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## Item 1: HPLC method

Three different HPLC systems were used sequentially in time, and connected to the mass spectrometer in parallel. All columns were purchased from Phenomenex (Torrance, CA).

The following table lists the parameters of each HPLC system:

	<b>System 1</b>	<b>System 2</b>	<b>System 3</b>
<b>Mobile Phase</b>	<b>A<sup>a</sup>:</b> 99.9% water 0.1% acetic acid  <b>B<sup>b</sup>:</b> 99.9% acetonitrile 0.1% acetic acid	<b>A:</b> 79.75% water 20% acetonitrile 0.25% ammonium hydroxide 10 mM ammonium acetate  <b>B:</b> 79.75% acetonitrile 20% water 0.25% ammonium hydroxide 10 mM ammonium acetate	<b>A:</b> 95% water 5% acetonitrile 5 mM ammonium acetate  <b>B:</b> 95% acetonitrile 5% water 5 mM ammonium acetate
<b>Column</b>	Luna phenyl-hexyl (4.6x50 mm, 5 $\mu$ m)	Luna Amino (4.6x50 mm, 5 $\mu$ m)	Synergi Polar-RP (4.6x50 mm, 4 $\mu$ m)
<b>Gradient</b>	From 0%B and 1 mL/min to 90%B and 2 mL/min in 0.7 minutes	From 100%B and 1.5 mL/min to 0%B and 2.5 mL/min in 1.6 minutes	From 5%B and 1 mL/min to 95%B and 2 mL/min in 2.65 minutes
<b>Total Run Time (minutes)</b>	1.4	3.4	3.75
<b>Injection Volume (<math>\mu</math>L)</b>	5	10	10

<sup>a</sup> A: Aqueous phase

<sup>b</sup> B: Organic phase.

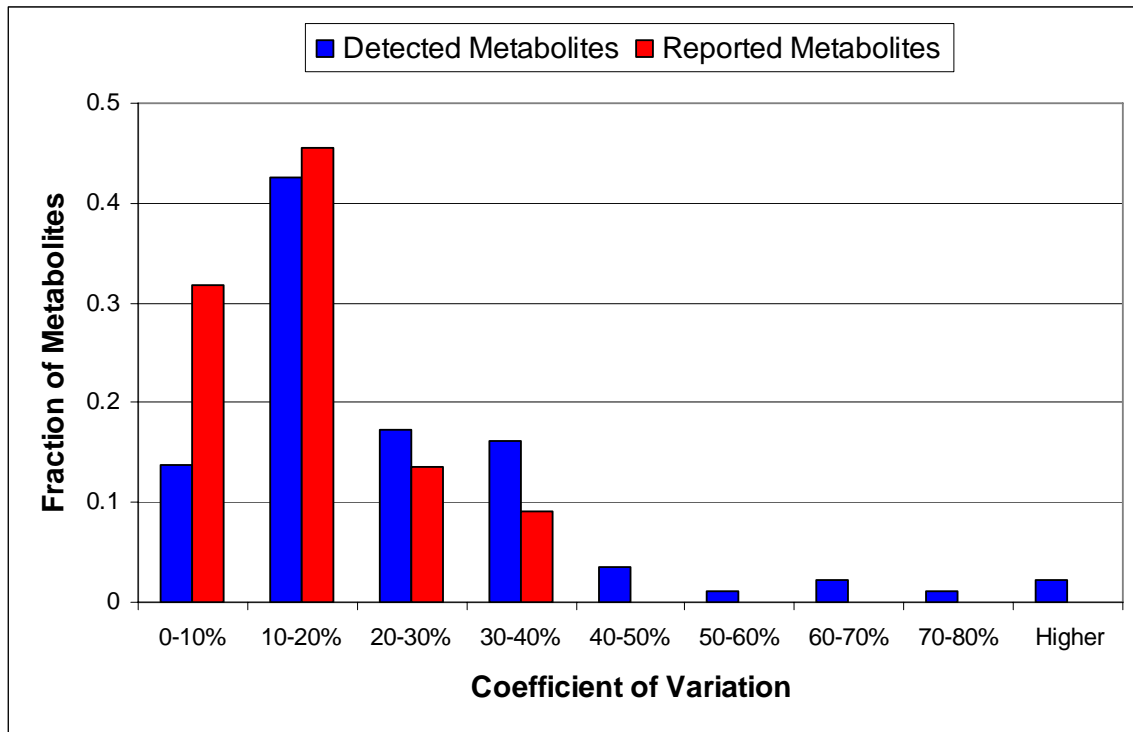
## **Item 2: Mass spectrometry method**

A Turbo electrospray ionization source was used. The ion spray potentials were 5,000 volt in the positive mode and 4,200 volt in the negative mode. Zero air was used for the nebulizer and bath gases, and N<sub>2</sub> was used for the curtain and collision gases. The gas pressures used were 50 psi for the nebulizer gas, 60 psi for the bath gas, 20 psi for the curtain gas and 7 psi for the collision gas. The bath gas temperature was 400 °C.

### **Item 3: Metabolite interferences**

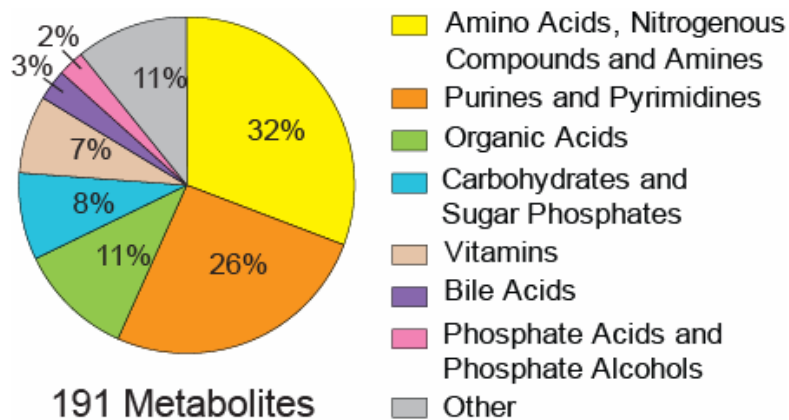
The HPLC-MS/MS method was unable to distinguish between a few of the reported metabolites and other tested metabolites, due to a combination of isobaric overlap and insufficient chromatographic resolution. Leucine and isoleucine were indistinguishable, and are therefore always mentioned together in the text. In the other instances of sets of indistinguishable metabolites, one of the metabolites in the set was likely to be present in the samples in much higher concentrations than the rest of the set, based on published concentrations in human plasma and given the nature of an oral glucose tolerance test. In these instances, it was assumed that the effect of the non-prevalent metabolites on the measurement was negligible, and only the prevalent metabolite was reported in the main text. The sets of indistinguishable metabolites involving a reported metabolite are listed below, with the prevalent metabolite first: {glucose, galactose, fructose}, { $\beta$ -hydroxybutyrate, malonate}, and {valine, guanidinoacetate}.

#### Item 4: Reproducibility of metabolite measurement in plasma



The variation associated with plasma sample preparation and LC-MS/MS analysis was assessed by splitting one plasma sample into 7 parts and processing each part separately. The coefficient of variation (CV) equals the standard deviation of metabolite level divided by the mean metabolite level. The CV distribution of metabolites reported in the main text (red bars, Item 6 below) is compared to the distribution of all detected metabolites (blue bars, Item 7 below).

## Item 5: Chemical classes of monitored metabolites



The chemical classification is based on the chemical taxonomy annotation in the Human Metabolome Database (Wishart, D.S. et al. HMDB: the Human Metabolome Database.

Nucleic Acids Res 35, D521-526 (2007).

## Item 6: Metabolites reported in the main text

Metabolite Name	Q1 <sup>a</sup>	Q3 <sup>b</sup>	DP <sup>c</sup>	CE <sup>d</sup>	IP <sup>e</sup>	HPLC <sup>f</sup>	Standard Source	Standard Catalog Number
Alanine	90.0	44.0	25	15	+	1	Sigma <sup>g</sup>	A-7627
Arginine	175.1	70.0	25	30	+	1	Sigma	A-5131
$\beta$ -hydroxybutyrate	103.0	59.0	-40	-15	-	3	Sigma	54920
Citrulline	174.1	131.0	-50	-15	-	3	Sigma	27510
Glucose	179.1	89.0	-50	-15	-	2	Sigma	49159
Glycerol	93.0	57.0	20	21	+	1	Shelton Scientific - IBI	IB15762
Glycochenodeoxycholic acid	448.3	74.0	-80	-60	-	3	Sigma	G0759
Glycocholic acid	464.3	74.0	-30	-60	-	3	Sigma	G2878
Hippuric acid	178.1	134.0	-50	-16	-	3	Sigma	112003
Histidine	156.1	110.0	25	23	+	1	Sigma	H-8125
Hypoxanthine	135.0	92.0	-50	-23	-	3	Sigma	56700
Isoleucine	132.1	86.0	50	20	+	1	Sigma	I-2752
Lactate	89.0	43.0	-40	-20	-	2	Sigma	69771
Leucine	132.1	86.0	50	20	+	1	Sigma	L-8000
Lysine	147.1	84.0	25	25	+	1	Sigma	G-3126
Malate	133.0	115.0	-40	-20	-	3	Sigma	M-0750
Methionine	150.1	61.0	40	30	+	1	Sigma	M-9625
Ornithine	133.1	70.0	40	30	+	1	Sigma	75480
Phenylalanine	166.1	120.0	50	17	+	1	Sigma	P-2126
Pyruvate	87.0	43.0	-30	-12	-	3	Sigma	107360
Taurochenodeoxycholic acid	498.3	80.0	-90	-90	-	3	Sigma	86335
Tyrosine	182.1	136.5	25	17	+	1	Sigma	T-3754
Valine	118.1	72.0	25	20	+	1	Sigma	V-0500
Xanthine	151.0	108.0	-40	-30	-	2	Sigma	95490

<sup>a</sup> Q1: precursor ion mass, in daltons (Da)

<sup>b</sup> Q3: product ion mass, in daltons (Da)

<sup>c</sup> DP: De-clustering Potential, in volts (V)

<sup>d</sup> CE: Collision Energy, in volts (V)

<sup>e</sup> IP: Ionization Polarity

<sup>f</sup> HPLC: the HPLC system in which the metabolite was measured. See the HPLC section (Item 1 above) for the parameters of each system.

<sup>g</sup> Sigma: Sigma-Aldrich Co.

**Item 7: Metabolites detected in plasma**  
**(metabolite #1-97)**

Column description:

**KEGG:** Compound ID from the Kyoto Encyclopedia of Genes and Genomes,  
<http://www.genome.ad.jp>

**Q1:** precursor ion mass, in daltons (Da)

**Q3:** product ion mass, in daltons (Da)

**HPLC:** the HPLC system in which the metabolite was measured

**CV:** Coefficient of variation of plasma sample analysis

The name used for a metabolite in the main text is in square brackets  
(if different from KEGG)

#	KEGG	Name	Q1	Q3	HPLC	CV
1	C01089	(R)-3-Hydroxybutanoate [ $\beta$ -hydroxybutyrate]	103.0	59.0	3	27
2	C00186	(S)-Lactate [Lactate]	89.0	43.0	2	7
3	C00149	(S)-Malate [Malate]	133.0	115.0	3	32
4	C00026/ C06104	2-Oxoglutarate/Adipate	145.1	101.0	3	20
5	C00632	3-Hydroxyanthranilate	154.0	136.2	1	54
6	C00197	3-Phospho-D-glycerate	185.0	97.0	2	78
7	C01015	4-Hydroxy-L-proline	132.1	86.2	1	15
8	C00847	4-Pyridoxate	182.1	138.0	3	15
9	C01996	Acetylcholine	146.1	87.0	1	18
10	C00008	ADP	426.0	79.0	2	66
11	C01551	Allantoin	159.0	116.0	1	13
12	C00020	AMP	346.1	79.0	2	85
13	C00108	Anthranilate	138.0	120.0	1	18
14	C00002	ATP	506.0	159.0	3	34
15	C00719	Betaine	118.1	58.0	1	14
16	C00486	Bilirubin	583.3	285.0	3	69
17	C00114	Choline	104.1	60.0	1	18
18	C00417	cis-Aconitate	173.0	129.0	3	32
19	C00158/ C00311	Citrate/Isocitrate	191.0	111.0	3	
20	C00300	Creatine	132.1	90.0	1	16
21	C00791	Creatinine	114.1	44.0	1	14
22	C01094/ C00085/ C00103/ C00668	D-Fructose 6-phosphate/D-Glucose 1-phosphate/alpha-D-Glucose 6-phosphate/D-Fructose 1-phosphate	259.0	97.0	2	18
23	C00191	D-Glucuronate	193.0	113.0	3	27
24	C00117/ C00199	D-Ribose 5-phosphate/D-Ribulose 5-phosphate	229.0	97.0	3	33
25	C00365	dUMP	307.0	79.0	3	29
26	C00122/ C01384	Fumarate/Maleate	115.0	71.0	2	18
27	C00035	GDP	442.0	79.0	3	34
28	C00341	Geranyl diphosphate	313.1	79.1	3	46



29	C00031/ C00124	Glucose	179.1	89.0	2	8
30	C00116	Glycerol	93.0	57.0	1	5
31	C00111	Glycerone phosphate	169.0	97.0	3	35
32	C05466	Glycochenodeoxycholate [GCDCA]	448.3	74.0	3	16
33	C01921	Glycocholate [GCA]	464.3	74.0	3	15
34	C00044	GTP	522.0	159.0	3	
35	C01586	Hippurate [Hippuric acid]	178.1	134.0	3	24
36	C00544	Homogentisate	167.0	123.0	3	30
37	C00262	Hypoxanthine	135.0	92.0	3	19
38	C00130	IMP	347.0	79.0	2	18
39	C00294	Inosine	267.1	135.0	3	30
40	C00956	L-2-Aminoadipate	160.1	116.0	2	33
41	C00243	Lactose	341.1	161.0	2	8
42	C00041	L-Alanine [Alanine]	90.0	44.0	1	16
43	C00062	L-Arginine [Arginine]	175.1	70.0	1	13
44	C00152	L-Asparagine	133.1	74.0	1	14
45	C00049	L-Aspartate	134.0	74.0	1	15
46	C00327	L-Citrulline [Citrulline]	174.1	131.0	3	30
47	C00097	L-Cysteine	122.0	76.0	1	27
48	C00407/ C00123	Leucine/Isoleucine	132.1	86.2	1	8
49	C00025	L-Glutamate	148.1	84.0	1	19
50	C00064	L-Glutamine	147.1	84.0	1	14
51	C00135	L-Histidine [Histidine]	156.1	110.0	1	14
52	C00155	L-Homocysteine	136.0	90.0	1	17
53	C00328	L-Kynurenine	207.1	144.0	3	16
54	C00047	L-Lysine [Lysine]	147.1	84.0	1	15
55	C00073	L-Methionine [Methionine]	150.1	61.0	1	34
56	C00077	L-Ornithine [Ornithine]	133.1	70.0	1	17
57	C00079	L-Phenylalanine [Phenylalanine]	166.1	120.2	1	6
58	C00148	L-Proline	116.1	70.0	1	11
59	C00065	L-Serine	106.0	60.0	1	19
60	C00188	L-Threonine	120.1	74.0	1	18
61	C00078	L-Tryptophan	205.1	188.3	1	3
62	C00082	L-Tyrosine [Tyrosine]	182.1	136.3	1	7
63	C02170	Methylmalonate	117.0	73.0	2	
64	C03406	N-(L-Arginino)succinate	291.1	70.0	1	46
65	C01026	N,N-Dimethylglycine	104.1	58.0	1	19
66	C00003	NAD+	662.1	540.0	2	
67	C03626	NG,NG-Dimethyl-L-arginine (asymmetric)	201.1	156.0	3	36
68	C03626	NG,NG-Dimethyl-L-arginine (asymmetric/symmetric)	203.1	70.3	1	36
69	Not found	NG,NG-Dimethyl-L-arginine (symmetric)	201.1	131.0	3	
70	C03884	Ngamma-Monomethyl-L-arginine	189.1	70.0	1	
71	C00153	Nicotinamide	123.0	80.0	1	13
72	C00295	Orotate	155.0	111.0	3	31
73	C01103	Orotidine 5'-phosphate	367.0	323.1	2	
74	C00209	Oxalate	89.0	61.0	3	33
75	C00036	Oxaloacetate	131.0	87.0	3	24
76	C00127	Oxidized glutathione	611.2	306.0	2	

77	C00864	Pantothenate	218.1	88.0	3	46
78	C00166	Phenylpyruvate	163.0	91.0	2	29
79	C03722	Pyridine-2,3-dicarboxylate	166.0	122.0	2	25
80	C00314	Pyridoxine	170.1	152.2	1	7
81	C00022	Pyruvate	87.0	43.0	3	
82	C00780	Serotonin	177.1	160.0	1	25
83	C00093	sn-Glycerol 3-phosphate	171.0	79.0	2	9
84	C00042	Succinate	117.0	73.0	3	33
85	C00245	Taurine	126.0	108.0	1	14
86	C05465	Taurochenodeoxycholate [TCDCA]	498.3	80.0	3	17
87	C00068	Thiamin diphosphate	423.0	303.2	2	9
88	C01104	Trimethylamine N-oxide	76.1	58.0	1	19
89	C00015	UDP	403.0	79.0	3	36
90	C00029/ C00052	UDPglucose/UDP-D-galactose	565.1	323.0	2	132
91	Not found	Unidentified peak	149.0	87.0	3	26
92	C00106	Uracil	111.0	42.0	2	24
93	C00366	Urate	167.0	124.0	3	24
94	C00299	Uridine	243.1	110.0	2	16
95	C00075	UTP	483.0	159.0	3	
96	C00183/ C00581	Valine	118.1	72.0	1	7
97	C00385	Xanthine	151.0	108.0	2	11

**Item 8: Metabolites monitored but not detected in plasma  
(metabolite #98-191)**

Column description:

**KEGG:** Compound ID from the Kyoto Encyclopedia of Genes and Genomes,  
<http://www.genome.ad.jp>

**Q1:** precursor ion mass, in daltons (Da)

**Q3:** product ion mass, in daltons (Da)

**HPLC:** the HPLC system in which the metabolite was measured

#	KEGG	Name	Q1	Q3	HPLC
98	C00631	2-Phospho-D-glycerate	185.0	79.1	3
99	C01179	3-(4-Hydroxyphenyl)pyruvate	179.0	107.0	3
100	C00575	3',5'-Cyclic AMP	328.1	134.0	2
101	C00942	3',5'-Cyclic GMP	344.0	150.0	3
102	C05145	3-Aminoisobutanoate	104.1	86.0	1
103	C03227	3-Hydroxy-L-kynurenine	223.1	162.0	3
104	C05594	3-Methoxy-4-hydroxyphenylethyleneglyco	183.1	150.0	2
105	Not found	3-Nitro-L-tyrosine	227.1	181.3	1
106	C02642	3-Ureidopropionate	133.1	115.0	1
107	C00334	4-Aminobutanoate	104.1	87.0	1
108	C00156	4-Hydroxybenzoate	137.0	93.0	3
109	C03479	5-Formyltetrahydrofolate	472.2	315.0	2
110	C05635	5-Hydroxyindoleacetate	190.1	146.0	2
111	C00643	5-Hydroxy-L-tryptophan	221.1	204.0	1
112	C00440	5-Methyltetrahydrofolate	458.2	329.0	2
113	C00164	Acetoacetate	101.0	57.0	2
114	C00024	Acetyl-CoA	403.6	79.0	3
115	C00147	Adenine	134.1	107.0	2
116	C00212	Adenosine	266.1	134.0	2
117	C03794	Adenylosuccinate	462.1	79.0	3
118	C00072	Ascorbate	175.0	115.0	3
119	C00120	Biotin	243.1	200.0	2
120	C00487	Carnitine	163.1	85.0	1
121	C00386	Carnosine	227.1	110.0	1
122	C00112	CDP	402.0	158.9	3
123	C02528	Chenodeoxycholate	391.3	373.5	2
124	C00695	Cholate	407.3	343.0	3
125	C00055	CMP	322.1	79.0	2
126	Not found	Cotinine	177.1	80.0	1
127	C00063	CTP	482.0	159.0	3
128	C02823	Cyanocobalamin	678.3	147.3	1
129	Not found	Cystamine	153.0	108.0	1
130	C01678	Cysteamine	78.0	61.0	1
131	C00475	Cytidine	244.1	112.0	1
132	C00380/ C00388	Cytosine/Histamine	112.0	95.0	1
133	C00705	dCDP	386.0	79.0	3

134	C00239	dCMP	306.1	79.0	3
135	C00458	dCTP	466.0	159.0	3
136	C00559	Deoxyadenosine	252.1	136.3	1
137	C00881	Deoxycytidine	228.1	73.0	1
138	C00526	Deoxyuridine	229.1	113.0	1
139	C00095	D-Fructose	179.1	89.0	2
140	C05378/ C00665/ C00660	D-Fructose 1,6-bisphosphate/D-Fructose 2,6-bisphosphate/D-Glucose 1,6-bisphosphate	339.0	79.0	3
141	C00577	D-Glyceraldehyde	89.0	59.0	2
142	C00415	Dihydrofolate	442.2	176.0	2
143	C03758	Dopamine	154.1	137.2	1
144	C00794	D-Sorbitol	181.1	89.0	2
145	C00364	dTMP	321.1	195.0	3
146	C00459	dTTP	481.0	383.0	3
147	C00460	dUTP	467.0	370.0	3
148	C05589	Epinephrine/Normetanephrine	184.1	166.3	1
149	C00346	Ethanolamine phosphate	142.0	44.0	1
150	C00504	Folate	440.1	311.0	3
151	C00051	Glutathione	306.1	143.0	2
152	C00037	Glycine	76.0	30.5	1
153	C00144	GMP	362.1	79.0	3
154	C00242	Guanine	150.0	133.0	2
155	C00387	Guanosine	282.1	150.1	2
156	C01817	Homocystine	267.1	132.0	3
157	C05582	Homovanillate	181.1	137.1	2
158	C01717	Kynurenate	188.0	144.0	2
159	C01724	Lanosterol	425.4	212.1	2
160	C02291	L-Cystathionine	221.1	134.0	3
161	C00263	L-Homoserine	120.1	74.2	1
162	C05588	L-Metanephrine	198.1	180.5	1
163	C05589	L-Normetanephrine	184.1	134.0	1
164	C00083	Malonyl-CoA	425.6	79.0	3
165	C01598	Melatonin	233.1	174.3	1
166	Not found	Methylhydroxyisobutyrate	119.1	87.0	1
167	C00137	myo-Inositol	179.1	81.0	2
168	C00004	NADH	664.1	408.0	2
169	C00006	NADP+	742.1	620.0	2
170	C00005	NADPH	744.1	408.0	2
171	C05926	Neopterin	252.1	192.0	2
172	C00253	Nicotinate	122.0	78.0	3
173	C01185	Nicotinate D-ribonucleotide	334.0	290.0	2
174	C05127	N-Methylhistamine	126.1	109.0	1
175	C00074	Phosphoenolpyruvate	167.0	79.0	2
176	C00163	Propanoate	73.0	55.0	3
177	C00584	Prostaglandin E2	351.2	315.3	3
178	C00018	Pyridoxal phosphate	246.0	97.0	3
179	C00021	S-Adenosyl-L-homocysteine	385.1	136.3	1
180	C07588	Salicylic acid	194.1	150.0	3
181	C00315	Spermidine	146.2	72.0	1
182	C00750	Spermine	203.2	129.3	1

183	C00089	Sucrose	341.1	179.0	3
184	C05122	Taurocholate	514.3	123.8	3
185	C00214	Thymidine	243.1	127.0	1
186	C00178	Thymine	125.0	42.0	2
187	C00167	UDPglucuronate	579.0	403.0	2
188	C00105	UMP	323.0	79.0	3
189	C01762	Xanthosine	285.1	153.0	1
190	C00655	Xanthosine 5'-phosphate	363.0	79.0	3
191	C02470	Xanthurenic acid	204.0	160.0	2