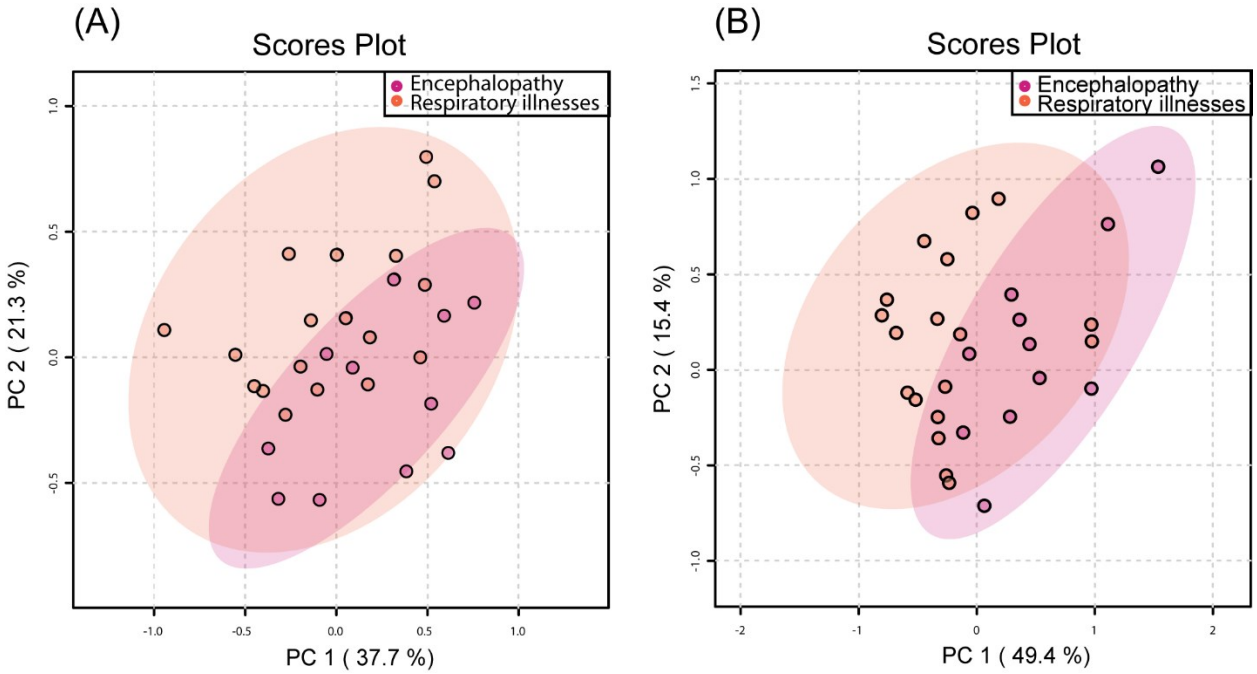

Figure S5: : (A)(B)PCA score plots derived from 1D CPMG and 1D diffusion edited ^{1}H NMR spectra of serum samples between respiratory illnesses and encephalopathy, respectively.

Metabolite	ppm	p-value	VIP	Correlation	FDR	AUROC
Lactate	1.332	< 0.0001	4.85	0.42	0.01	0.99
3 Hydroxybutyrate	1.204	< 0.0001	4.54	0.44	0.05	0.99
3-Hydroxyisovalerate	1.274	< 0.0001	4.45	0.51	0.02	0.95
Proline	4.138	< 0.0001	4.36	0.43	0.03	0.97
1,2-Propanediol	1.146	< 0.0001	4.1	0.41	0.06	0.96
Creatine	3.04	0.01	3.85	0.47	0.03	0.93
Citrate	2.553	< 0.0001	3.25	-0.48	0.02	0.92
2 Hydroxybutyrate	0.903	0.03	3.11	0.48	0.05	0.82
Glycine	3.564	0.04	2.65	0.44	0.08	0.92
Phenylalanine	7.333 , 7.381 , 7.432	< 0.0001	2.42	0.52	0.004	0.92
Glycoprotein	2.01	< 0.0001	2.26	0.86	0.002	0.83
Myoinositol	3.625	< 0.0001	2.21	-0.45	0.002	0.89
Glutamine	2.145,2.459	< 0.0001	2.12	0.45	0.05	0.80
Choline	3.209,4.070	< 0.0001	2.03	-0.73	0.03	0.89
Carnitine	3.233	0.0007	1.56	-0.42	0.09	0.84
Pyruvate	2.36	< 0.0001	2.01	0.34	0.001	0.89
Isoleucine	0.944,1.015	0.0009	1.58	-0.44	0.03	0.70
Lysine	1.452,3.030	0.0012	1.56	0.47	0.05	0.80
Aspartic acid	2.675,2.807	0.02	1.56	0.48	0.06	0.74
Alanine	1.4	< 0.0001	1.53	-0.49	0.01	0.82
Valine	0.996 , 1.047	0.001	1.52	-0.25	0.04	0.72
Threonine	4.261	< 0.0001	1.5	-0.62	0.02	0.50
Glutamate	2.357	0.04	1.51	0.64	0.08	0.89
Methionine	2.648. 3.87	0.0001	1.51	-0.45	0.07	0.76
Leucine	0.972,0.961,3.74	0.02	1.49	-0.41	0.06	0.77
Tyrosine	7.199	< 0.0001	2.32	-0.44	0.05	0.67
LIPID molecules						
CH3 of Bile acid	0.7	0.01	2.46	0.5	0.06	0.86
CH3 of HDL	0.86	0.05	3.46	-0.7	0.05	0.98
CH3 of LDL	0.88	0.01	1.95	-0.85	0.03	0.95
CH3 of VLDL	0.89	<0.0001	2.78	0.45	0.09	0.98
(CH2)n of HDL	1.26	< 0.0001	3.33	-0.74	0.06	0.94
CH2 of LDL	1.28	<0.0001	4.48	-0.46	0.06	0.91
CH2 of VLDL	1.29	< 0.0001	2.99	0.74	0.07	0.90
CH2CH2CH2CO	1.34,1.38	< 0.0001	2.34	-0.47	0.07	0.87
CH2CH2CO	1.58,1.62	< 0.0001	1.96	-0.77	0.05	0.52
NAG	2.01	< 0.0001	1.36	0.73	0.03	0.97
N(CH3)3 of phospholipid with choline headgroup	3.21	< 0.0001	1.07	-0.51	0.09	0.95

Table SI 1: List of significant small metabolites and lipid molecules identified using correlations, VIP obtained by the discriminant analysis (OPLSDA) of CPMG of CPMG and diffusion edited 1H NMR spectra, and performing unpaired t-test. p values that are are adjusted with Bonferroni correction, FDR (false discovery rate).The cut off criterion being correlation >0.4, VIP >1, p value < 0.05, FDR <0.1.

Fatty acyl chain of various lipid types (trigylcerides, phospholipids, cholestryl esters all as a part of lipoprotein(HDL,LDL,VLDL)			
S.No.	**Metabolites**	**Chemical shift (ppm)**	**Labeled as**
1	CH_3 OF BILE ACIDS	0.7	L1
2	CH_3 of HDL	0.86	L2
3	CH_3 of LDL	0.88	L3
4	CH_3 of VLDL	0.89	L4
5	$(CH_2)n$ OF HDL	1.26	L5
6	CH_2 OF LDL	1.28	L6
7	CH_2 OF VLDL	1.29	L7
8	$CH_2CH_2CH_2CO$	1.34,1.38,1.42,1.46	L8
9	CH_2CH_2CO	1.58,1.62	L9
10	NAG	2.01	L10
11	CH_2CO	2.18,2.26,2.3	L 11
12	$NCHCH_2CHN$	2.78,2.82	L12
Choline head group protons from phopholipids, mainly in lipoproteins			
	Metabolites	**Chemical shift (ppm)**	**Labeled as**
13	$N(CH_3)_3$	3.26/3.21	L13
14	$N–CH_2$	3.54	L14

SI Table 2: Assignment of fatty acyl chains and lipoprotein complexes

Metabolite	ppm	p-value	VIP	Correlation	FDR	AUROC
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SI Table 3: Assignment of fatty acyl chains and lipoprotein complexes