Analytical and Bioanalytical Chemistry

Supporting information

AN UNTARGETED METABOLOMIC STRATEGY BASED ON LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY TO STUDY HIGH GLUCOSE-INDUCED CHANGES IN CULTURED HUMAN PROXIMAL TUBULAR CELLS

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Experimental conditions

Initial procedure for sample preparation of the intracellular fluid

To begin with the extraction optimization of intracellular fluid, cell pellets from *section 2.2* were extracted with 400 µL of 75 % (v/v) MeOH in water, vortexed for 30 s and left still for 5 min in an ultrasonic bath followed by a centrifugation step (14000*g* for 5 min at 4 ºC). The supernatant was separated into two equal parts of 200 µL each and were evaporated for 3.5 h till dryness. 100 µL of 80 % (v/v) acetonitrile in water were added to the dried samples to be analyzed by HILIC and 100 µL of water were added to the dried samples to be analyzed by RPLC, they were vortexed for 30 s, centrifuged at 14000*g* for 5 min at 4 ºC and supernatants were placed in glass inserts for further analyses.

Initial conditions for intracellular fluid LC-MS analysis

All initial LC-MS conditions are the same as the ones included in *section 2.4*, except for the fragmentator which was set at 100 V both in HILIC and RPLC and that analyses were performed in positive ESI mode for both chromatographic modes.

Figures

Figure S1. PCA comparing different data normalization methods: A) dataset normalized to protein content, and B) dataset normalized to cell number.

Figure S2. PCA without QCs for the four analytical sequences. A) intracellular fluid (HILIC), B) intracellular fluid (RPLC), C) extracellular fluid (HILIC), and D) extracellular fluid (RPLC).

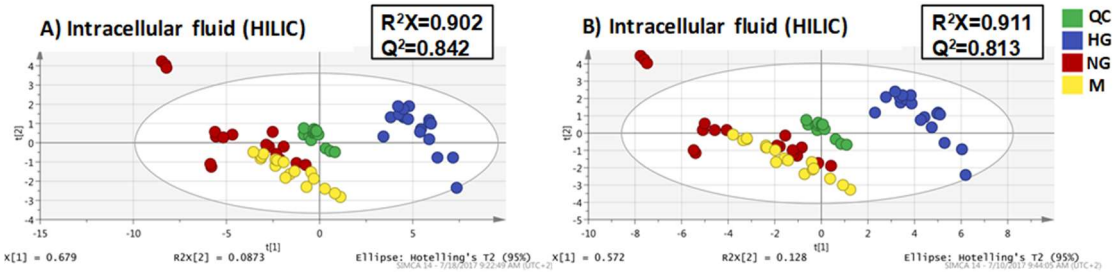
Figure S3. Permutation tests of all PLS-DA models.

Figure S4. Box-plots of the five metabolites of the intracellular fluid unequivocally and tentatively identified.

Figure S5. Box-plots of the five metabolites of the extracellular fluid unequivocally and tentatively identified.

Figure S6. Box-plots of the five metabolites of the both fluids unequivocally and tentatively identified.

Figure S1.



Normalized to protein content

Normalized to the cell number

Figure S2.

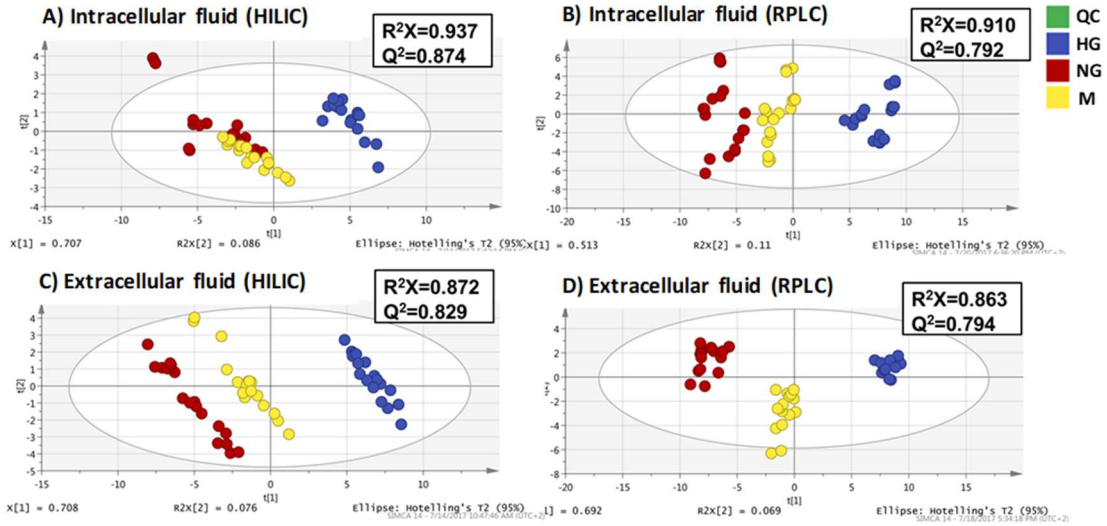

A) Intracellular fluid (HILIC)
R²X=0.937
Q²=0.874
B) Intracellular fluid (RPLC)
R²X=0.910
Q²=0.792
QC
HG
NG
M
C) Extracellular fluid (HILIC)
R²X=0.872
Q²=0.829
D) Extracellular fluid (RPLC)
R²X=0.863
Q²=0.794
t[1]
t[2]
R2X[2] = 0.086
R2X[2] = 0.11
R2X[2] = 0.076
R2X[2] = 0.069
Ellipse: Hotelling's T2 (95%)

Figure S3.

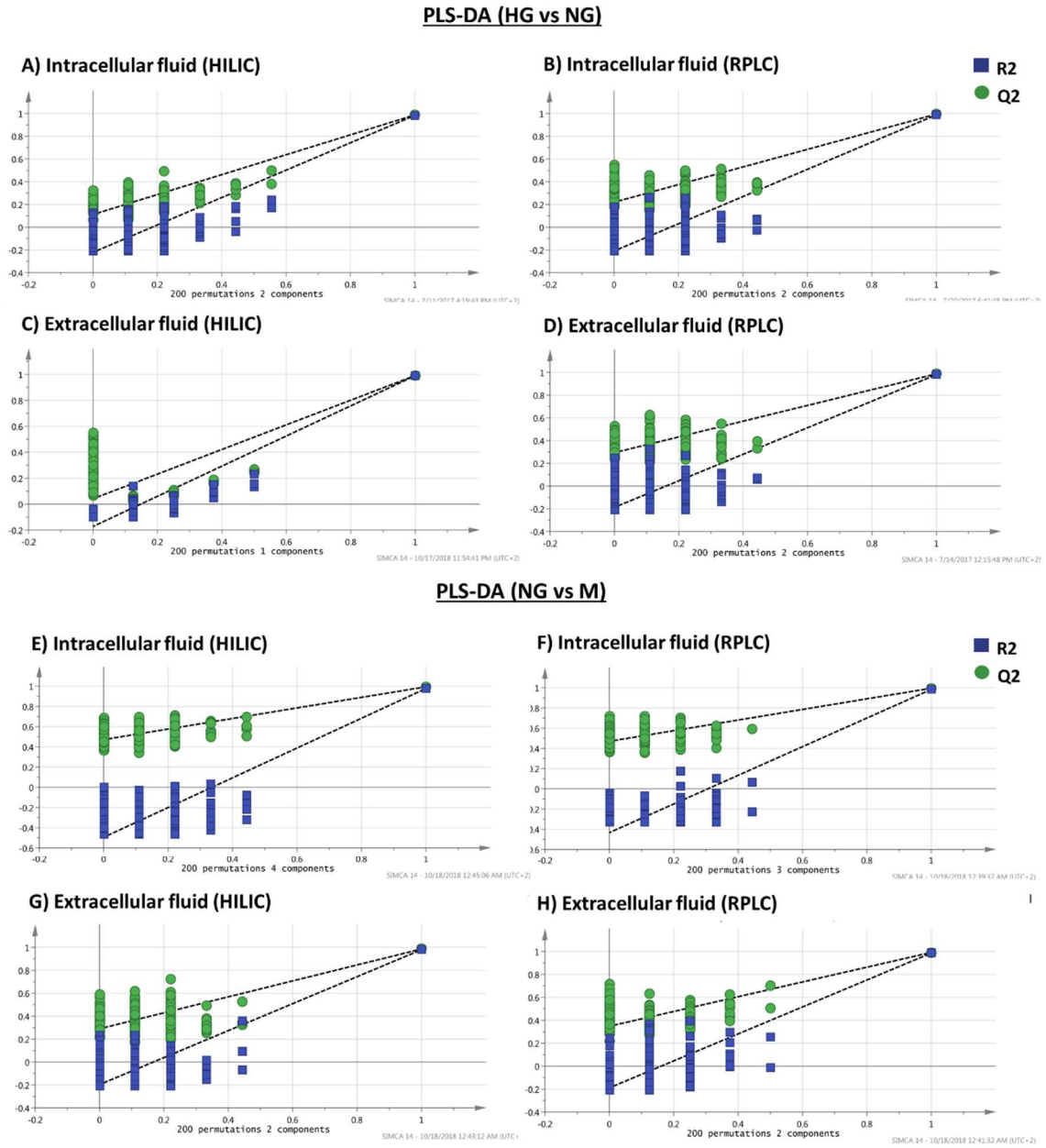

PLS-DA (HG vs NG)
A) Intracellular fluid (HILIC)
B) Intracellular fluid (RPLC)
R2
Q2
200 permutations 2 components
C) Extracellular fluid (HILIC)
200 permutations 1 components
D) Extracellular fluid (RPLC)
200 permutations 2 components
PLS-DA (NG vs M)
E) Intracellular fluid (HILIC)
F) Intracellular fluid (RPLC)
200 permutations 4 components
200 permutations 3 components
G) Extracellular fluid (HILIC)
H) Extracellular fluid (RPLC)
200 permutations 2 components
200 permutations 2 components

Figure S4.

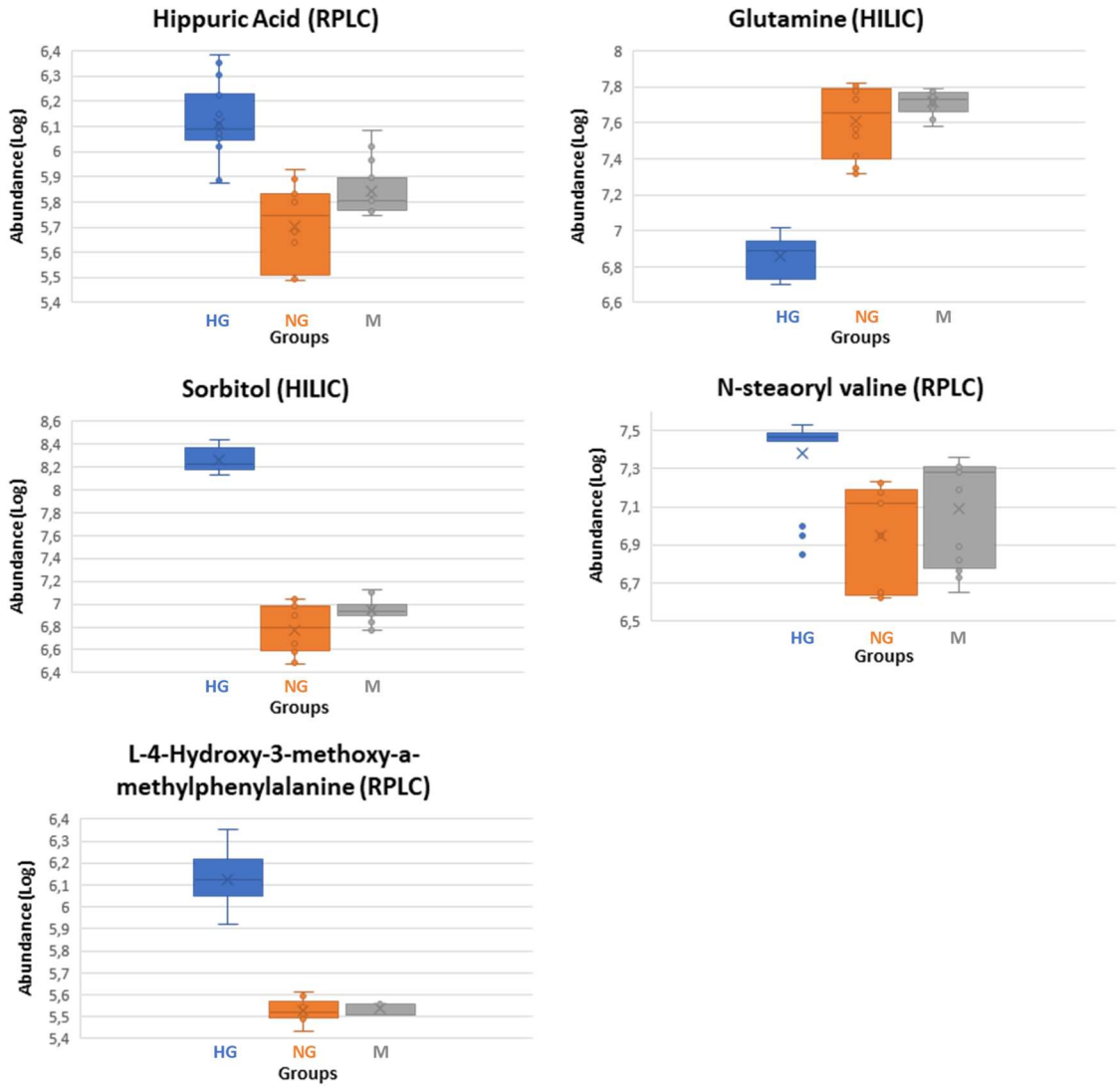

Hippuric Acid (RPLC)
Abundance (Log)
6,4
6,3
6,2
6,1
6
5,9
5,8
5,7
5,6
5,5
5,4
HG
NG
M
Groups
Glutamine (HILIC)
Abundance (Log)
8
7,8
7,6
7,4
7,2
7
6,8
6,6
HG
NG
M
Groups
Sorbitol (HILIC)
Abundance (Log)
8,6
8,4
8,2
8
7,8
7,6
7,4
7,2
7
6,8
6,6
6,4
HG
NG
M
Groups
N-steaoryl valine (RPLC)
Abundance (Log)
7,5
7,3
7,1
6,9
6,7
6,5
HG
NG
M
Groups
L-4-Hydroxy-3-methoxy-a-methylphenylalanine (RPLC)
Abundance (Log)
6,4
6,3
6,2
6,1
6
5,9
5,8
5,7
5,6
5,5
5,4
HG
NG
M
Groups

Figure S5.

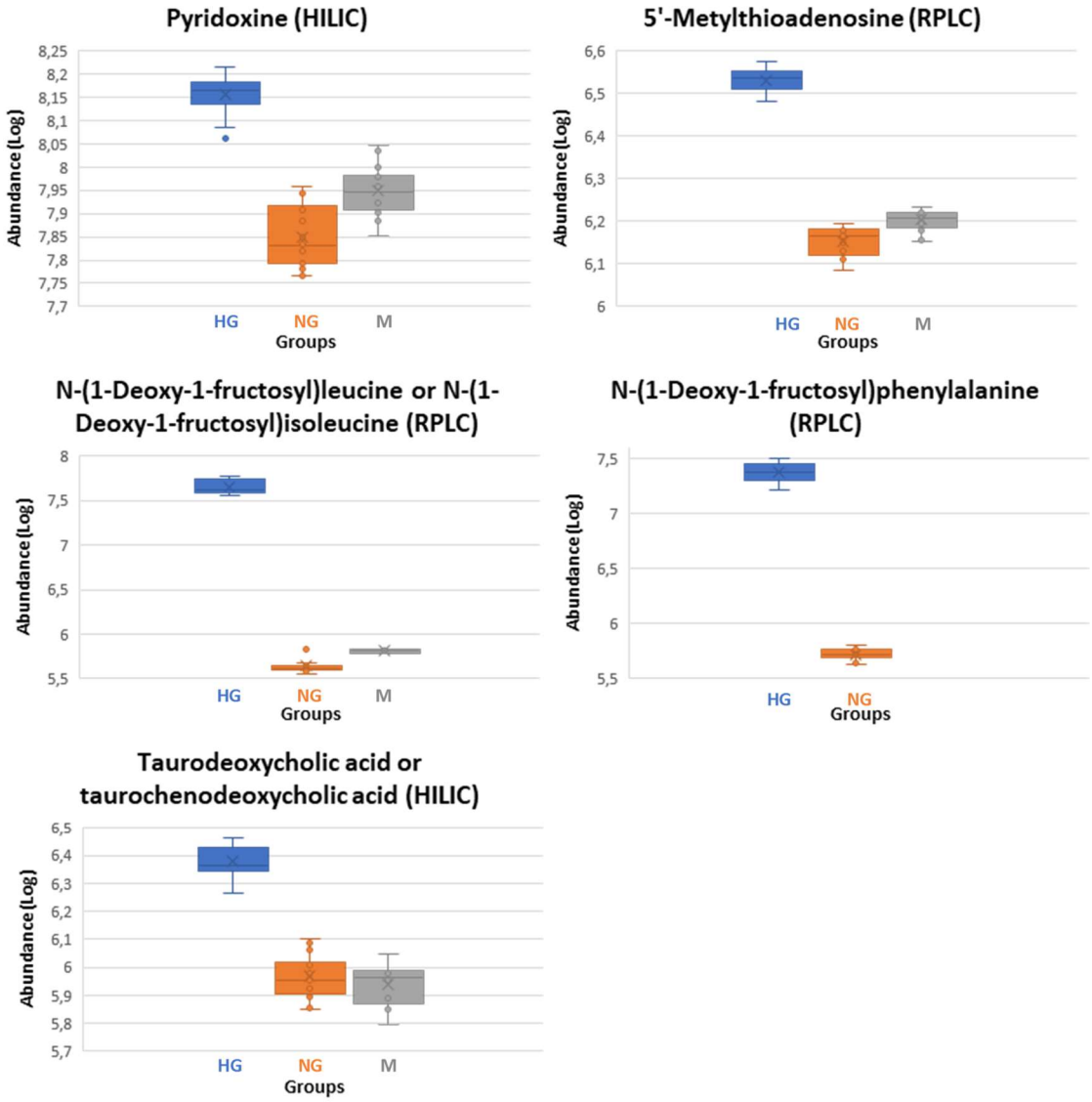

Pyridoxine (HILIC)
Abundance (Log)
8,25
8,2
8,15
8,1
8,05
8
7,95
7,9
7,85
7,8
7,75
7,7
HG
NG
M
Groups
5'-Metylthioadenosine (RPLC)
Abundance (Log)
6,6
6,5
6,4
6,3
6,2
6,1
6
HG
NG
M
Groups
N-(1-Deoxy-1-fructosyl)leucine or N-(1-Deoxy-1-fructosyl)isoleucine (RPLC)
Abundance (Log)
8
7,5
7
6,5
6
5,5
HG
NG
M
Groups
N-(1-Deoxy-1-fructosyl)phenylalanine (RPLC)
Abundance (Log)
7,5
7
6,5
6
5,5
HG
NG
Groups
Taurodeoxycholic acid or taurochenodeoxycholic acid (HILIC)
Abundance (Log)
6,5
6,4
6,3
6,2
6,1
6
5,9
5,8
5,7
HG
NG
M
Groups

Figure S6.

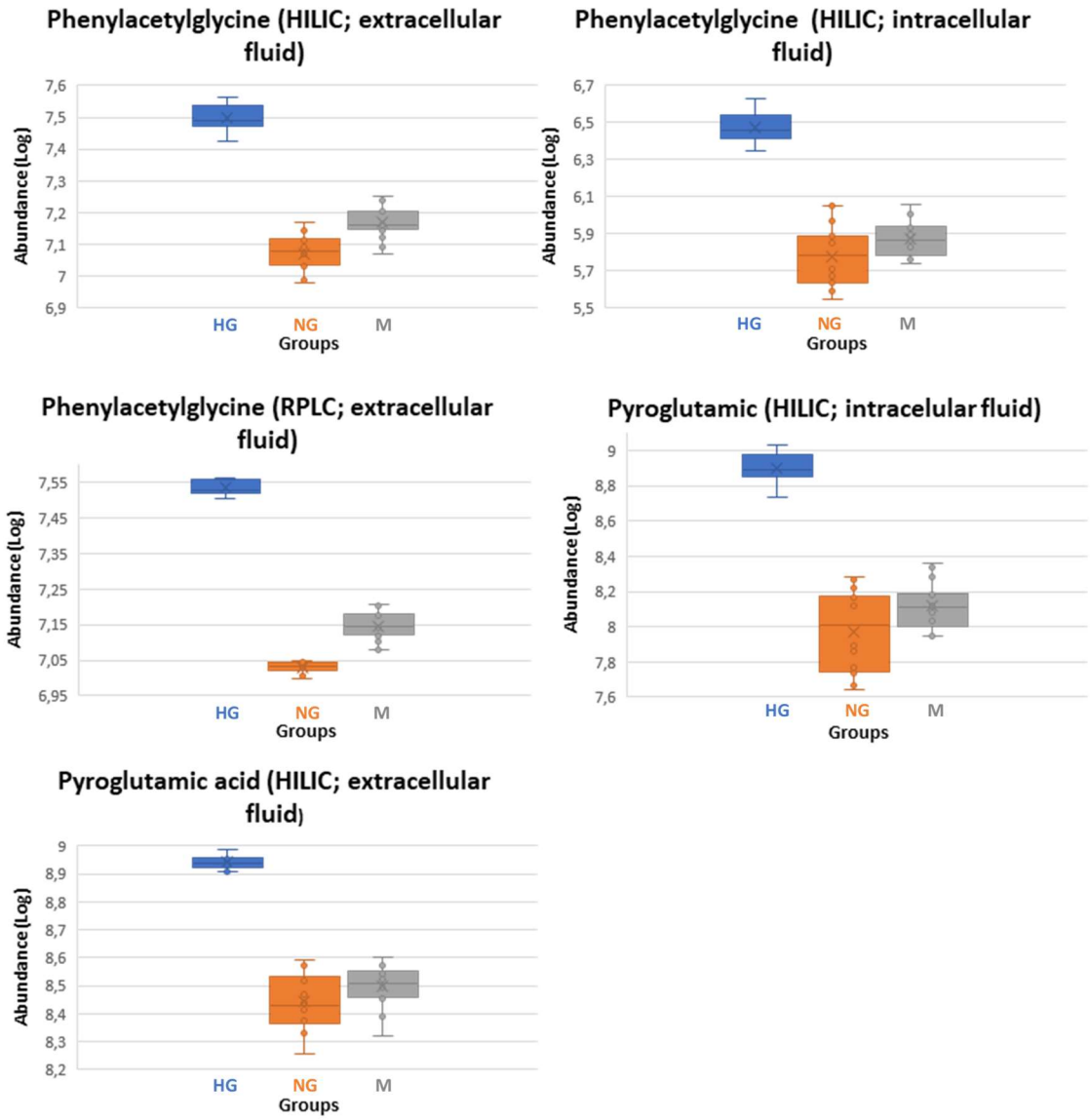

Phenylacetylglycine (HILIC; extracellular fluid)
Abundance (Log)
7,6
7,5
7,4
7,3
7,2
7,1
7
6,9
HG
NG
M
Groups
Phenylacetylglycine (HILIC; intracellular fluid)
Abundance (Log)
6,7
6,5
6,3
6,1
5,9
5,7
5,5
HG
NG
M
Groups
Phenylacetylglycine (RPLC; extracellular fluid)
Abundance (Log)
7,55
7,45
7,35
7,25
7,15
7,05
6,95
HG
NG
M
Groups
Pyroglutamic (HILIC; intracelular fluid)
Abundance (Log)
9
8,8
8,6
8,4
8,2
8
7,8
7,6
HG
NG
M
Groups
Pyroglutamic acid (HILIC; extracellular fluid)
Abundance (Log)
9
8,9
8,8
8,7
8,6
8,5
8,4
8,3
8,2
HG
NG
M
Groups

Table S1. Unknown molecular features which were statistically significant for any of the four analytical sequences.

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP		Trend*
				HG *vs* NG	HG *vs* NG	
Intracellular fluid (HILIC)						
1	2.66	148.0734	-	1.25	0.93	↑
2	4.81	758.4309	-	1.25	0.78	↑
3	7.26	272.1126	-	1.53	0.74	↑
4	7.62	581.3795	-	1.36	0.68	↑
5	7.62	296.0426	259.0822, 223.0121, 189.9783, 149.0022	1.33	0.23	↑
6	7.62	807.5493	-	1.39	0.63	↑
7	7.63	447.0679	-	1.29	0.78	↑
8	7.63	598.0909	-	1.26	0.83	↑
9	7.63	425.1665	-	1.37	0.55	↑
10	7.64	280.0683	128.0363, 146.9625	1.40	0.78	↑
11	7.65	309.1076	-	2.01	0.45	↑
12	7.65	471.1899	198.0131, 451.0046, 306.9910, 362.9814	1.42	0.67	↑
13	7.81	187.0021	-	1.21	0.94	↑
14	11.64	374.0922	229.1363,285.1041, 248.9958, 137.0751	1.32	0.32	↑
15	11.64	308.1201	229.1359, 137.0760, 117.0373, 266.9887	1.46	0.81	↑
16	14,31	314.1209	145.0616, 127.0518, 109.0403	1.27	0.77	↓
17	14.31	488.2177	305.1180, 145.0639, 227.0870, 341.1378	1.10	0.97	↓
Intracellular fluid (RPLC)						
18	0.82	302.0502	174.0116	1.52	0.79	↑
19	1.14	262.0894	122.0602, 160.0057, 214.1103, 179.0990	1.30	0.74	↑
20	1.29	471.2808	-	1.28	0.21	↑
21	1.44	514.3223	258.1521, 138.0466, 498.3221	1.36	0.48	↑
22	1.79	676.3577	281.1392, 504.3969, 171.0762, 129.1048	1.16	0.60	↑
23	2.02	693.3844	-	1.23	0.28	↑
24	4.18	348.1436	188.0679, 146.0613, 159.0922, 117.0637	1.26	0.50	↑
25	4.23	722.2880	-	1.14	0.71	↑
26	4.32	287.1844	143.1195, 157.8862, 129.0979	1.43	0.33	↑
27	5.21	345.1902	187.1087, 127.0874, 142.0884, 169.0973	1.18	0.46	↑
28	5.33	606.2323	230.0784, 345.0946, 328.0754, 201.1194	1.14	0.31	↑
29	5.41	391.2107	279.1338	1.29	0.88	↑

30	5.69	321.1690	187.1046	1.24	0.54	↑
31	5.78	851.4043	-	1.11	0.73	↑
32	6.30	466.1909	129.0994, 102.0574	1.58	0.44	↑
33	6.49	480.2062	322.1741, 147.1110, 130.0898	1.59	0.75	↑
34	6.49	321.1687	253.1559, 270.1896, 201.8895, 114.0312	1.57	0.79	↑
35	6.49	159.0349	114.0386, 102.0098, 118.09864	1.55	0.67	↑
36	6.68	341.2309	229.1560, 183.1514	1.20	0.85	↑
37	7.23	480.2059	463.2520, 295.0297, 129.1016	1.82	0.73	↑
38	7.70	572.3173	-	1.17	0.14	↑
39	7.91	439.1416	-	1.22	0.75	↑
40	7.95	537.3520	120.0790, 508.6301, 484.6276, 521.2505	1.12	0.53	↑
41	7.98	660.2289	614.0418, 561.7185, 581.3516	1.20	0.30	↑
42	7.99	352.1099	160.0434, 174.0585, 128.0526, 263.1236, 309.1235	1.55	0.82	↑
43	7.99	159.0351	114.0372, 102.0132	1.53	0.79	↑
44	8.28	439.1418	160.0408, 217.0632, 100.0753, 176.0653	1.26	0.23	↑
45	10.26	685.3406	120.0794, 226.1537, 412.1598, 664.8122	1.14	0.33	↑
46	10.48	366.1261	-	1.85	0.34	↓
47	10.56	815.0357	776.6065, 173.1272, 758.7957, 602.3212	1.24	0.91	↑
48	14.89	442.2257	-	1.19	0.69	↑
Extracellular fluid (HILIC)						
49	0.79	394.2886	277.1832, 325.1807, 135.0712, 351.3373, 247.1749	1.11	0.77	↑
50	0.8	376.2863	305.1168, 227.0871, 162.0785, 180.0871, 287.0871	1.15	0.59	↑
51	0.81	366.2565	272.0810, 283.2609, 299.2612	1.15	0.70	↑
52	0.97	626.3375	172.9893, 285.1105, 356.9981	1.15	0.99	↑
53	2.53	412.1894	-	1.23	0.50	↑
54	2.53	75.0325	-	1.32	0.98	↑
55	4.08	308.1209	204.9890, 266.9862, 229.1300	1.20	0.95	↑
56	4.98	422.0732	278.9941, 316.9850, 112.9892	1.33	1.04	↑
57	7.39	354.0586	195.0517, 273.0965, 289.0917	1.22	0.76	↑
58	7.86	296.0420	254.9960, 230.832, 128.0412, 274.9964	1.14	0.18	↑
59	7.87	425.1663	-	1.63	1.08	↑
60	7.88	280.0682	128.0407, 146.9652	1.41	0.65	↑
61	7.89	944.6605	-	1.15	0.60	↑
62	7.90	453.1801	-	1.21	0.00	↑
63	8.15	161.8513	-	1.37	0.94	↑

64	11.28	445.1746	-	1.17	0.95	↑
65	11.42	793.5629	-	1.13	1.05	↑
66	11.58	297.1436	-	1.24	0.20	↑
Extracellular fluid (RPLC)						
67	0.63	196.0924	100.0808, 110.0639, 134.6824	1.40	0.28	↑
68	0.79	236.1207	122.0712, 110.0694, 164.1018, 207.1207	1.41	0.98	↑
69	2.28	599.2759	583.2780, 264.1288, 395.8990, 463.0221	1.10	0.90	↑
70	2.39	321.1621	286.1252, 304.1353, 322.1495, 130.0854	1.18	0.98	↑
71	2.87	260.1362	142.0492, 130.0507, 114.0564	1.10	1.08	↑
72	3.18	321.1684	130.0844, 147.1132, 176.0699, 2014.1375, 120.0804	1.11	1.02	↑
73	3.27	349.1739	158.0898, 175.1138, 112.0891	1.15	0.94	↑
74	3.86	294.1215	120.0808, 166.0863, 186.0915	1.12	1.09	↑
75	4.14	453.1037	203.0808, 185.0705, 151.9828	1.53	1.00	↑
76	4.35	135.0688	118.9208, 118.0701, 107.0121, 109.0437, 120.0736	1.17	0.99	↑
77	4.56	321.1329	130.0510, 176.0715, 147.0775, 277.1010	1.16	1.06	↑
78	4.56	173.0470	172.0377, 144.0464, 114.9999,130.0849, 133.9231, 139.0080, 152.9794	1.16	0.75	↑
79	5.16	90.0468	-	1.25	0.94	↑
80	5.16	272.0502	188.0368, 232.0274, 161.0255	1.22	0.81	↑
81	5.16	288.0222	149.0039, 232.0287, 122.0714, 188.0454	1.24	0.92	↑
82	5.20	333.1323	188.0696, 146.0589, 215.1262, 130.0497	1.12	1.03	↑
83	5.81	443.1182	269.0636, 176.0749, 147.0607, 402.6194	1.29	1.06	↑
84	6.07	334.0991	289.1010, 128.0521, 159.0908, 176.0703, 243.0981	1.75	0.90	↑
85	6.60	332.0847	215.1296, 315.0787, 155.0786, 199.0739	1.35	1.06	↑
86	6.90	276.0600	132.0096, 213.0706	1.26	1.04	↑
87	7.00	542.1511	512.1337, 301.1184, 257.1279, 160.0441	1.15	0.74	↑
88	7.02	159.0354	114.0375, 142.0295	1.16	0.99	↑
89	7.02	415.0872	241.0298, 151.9833, 122.0294, 176.0684	1.18	1.00	↑
90	7.02	240.0238	114.0369, 160.0427, 130.0865	1.17	0.99	↑
91	7.02	628.0435	-	1.12	1.06	↑
92	7.47	159.0352	114.0378	1.11	0.99	↑
93	7.47	574.1247	241.0319, 416.0960, 160.0431, 217.0645, 335.1054	1.10	0.96	↑
94	8.24	159.0352	114.0371, 142.0318	1.45	0.94	↑
95	8.70	292.1431	211.0625, 143.0627	1.12	0.78	↑
96	11.26	527.1726	227.5030, 131.0783, 201.1099, 377.0110	1.46	1.08	↑
97	12.21	236.1207	160.0413, 309.1310, 174.0588, 128.0550, 219.0516	1.22	1.05	↑

98	12.50	358.1421	223.0476, 256.2228, 108.0555, 350.2162	1.84	1.01	↑
99	14.88	229.2404	112.8952, 212.2344, 148.0868, 172.9336	1.13	0.60	↑
100	17.02	487.2589	119.0836, 203.9898, 145.1113, 365.1391	1.24	0.74	↑
101	17.07	257.2714	-	1.10	0.97	↑
102	19.08	278.1521	149.0228	1.16	1.06	↑
103	27.78	227.1898	-	1.38	1.02	↑
104	27.94	394.2134	-	1.10	0.97	↑

*↑: The metabolite (on average) is more abundant in HG vs NG; ↓: The metabolite (on average) is less abundant in HG vs NG