Supporting Information

NMR-Guided Mass Spectrometry for Absolute Quantitation of Human Blood Metabolites

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This file contains 12 pages, S1 to S12. The first page is the Title page; pages S2 to S5 contain Table S1, Table S2, Table S3 and Table S4, respectively; pages S6 to S8 contain Figure S1 and pages S9 to S12 contain Figures S2 to S5, respectively.

Table S1: Demographical parameters for the serum samples used for absolute quantitation of metabolites by mass spectrometry

Serum sample	Race	Gender	Age (Years)
Serum 1	Caucasian	Female	45
Serum 2	African American	Female	41
Serum 3	Caucasian	Male	28
Serum 4	Caucasian	Female	26
Serum 5	African American	Male	30
Serum 6	Caucasian	Male	36
Serum 7	Caucasian	Male	31
Serum 8	Caucasian	Female	21

Table S2: List of common metabolites detected by NMR and MS in the serum samples. Eight metabolites, highlighted in bold, were either overlapped in NMR spectrum, exhibited multiple MRM transitions for the same metabolite, or displayed poor chromatographic peak shapes and hence were omitted from further analysis.

	NMR	**MS (Q1/Q3)**
1	Glycine	Glycine (76.0 / 30.2)
2	Sarcosine	Sarcosine (89.9 / 44.0)
3	Alanine	Alanine (90.0 / 44.0)
4	Choline	Choline (104.1 / 60.0)
5	Dimethylglycine	Dimethylglycine (104.1 / 58.0)
6	Serine	Serine (106.0 / 60.0)
7	Creatinine	Creatinine (114.1 / 44.0)
8	Proline	Proline (116.1 / 70.0)
9	Betaine	Betaine (118.0 / 58.0)
10	Valine	Valine (118.1 / 72.0)
11	Threonine	Threonine (120.1 / 74.0)
12	Pyroglutamic Acid	Pyroglutamic Acid (130.0 / 83.4)
13	Creatine	Creatine (132.1 / 90.0)
14	Leucine	Leucine (132.1 / 86.0)
15	Isoleucine	Isoleucine (132.1 / 86.0)
16	Ornithine	Ornithine (133.1 / 70.0)
17	Asparagine	Asparagine (133.1 / 74.0)
18	Aspartic Acid	Aspartic Acid (134.1 / 74.0)
19	Glutamine	Glutamine (147.1 / 84.0)
20	Lysine	Lysine (147.1 / 84.0 (2)
21	**Glutamic acid**	**Glutamic acid (148.1 / 84.0)**
22	Methionine	Methionine (150.1 / 61.0)
23	Histidine	Histidine (156.1 / 110.0)
24	Carnitine	Carnitine (162.1 / 85.0)
25	Phenylalanine	Phenylalanine (166.1 / 120.0)
26	**Arginine**	**Arginine (175.1 / 70.0)**
27	**Mannose**	**Mannose (181.0 / 99.0)**
28	Tyrosine	Tyrosine (182.1 / 136.0)
29	Acetylcarnitine	Acetylcarnitine (204.1 / 85.0)
30	Tryptophan	Tryptophan (205.1 / 146.0)
31	Uridine	Uridine (245.1 / 113.1)
32	lactate	lactate (89.0 / 43.0)
33	Glucose	Glucose (179.0 / 89.0)
34	**3-Hydroxybutyric acid (3HBA)**	**Malonic Acid/3HBA (103.0 / 59.0)**
35	**Fumaric Acid**	**Fumaric Acid/Maleic Acid (114.9 / 71.0)**
36	**Hypoxanthine**	**Hypoxanthine (135.0 / 92.0)**
37	**Xanthine**	**Xanthine (151.0 / 108.0)**
38	**2-Hydroxyisovaleric Acid**	**2/3-Hydroxyisovaleric Acid (117.0 / 71.0)**

Table S3: NMR derived metabolites concentrations (μM) for the 8 blood serum samples using a single internal reference, TSP.

Metabolite	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6	Serum 7	Serum 8
Glycine	301	357	308	364	303	252	363	423
Sarcosine	1.4	1.2	1.5	1.7	1.2	1.2	1.4	1.7
Alanine	372	254	294	248	265	224	286	451
Choline	15.1	15.4	15.8	14.6	13.2	25.9	18.2	19.9
Dimethylglycine	2.4	2.1	3.1	2.6	2.1	2.1	2.4	2.6
Serine	88.8	101	120	127	98.7	97.0	110	158
Creatinine	59.5	54.9	65.1	50.7	66.3	60.9	60.5	55.9
Proline	346	213	360	307	282	192	327	617
Betaine	54.9	36.0	44.7	44.1	38.4	48.9	45.9	55.0
Valine	148	89.8	123	113	112	112	123	166
Threonine	134.7	142.6	187.7	191.1	209.1	110.2	144.9	192.0
Pyroglutamic acid	343	197	402	365	407	129	367	497
Creatine	16.1	21.6	56.9	31.9	17.1	11.1	20.4	42.9
Leucine	80.2	54.5	84.5	53.3	70.5	60.2	65.5	105
Isoleucine	47.8	27.4	47.5	31.9	40.8	26.4	34.8	60.9
Ornithine	41.1	26.7	53.3	32.4	49.5	51.3	46.6	49.7
Asparagine	49.2	46.3	78.5	50.9	60.2	57.4	66.3	91.5
Aspartic Acid	20.9	32.6	19.0	13.7	19.0	22.5	14.2	22.1
Glutamine	256	256	344	302	291	443	285	389
Lysine	80.4	58.1	111	73.7	84.3	105	77.3	159
Methionine	22.5	19.5	31.0	19.4	20.1	20.1	21.3	41.0
Histidine	32.1	29.5	41.0	29.1	28.5	42.2	33.4	45.9
Carnitine	30.5	27.3	44.7	37.7	38.2	34.1	39.6	42.9
Phenylalanine	64.5	59.1	74.4	50.7	52.5	64.1	54.3	93.1
Tyrosine	63.9	39.4	60.3	49.0	47.1	31.5	50.7	96.0
Acetylcarnitine	1.5	1.5	2.1	2.1	1.7	2.1	1.4	1.7
Tryptophan	30.3	28.5	38.2	31.4	33.6	39.1	41.3	46.8
Uridine	2.1	3.9	6.2	5.1	3.8	6.7	3.6	3.3
lactate	1950	1790	1460	862	1840	2340	1060	1780
Glucose	3730	4180	3400	3800	3270	3710	3450	4340

Table S4: MS derived concentrations (μM) for the 8 blood serum samples using the NMR derived concentrations from Serum 7 as the reference.

Metabolite	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6	Serum 7	Serum 8
Glycine	336	349	322	388	297	250.	363	374
Sarcosine	1.5	1.3	1.8	1.8	1.5	1.3	1.4	1.3
Alanine	392	277	320	280.	287	237	286	421
Choline	15.9	14.8	14.6	14.3	12.9	19.5	18.2	18.2
Dimethylglycine	2.7	2.3	3.3	3.0	2.1	2.5	2.4	2.7
Serine	99.2	110.	127	145	103	110	110.	172
Creatinine	64.0	53.1	63.8	53.1	64.3	59.5	60.5	50.6
Proline	349	211	345	302	287	170	327	571
Betaine	56.9	38.8	46.6	47.5	40.1	43.5	45.9	53.5
Valine	156	86.6	126	123	109	103	123	136
Threonine	131	135	159	196	198	104	145	182
Pyroglutamic acid	313	242	404	264	339	325	367	342
Creatine	17.8	21.3	50.6	32.6	16.6	11.3	20.4	35.9
Leucine	82.8	50.0	84.9	56.5	68.4	60.4	65.5	96.5
Isoleucine	49.0	26.4	48.1	34.5	41.1	27.7	34.8	55.3
Ornithine	44.4	28.7	57.0	38.1	46.4	45.5	46.6	46.1
Asparagine	51.7	46.7	85.1	53.8	59.2	58.2	66.3	88.3
Aspartic Acid	21.9	30.0	19.1	14.7	18.9	22.0	14.2	20.9
Glutamine	283	238	312	310.	294	293	285	330.
Lysine	84.3	55.7	107	80.5	78.5	83.0	77.3	122
Methionine	22.1	17.6	30.6	21.1	18.7	17.8	21.3	34.7
Histidine	32.9	27.3	42.2	29.6	29.5	33.1	33.4	40.9
Carnitine	38.8	30.8	45.5	38.6	39.5	34.6	39.6	43.7
Phenylalanine	66.9	57.3	76.4	53.1	53.3	60.0	54.3	93.6
Tyrosine	71.6	41.0	69.6	57.8	54.7	32.6	50.7	88.8
Acetylcarnitine	1.4	1.5	1.6	1.8	1.5	1.5	1.4	1.5
Tryptophan	32.1	27.9	38.8	33.6	35.6	38.0	41.3	43.5
Uridine	2.2	4.2	5.6	5.2	3.8	6.1	3.6	3.5
lactate	1680	1580	1460	993	1690	2100	1060	1430
Glucose	3440	3630	3360	3760	2960	3440	3450	3540

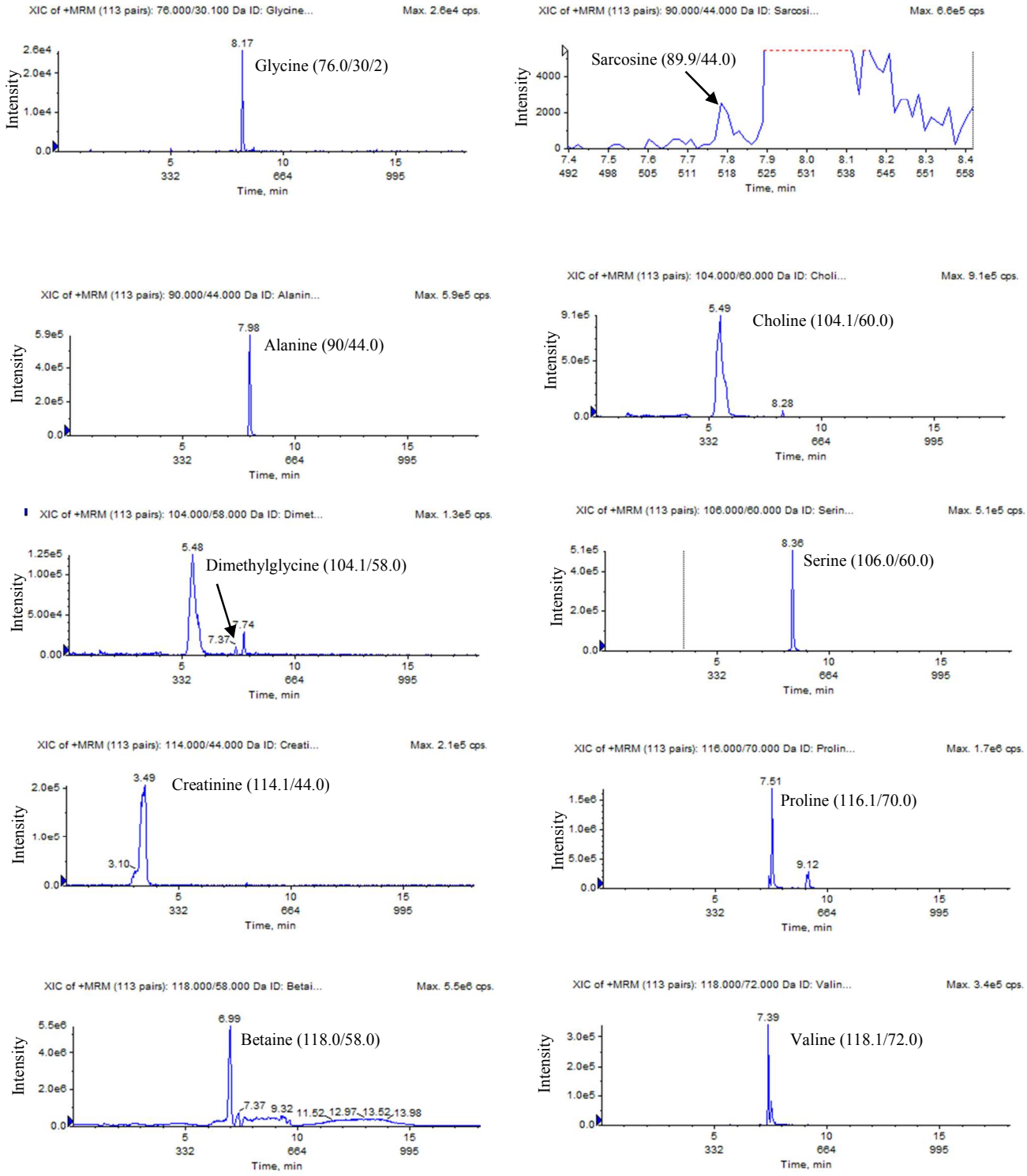

Figure S1: Typical MS/MS ion chromatograms for metabolites used for NMR-guided absolute quantitation in MS.

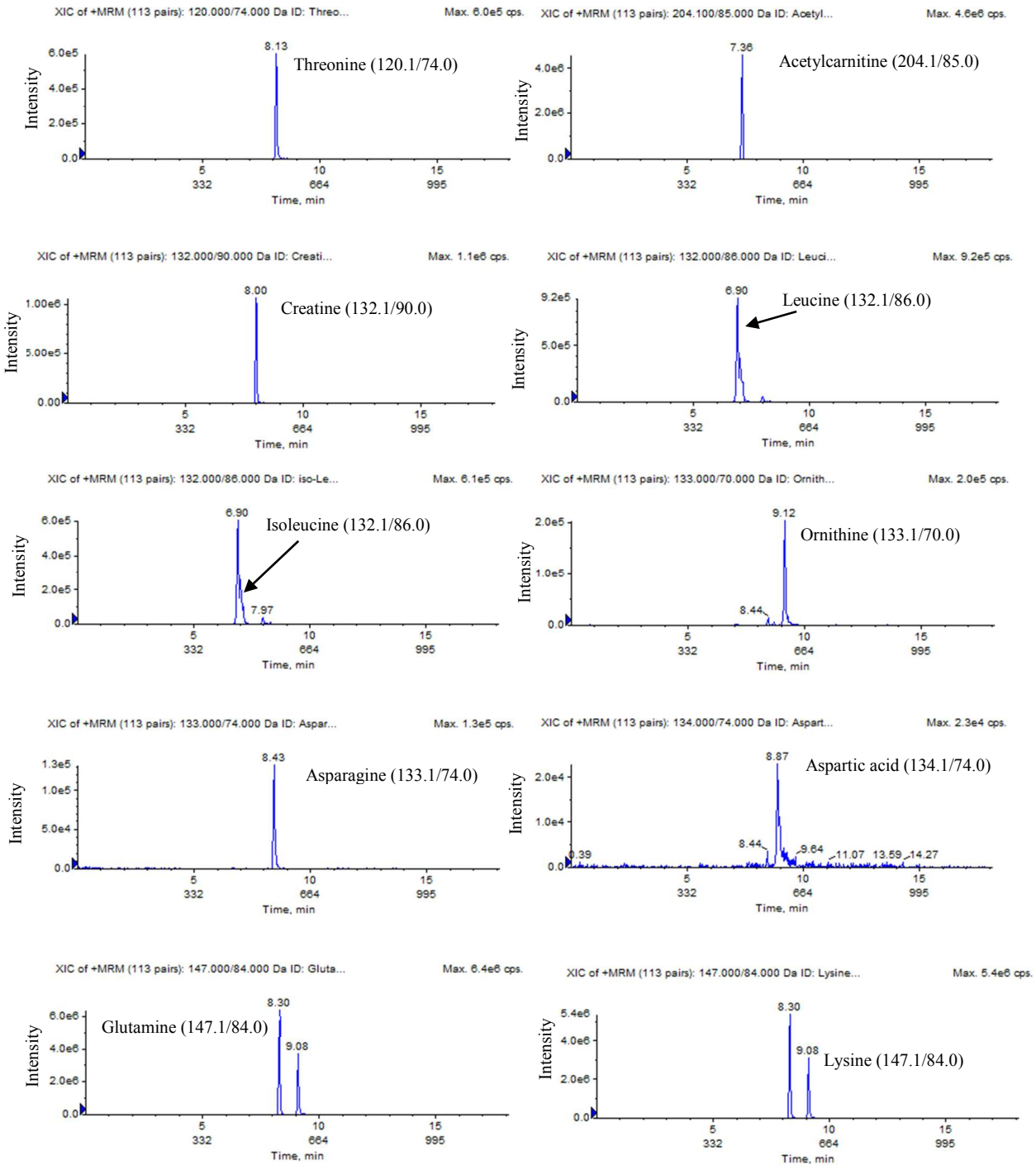

Figure S1: Continued.

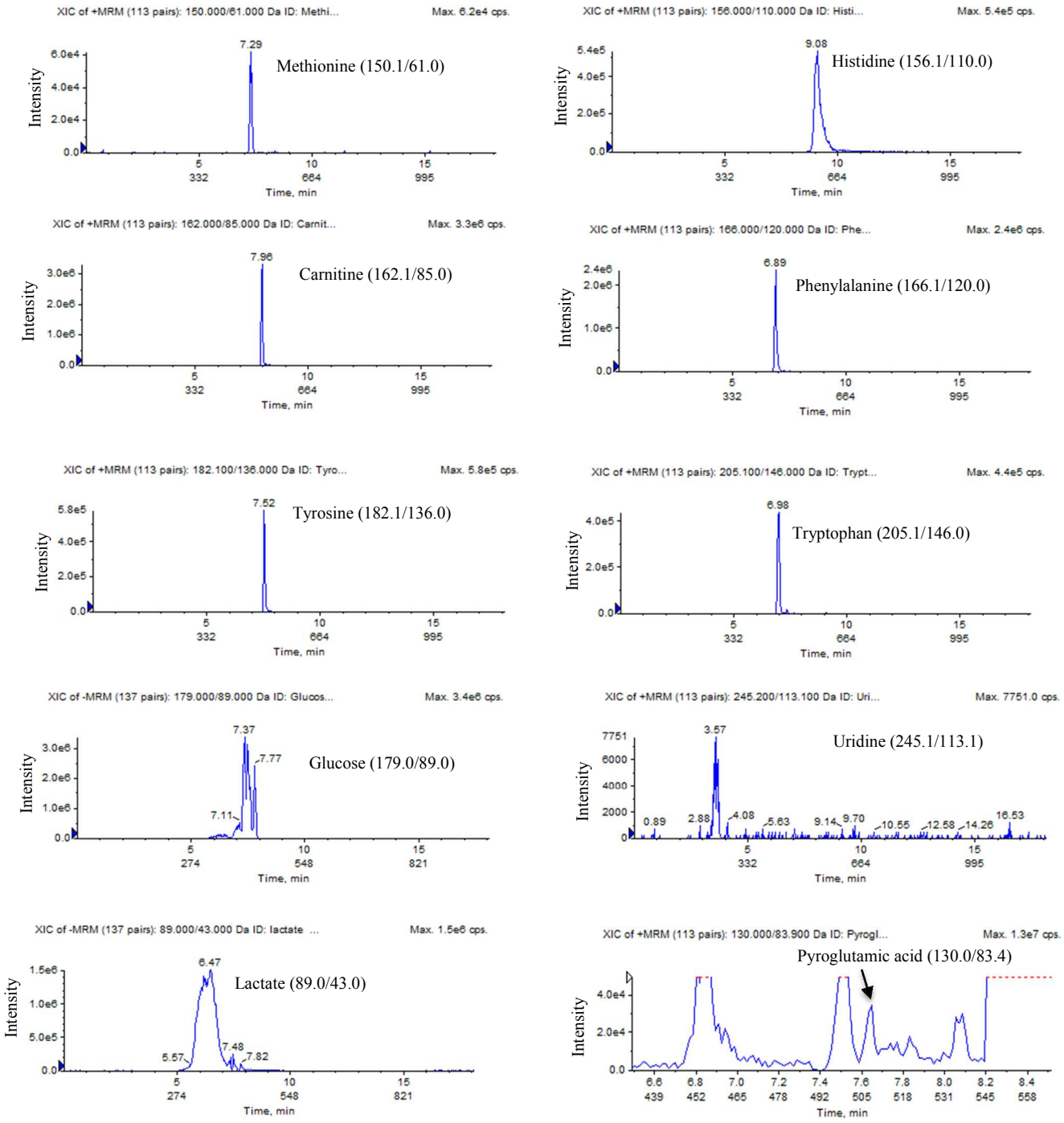

Figure S1: Continued.

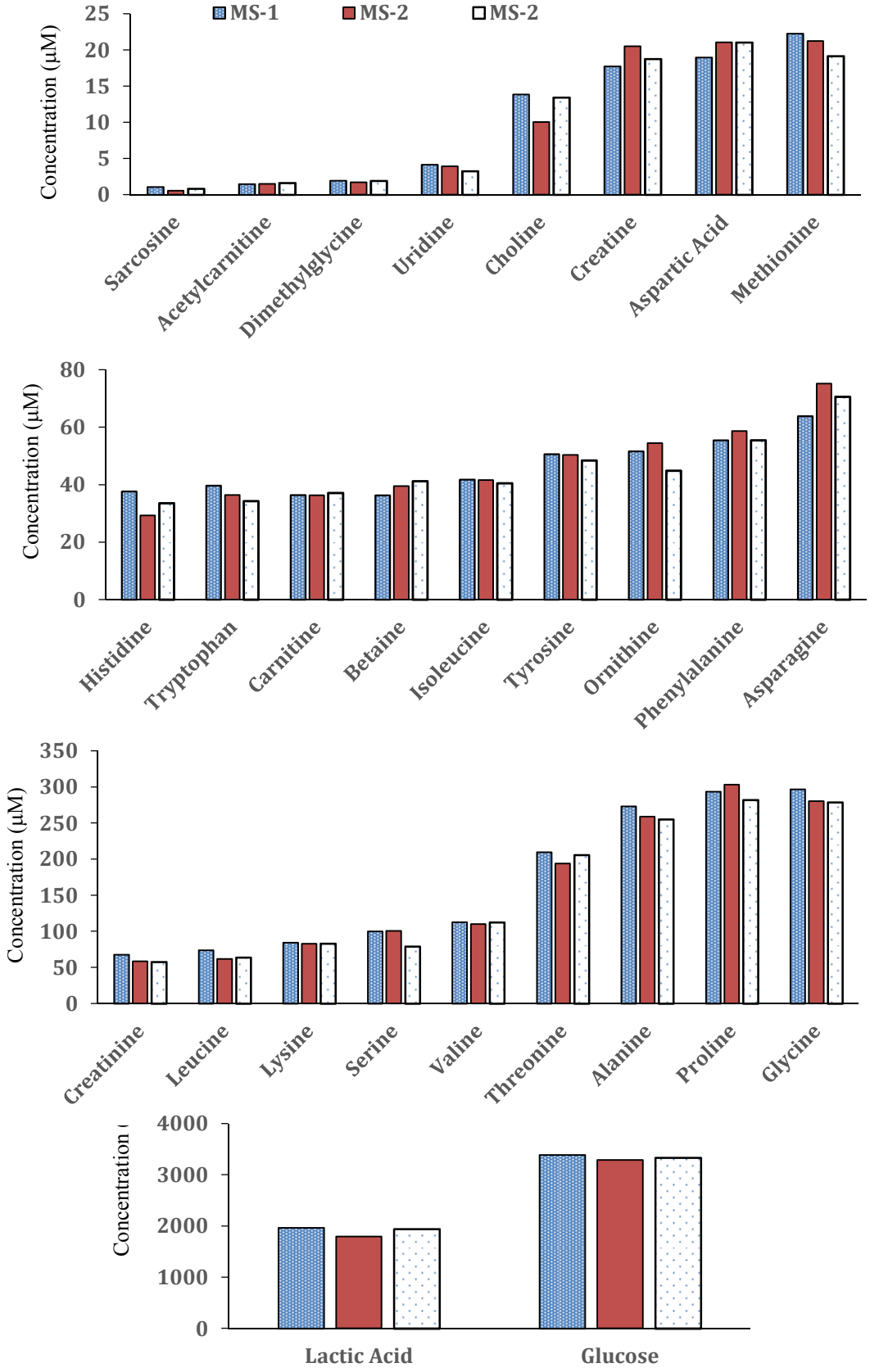

Figure S2: Comparison of absolute concentrations of 28 metabolites for the same serum as in Figure 2 obtained using NMR-guided MS, and using different types of serum samples as the reference. The MS data was obtained on an AB Sciex QTrap 6500+ MS. MS-1: one of the 8 serum samples was used as the reference; MS-2: a pooled sample from 8 serum samples was used as the reference; and MS-3: a commercial pooled human serum was used as the reference. All three references provided similar metabolite concentrations with an average CV of 6.3 %. Further, the results were similar for all eight serum samples. Glutamine and pyroglutamic acid were omitted due to their errors arising from glutamine

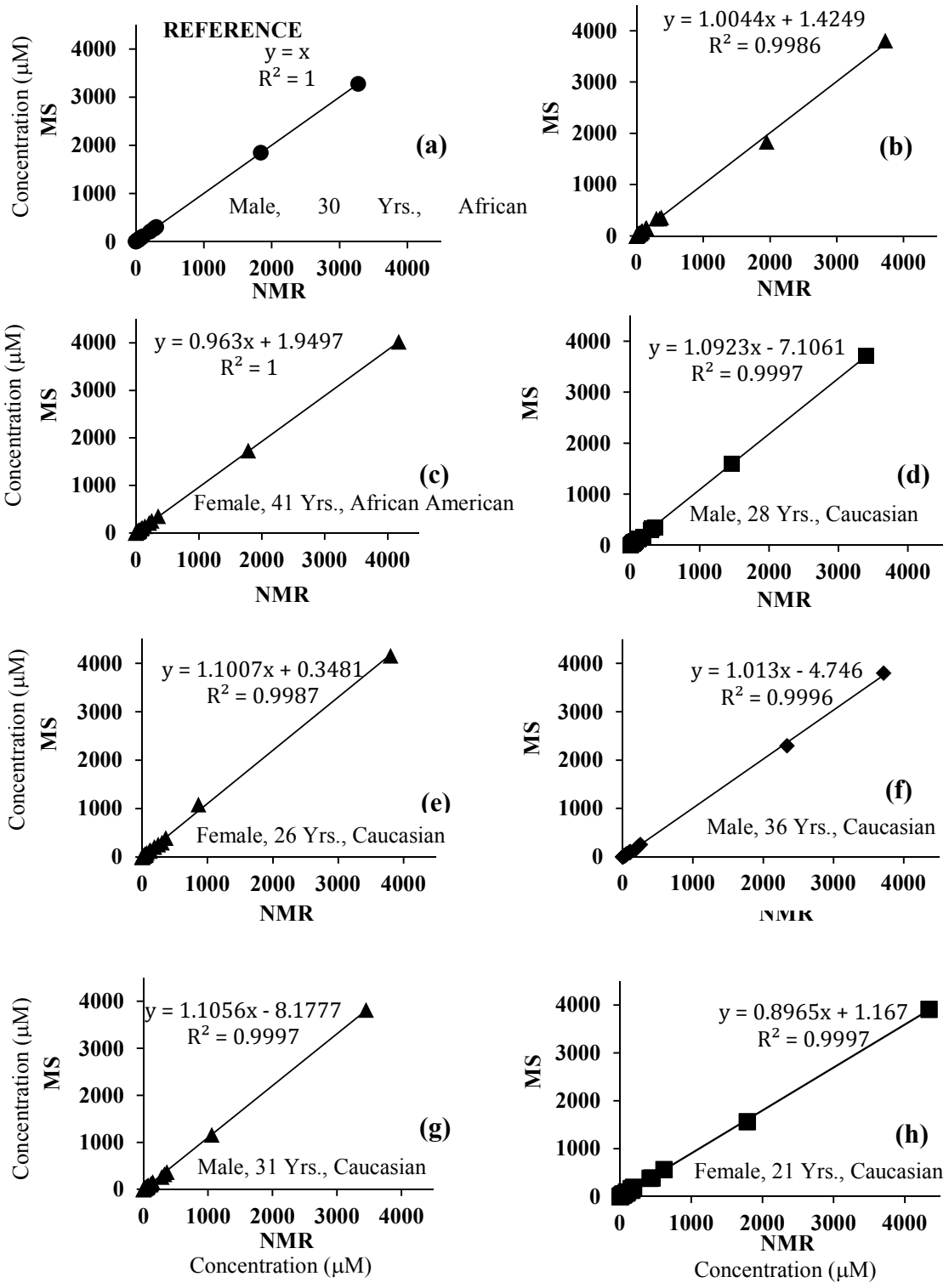

Figure S3: Correlation of concentrations all 30 metabolites including lactate and glucose for metabolites derived from NMR and NMR-guided MS for 8 healthy human serum samples. Here, MS concentrations for one serum sample were set equal to those derived from NMR for the same serum sample (Reference # 5; Fig. 3a; R^2=1); these concentrations, along with their MRM peak areas were used as references for quantitation of the same metabolites in the remaining serum samples (Figs. 3b-3h). Virtually, identical results were obtained irrespective of the serum sample that was used as the reference.

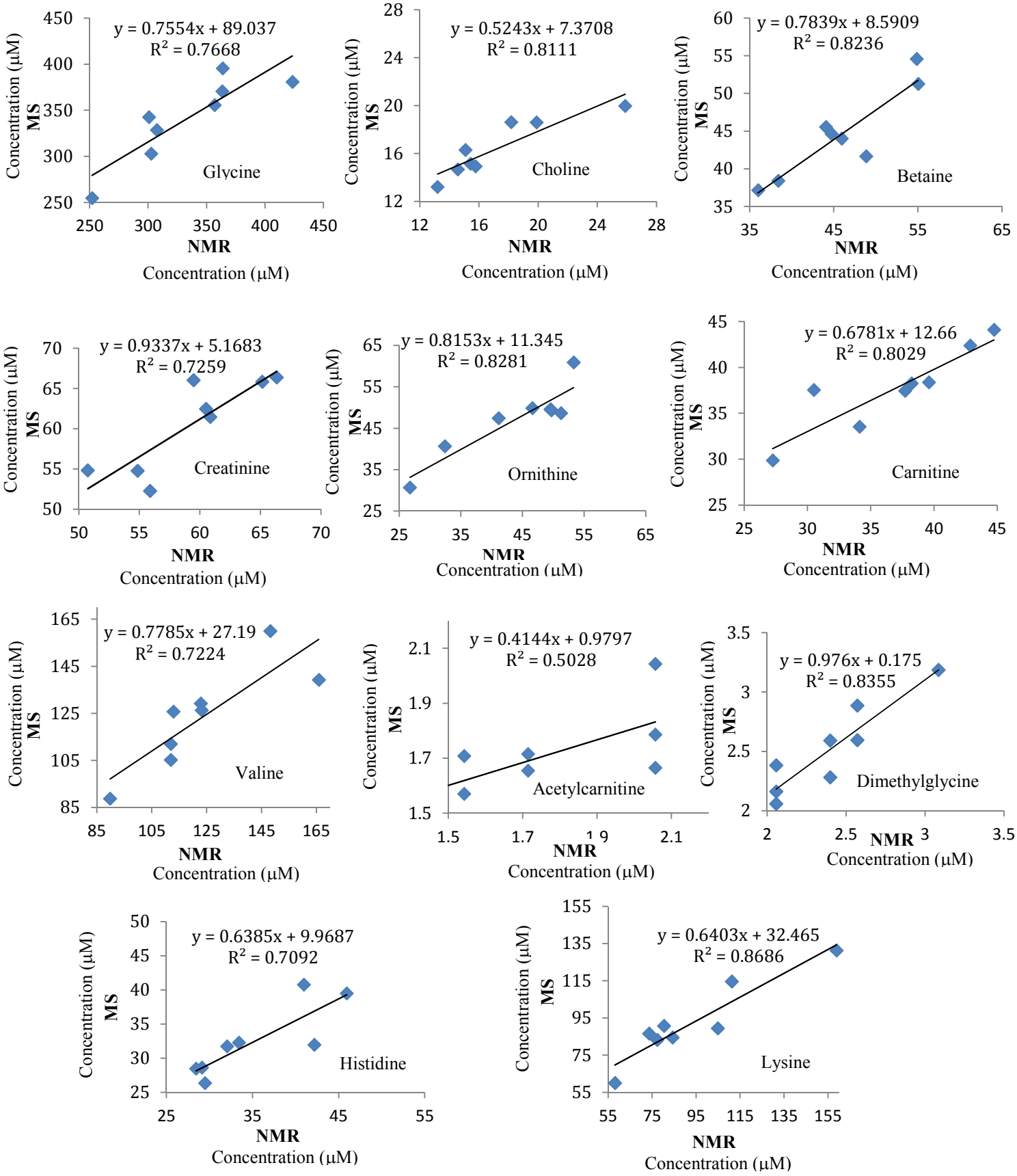

Figure S4: Correlation of absolute concentrations for metabolites derived from NMR and NMR-guided MS for 8 healthy human (4 male; 4 female) serum samples. Here, MS concentrations for one serum sample were set equal to those derived from NMR and used as the references for quantitation of the same metabolites in the remaining serum samples. Virtually identical results were obtained irrespective of the serum sample used as the reference.

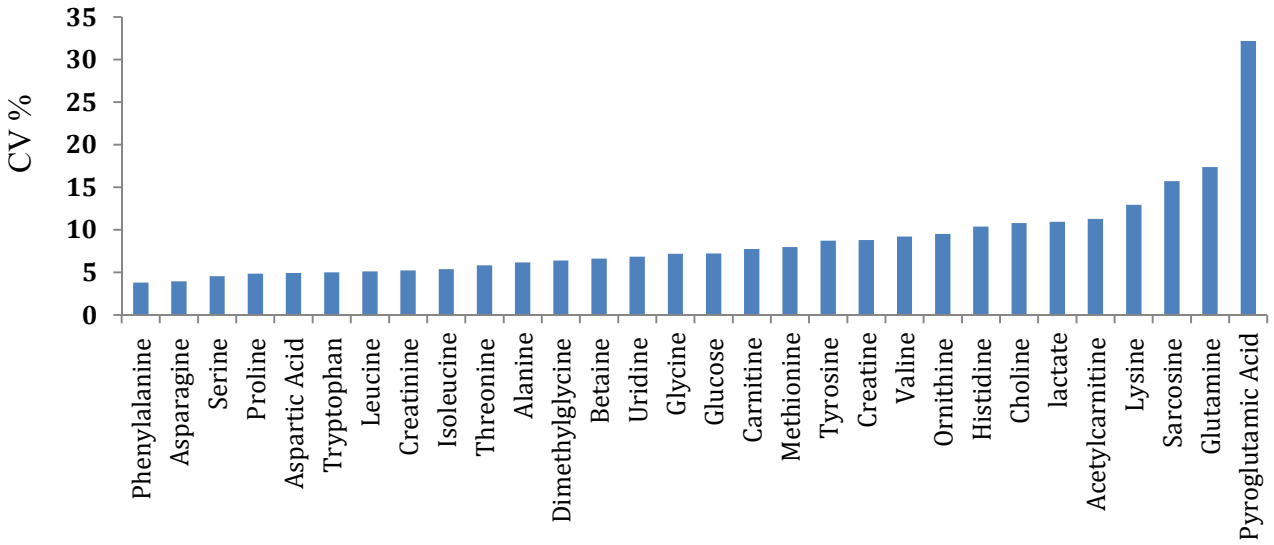

Figure S5: Coefficient of variation of concentrations of metabolites from the same sample obtained, separately, using different samples as NMR reference. Average CV for all except three metabolites was 7.2%, while that for more than 50% of the metabolites was 5.6%. The CVs for glutamine, pyroglutamic acid and sarcosine was more than 15% and are in accordance with their poor correlations between NMR and NMR guided MS concentrations (Figure 6).