Supplemental Table 1: Isotope Labeled Standards

Metabolite	Isotope Description, Purity	MW	Ref. Plasma Conc. (μg/ml)	Spike in (μg/ml)	Polarity	LC Column
Hypoxanthine	(13C5, 99%)	136	0.05-2	0.5	negative	2,3
Trimethylamine N-Oxide	(D9, 98%)	75.06	2.85	0.1	positive	1
Inosine	(U-15N4, 95%)	268.08	0.01-5	0.1	negative	3
Taurine	(1,2-13C2, 98%)	125.14	5-13.5	1	positive	1
Xanthine	(1,3-15N2, 98%)	150.09	0.05-2.8	0.1	negative	2,3
L-Malic Acid	(13C4, 99%)	134.08	0.4-1.5	0.5	negative	3
L-Glutamic Acid	(U-13C5, 98%; 15N, 98%)	147.1	3.5-8.8	1	positive	1
L-Threonine	(U-13C4, 98%)	119.11	16.6-32	1	positive	1
Succinic Acid	(13C4, 99%)	118.08	1.0-14.5	0.5	negative	3

LC columns (see Methods):

1= Luna phenyl-hexyl column, reverse phase
2= Luna amino column, normal phase
3=Synergi Polar column, reverse phase

Spike in represents the indicated amount of each metabolite added to plasma prior to sample preparation, and absolute quantitation was then determined by the ratio of the endogenous analyte peak to the isotope standard peak (see Methods).

Supplemental Table 2 (see Tables 2 and 3).

Absolute quantitation for the subset of metabolites in Table 3 for which isotope labeled standards were available.
Note that data were acquired on a subset of available peripheral blood samples from n=26 subjects from the original PMI cohort.

Metabolite	**Baseline (ng/ml)**	**60 min (ng/ml)**	**Median % Δ 60 min vs. Baseline**	**P value Baseline vs. 60 min**
Hypoxanthine	102 (77, 136)	156 (134-193)	55.6 (51.5, 89.7)	0.001
Inosine	0.8 (0.6-1.0)	1.2 (1.0-1.3)	46.7 (10.5, 62.8)	0.003
Threonine	11,700 (10,000-13,200)	11, 020 (8,500-13,200)	-9.1 (-15.6,-4.2)	0.0003
TMNO	340 (140-560)	310 (100-460)	-26.3 (-32.7,-9.8)	0.004
Xanthine	5.8 (5.3-8.6)	7.8 (6.2-10)	27.8 (23.7,40.1)	0.0003

Data is displayed as median (interquartile range). Δ indicates median change in absolute quantities of metabolite levels

Supplemental Table 3 (see Tables 6 and 7).

A. Absolute quantitation for the subset of metabolites in Table 6 for which isotope labeled standards were available. Note that data were acquired on a subset of available matching coronary sinus and peripheral blood samples from n=10 subjects from the original PMI cohort.

Metabolite	CS Baseline (ng/ml)	CS 60 min (ng/ml)	P value Baseline vs. 60min	Peripheral Baseline (ng/ml)	Peripheral 60 min (ng/ml)	P value Baseline vs. 60min	P value ΔCS vs. ΔP (ng/ml)	Ratio ΔCS /ΔP
Hypoxanthine	88 (69-111)	176 (146-195)	0.0001	105 (88-137)	152 (133-193)	0.001	78 vs 49 (0.12)	1.6
Inosine	8 (7-9)	13 (10-17)	0.0008	8 (6-10)	11 (9-13)	0.004	4.6 v 3.1 (0.07)	1.5
Xanthine	54 (46-76)	84 (58 -102)	0.0002	58 (53-86)	78 (62-100)	0.0003	18 vs 19 (0.72)	1.0

B. Absolute quantitation for the subset of metabolites in Table 7 for which isotope labeled standards were available.

Metabolite	CS Baseline (ug/ml)	CS 60 min (ng/ml)	P value Baseline vs. 60min	Peripheral Baseline (ng/ml)	Peripheral 60 min (ng/ml)	P value Baseline vs. 60min	P value ΔCS vs. ΔP (ng/ml)	Ratio ΔCS /ΔP
Succinic Acid	316 (290,370)	363 (338,407)	0.02	299 (280-328)	303 (266-310)	0.42	47.2 vs 4.8 (0.01)	9.8
Taurine	4.8 (3.9-5.5)	5.7 (4.8-6.1)	0.017	4.9 (4.6-5.3)	5.3 (4.0-5.5)	0.50	0.8 vs 0.2 (0.12)	4
Malic Acid	181 (162,219)	236 (222-251)	0.0007	182 (40-212)	218 (184-235)	0.06	55.1 vs 21.3 (0.13)	2.6

Data is displayed as median (interquartile range). Δ indicates median change in absolute quantities of metabolite levels

Supplemental Table 4 (for metabolites in Figure 2)

Metabolite	CS Baseline (ug/ml)	CS 60 min (ng/ml)	P value Baseline vs. 60min	Peripheral Baseline (ng/ml)	Peripheral 60 min (ng/ml)	P value Baseline vs. 60min	P value ΔCS vs ΔP (ng/ml)	Ratio ΔCS /ΔP
Malic Acid	181 (162,219)	236 (222-251)	0.0007	182 (40-212)	218 (184-235)	0.06	55.1 vs 21.3 (0.13)	2.6
Glutamic Acid	8.1 (7.2-9.2)	9.6 (6.8-8.9)	0.001	8.9 (7.6-9.9)	9.5 (8.0-10.9)	0.12	1.5 vs 0.6 (0.10)	2.5

Supplemental Table 5: Data acquisition parameters for multiple reaction monitoring experiments for metabolites in the platform. All precursor and product ions are singly charged.

Metabolite	Polarity	Precursor ion mass	Daughter ion mass	Collision Energy (eV)
Glycine	positive	75.8	30.2	21
Trimethylamine-N-Oxide	positive	76.0	58.0	29
Cysteamine	positive	77.9	60.9	14
D9 Trimethylamine-N-Oxide	positive	85	66	31
Glyceraldehyde	negative	88.8	59.0	-10
Lactic Acid	negative	89.2	43.1	-12
Alanine	positive	90.0	44.1	15
Acetoacetate	negative	101.1	57.1	-15
Dimethyl Glycine	positive	103.9	58.1	10
Choline	positive	104.0	60.1	15
GABA	positive	104.0	69.0	20
Aminoisobutyric acid	positive	104.0	57.2	19
Serine	positive	105.9	60.1	15
Uracil	negative	111.0	42.2	-22
Cytosine/Histamine	positive	111.9	95.0	20
Creatinine	positive	113.9	44.0	26
Proline	positive	115.9	69.9	18
Succinate	negative	117.1	73.0	-15
Betaine	positive	117.9	58.0	29
Valine	positive	117.9	72.0	27
Guanidinoacetic Acid	positive	118.0	101.0	15

Methyl-OH-Isobutyric Acid	positive	119.0	87.0	10
Threonine	positive	120.0	74.0	18
Homoserine	positive	120.1	56.2	28
13C4 Succinic acid	negative	121	76	-18
Cysteine	positive	122.0	76.0	20
Nicotinic Acid	negative	122.1	78.1	-15
Niacinamide	positive	122.9	80.1	30
13C4 Threonine	positive	124	77	17
Thymine	negative	125.0	42.1	-27
Taurine	positive	125.7	107.9	20
1-methylhistamine	positive	126.0	109.0	20
13C2 Taurine	positive	128	46	29
Oxaloacetate	negative	130.9	87.0	-20
Creatine	positive	131.9	90.0	15
Hydroxyproline	positive	132.0	68.0	20
Leucine	positive	132.0	44.1	34
Isoleucine	positive	132.0	69.0	25
Asparagine	positive	132.9	74.0	22
N-Carbamyl-Beta-Alanine	positive	132.9	90.0	17
Malic Acid	negative	133.0	114.9	-20
Ornithine	positive	133.1	70.0	50
Aspartate	positive	134.0	74.1	21
Adenine	negative	134.0	107.1	-26
Hypoxanthine	negative	135.0	92.1	-23
Homocysteine	positive	135.8	90.0	20
4-Hydroxybenzoic acid	negative	136.9	93.1	-20
13C4 Malic acid	negative	137	74	-22

Anthranilic Acid	positive	138.0	120.0	27
13C5 Hypoxanthine	negative	140	96	-24
Phosphoethanolamine	positive	142.0	44.1	20
Alpha-Keto-Glutarate	negative	144.8	101.0	-15
Acetylcholine	positive	146.0	86.7	20
Spermidine	positive	146.2	72.2	22
Lysine/Glutamine	positive	146.9	130.0	16
Mevalonic acid lactone	negative	147.2	59.3	-19
Glutamic Acid	positive	147.9	102.0	17
Methionine	positive	149.9	103.9	17
Guanine	negative	150.0	133.0	-22
Xanthine	negative	151.0	108.0	-24
15N2 Xanthine	negative	153	109	-26
Dopamine	positive	153.9	136.7	17
3-OH-Anthanilic Acid	positive	154.0	136.0	15
13C5 Glutamic acid	positive	154	89	25
Orotic acid	negative	154.9	110.6	-15
Histidine	positive	155.7	110.0	23
Allantoin	positive	159.0	116.0	10
2-Aminodipic Acid	negative	160.0	116.0	-18
Carnitine	positive	162.8	103.0	27
Phenylalanine	positive	166.0	119.9	17
Quinollinic Acid	negative	166.0	122.0	-13
Homogentistic acid	negative	166.9	79.0	-15
Uric Acid	negative	167.0	124.0	-30.0
PEP	negative	168.0	79.1	-20
DHAP	negative	169.0	97.0	-15.0

Pyridoxine	positive	169.9	151.9	23
Glycerol-3-P	negative	171.0	79.0	-22
Citrulline	negative	174.0	131.2	-22
Arginine	positive	175.0	70.0	67
Ascorbic Acid	negative	175.1	115.1	-17
Cotinine	positive	176.9	79.8	34
Serotonin	positive	177.0	159.9	12
Hippuric acid	negative	177.9	133.9	-16
Fructose	negative	179.0	89.2	-15
Glucose/Galactose	negative	179.0	89.2	-15
Inositol	negative	179.0	161.0	-18
OH-Phenyl pyruvate	negative	179.0	107.1	-12
Sorbitol	negative	181.0	71.0	-25
Homovanillic Acid	negative	181.1	137.1	-12
Xanthosine 5' monophosphate	negative	181.1	181.1	-7
Tyrosine	positive	181.8	135.9	17
4-Pyridoxic Acid	negative	182.1	138.1	-21
4-Hydroxy-3-methoxyphenylglycol	negative	182.9	150.1	-21
Epinephrine	positive	183.7	165.8	12
Normetanephrine	positive	184.3	166.3	10
Glycerate-2-P	negative	184.8	79.1	-20
3-Phosphoglyceric Acid	negative	185.1	97.2	-22
Kynurenic Acid	negative	188.0	144.0	-40
5-Hydroxyindole-3-acetic Acid	negative	190.0	146.0	-16
Citric Acid	negative	191.0	87.0	-25
Isocitric Acid	negative	191.0	172.9	-18
Glucoronate	negative	192.9	102.9	-15

Metanephrine	positive	198.1	180.1	13
Erythrose-4-P	negative	199.2	97.0	-15
ADMA/SDMA	negative	203.1	70.3	40
Spermine	positive	203.3	112.2	30
Xanthurenic Acid	negative	204.0	160.0	-20
Tryptophan	positive	204.8	187.9	16
Kynurenine	negative	207.0	144.0	-33
Pantothenic Acid	negative	218.2	146.1	-22
L-5-Hydroxytryptophan	positive	221.0	203.8	17
Cystathionine	negative	221.0	133.8	-18
3-OH-Kynurenic Acid	negative	223.0	206.0	-13
3-Nitro-Tyrosine	positive	226.9	180.8	29
Carnosine	positive	227.3	110.2	32
2'-deoxycytidine	positive	228.1	112.1	18
2'-deoxyuridine	positive	229.0	113.1	10
Ribose-5-P/Ribulose-5-P	negative	229.3	96.9	-20
Thymidine	negative	241.1	125.1	-16
Cytidine	negative	242.1	110.0	18
Biotin	negative	243.0	166.0	-25
Thymidine	positive	243.1	127.2	17
Uridine	negative	243.1	111.0	16
Cytidine	positive	243.9	111.8	15
Pyridoxal-5-phosphate	negative	246.1	97.2	-20
g-Glu-Cy	positive	251.1	84.1	35
2'-deoxyadenosine	positive	252.0	136.2	28
Neopterin	negative	252.0	191.9	-22
F1P/F6P/G1P/G6P/	negative	259.1	97.1	-20

Adenosine	negative	266.0	134.2	-14
Inosine	negative	267.0	135.0	-30
Homocystine	negative	267.0	71.9	-25
15N4 Inosine	negative	271	139	-30
Guanosine	negative	282.1	150.1	-25
Xanthosine	positive	285.2	153.2	13
Argininosuccinate	positive	291.0	70.0	54
DCMP	negative	306.0	263.2	-25
Glutathione (reduced)	negative	306.1	272.1	-17
DUMP	negative	307.3	195.1	-25
Geranyl Pyrophosphate	negative	313.1	79.1	-37
DTMP	negative	321.3	195.1	-25
UMP	negative	323.1	97.1	-25
cAMP	negative	328.3	134.0	-25
Nicotinic Acid Mononucleotide	negative	334.1	79.2	-75
F1,6DiP/F2,6DiP	negative	339.0	96.7	-25
Sucrose	negative	341.1	179.1	-19
Lactose	negative	341.1	161.1	-11
cGMP/GMP	negative	343.8	150.1	-34
AMP	negative	346.0	79.0	-30
IMP	negative	347.1	97.0	-30
GMP	negative	362.1	79.1	42
OMP	negative	366.9	323.1	-19
S-(5'-adenosyl)-L-homocysteine	positive	385.0	134.3	30
DCDP	negative	386.2	158.8	-30
Chenodeoxycholic Acid	negative	391.2	391.2	-46
CDP	negative	402.0	159.0	-30

UDP	negative	403.5	158.9	-30
Cholic Acid	negative	407.4	343.4	-50
Thiamine Pyrophosphate	negative	424.2	303.2	-20
ADP	negative	426.0	159.0	-30
Folic Acid	negative	440.4	311.3	-30
Dihydrofolic Acid	negative	442.5	176.3	-30
GDP	negative	442.5	344.1	-30
Glycochenodeoxycholic Acid	negative	448.4	74	-50
5-Methyl THF	negative	458.5	329.2	-40
Adenylosuccinic Acid	negative	462.0	79.2	-82
Glycocholic Acid	negative	464.4	74	-50
DCTP	negative	466.1	368.1	-30
DUTP	negative	467.2	369.2	-30
5-Formyl-THF (Folinic Acid)	negative	472.4	315.0	-40
DTTP	negative	481.0	383.0	-30
CTP	negative	482.2	159.1	-30
UTP	negative	483.3	385.3	-30
Taurochenodeoxycholic Acid	negative	498.7	79.9	-75
ATP	negative	506	159	35
Taurocholic Acid	negative	514.4	123.8	-75
GTP	negative	522.2	158.9	-30
UDP-Glucose/UDP-Galactose	negative	565.5	323.2	-30
UDP-Glucuronic Acid	negative	579.2	403.3	-30
Billirubin	negative	583.2	285.0	-36
Glutathione (Oxidized)	negative	611.3	306.4	-30
NAD	negative	662.5	540.4	-15
NADP	negative	742.2	620.4	-15

NADPH	negative	744.0	408.0	-40
Cobalamin	positive	791.3	666.1	30

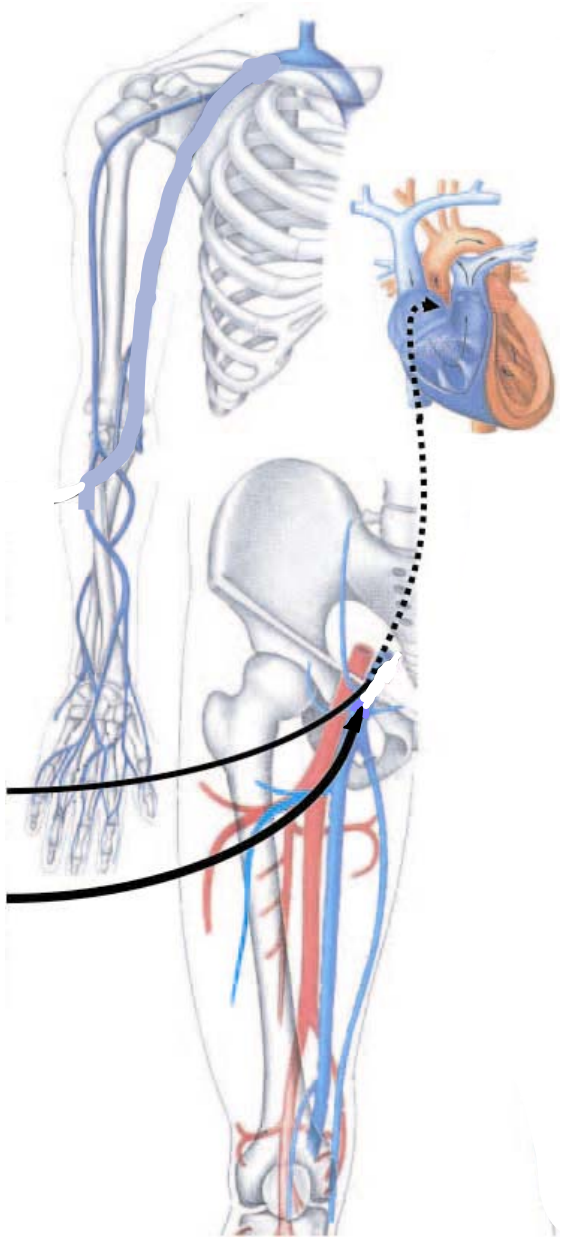
Supplemental Figure Legends

Supplemental Figure 1: Schematic representation of sites and timing of blood sample collection.

Supplemental Figure 2: Representative dose-response studies of isotope-labeled standards in normal pooled human plasma are shown. For each metabolite, the limit of quantitation was defined as a signal that was ten-fold greater than background (lowest dose with a closed circle). The absolute concentration of the endogenous plasma metabolite as assessed by the LC-MS method is denoted with an "X". The reported range of normal metabolite levels is denoted along the axis of each figure [(http://www.hmdb.ca/ and HMDB: The human metabolome database. Nucleic Acids Res. 2007 Jan;35(Database issue):D521-6]. Note is made that only one reference exists for plasma TMNO levels using a new methodology and thus reference level is denoted with a "?".

Supplemental Figure 3 (for comparison with Figure 3 in manuscript): Metabolites that were significantly changed from baseline at 60, 120 and 240 minutes in planned MI patients were assessed in an independent spontaneous MI cohort. White bars denote the differences in patients presenting with spontaneous MI as compared to control patients presenting to the cardiac catheterization suite with non-acute cardiovascular disease, based on studies incoroporating available isotople labeled standards for TMNO, threonine, and hypoxanthine.

Supplemental Figure 1



Coronary Sinus Samples
Time (min): Baseline, 10, 60

Femoral Vein Samples
Time (min): Baseline, 10, 60, 120, 240, 1440

Figure 2

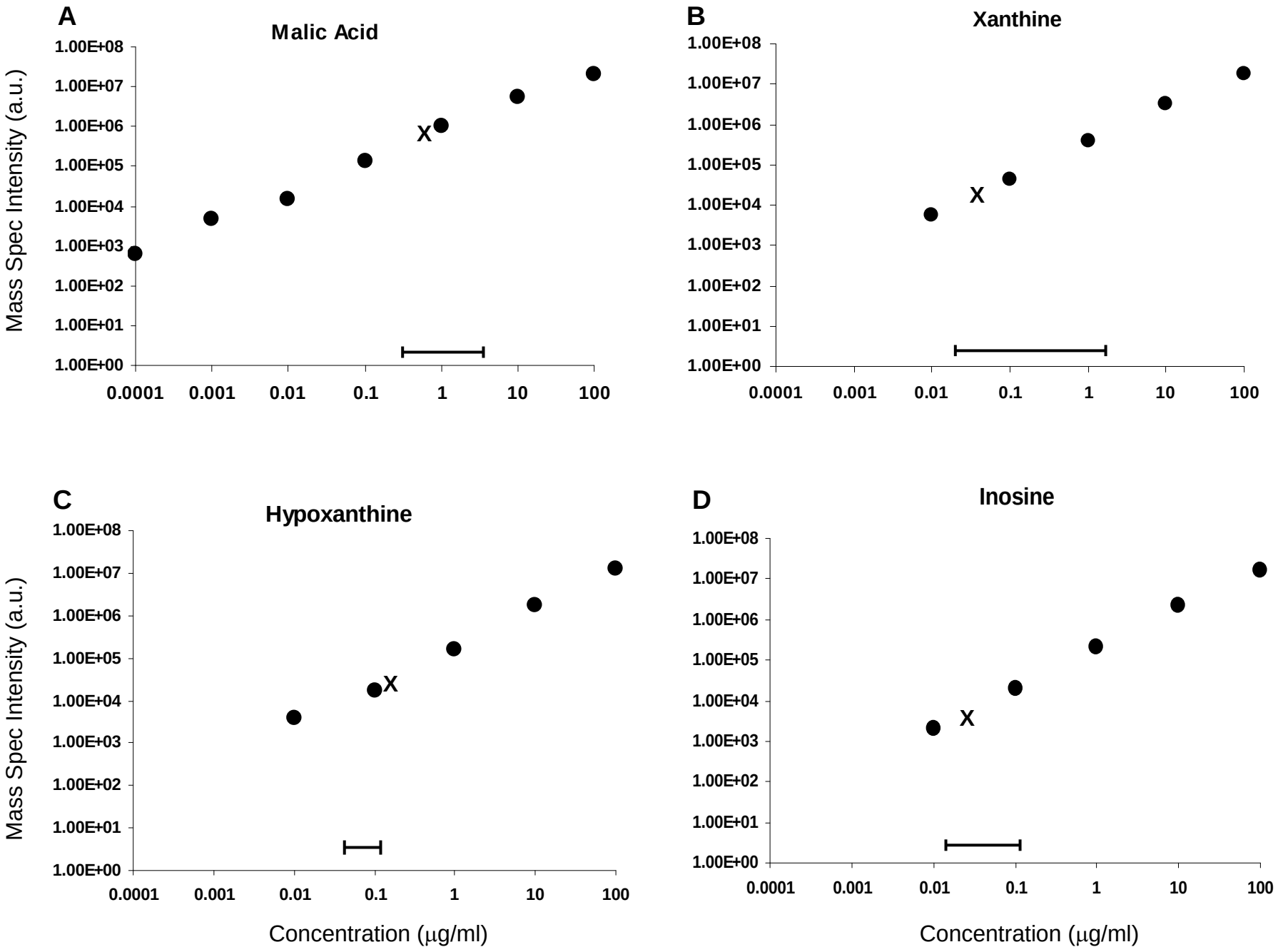

A
Malic Acid
B
Xanthine
C
Hypoxanthine
D
Inosine
Mass Spec Intensity (a.u.)
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0.0001
0.001
0.01
0.1
1
10
100
X
Concentration (μg/ml)

Figure 2

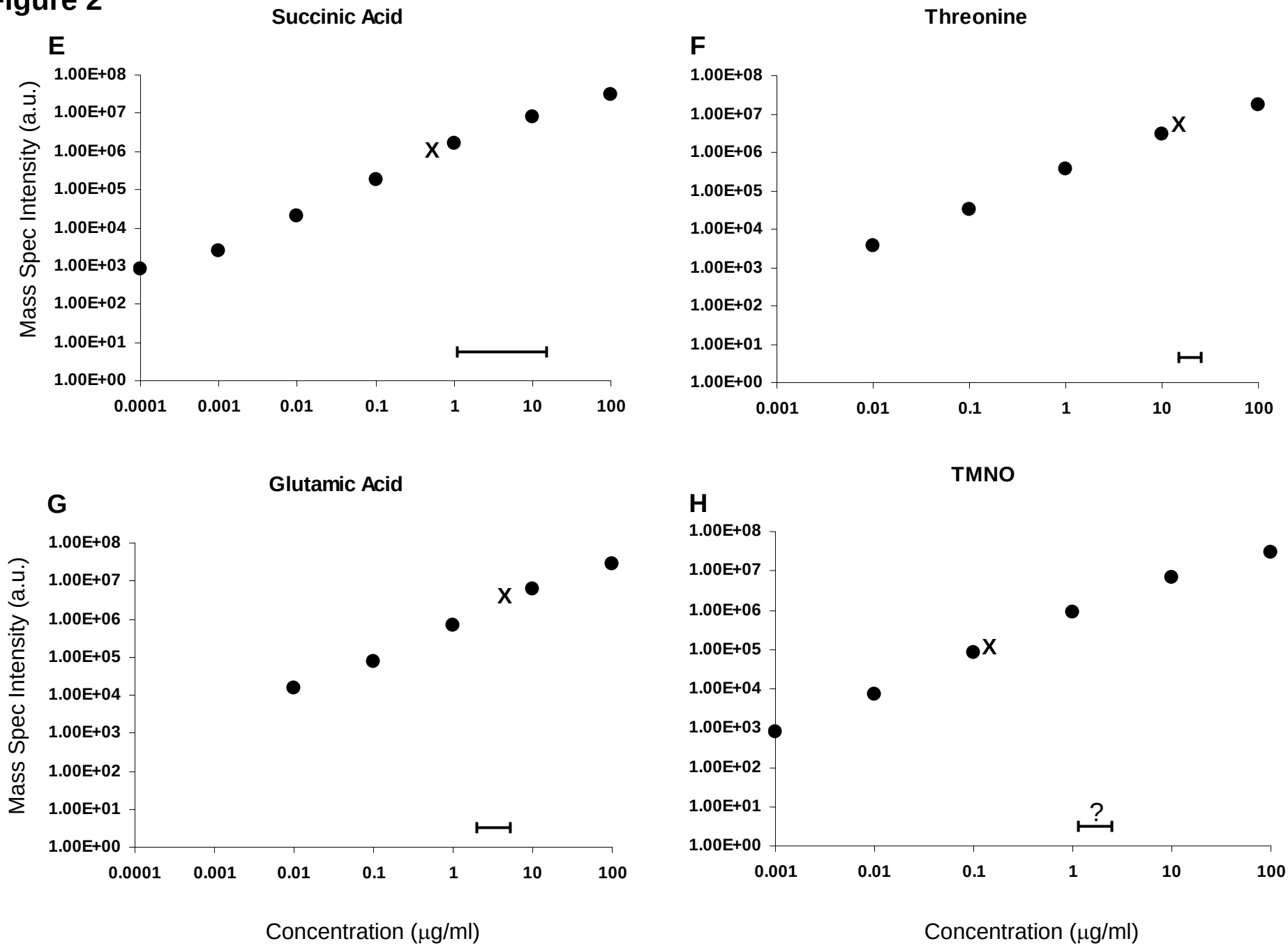

Succinic Acid
E
Mass Spec Intensity (a.u.)
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0.0001
0.001
0.01
0.1
1
10
100
X
Threonine
F
X
Glutamic Acid
G
X
TMNO
H
X
?
Concentration (μg/ml)
Concentration (μg/ml)

Figure 2

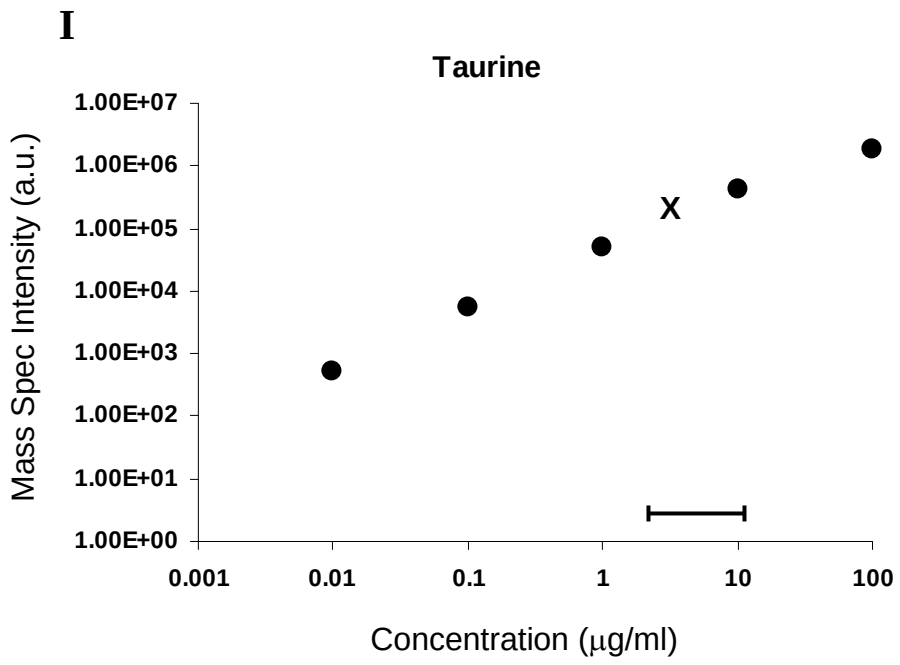
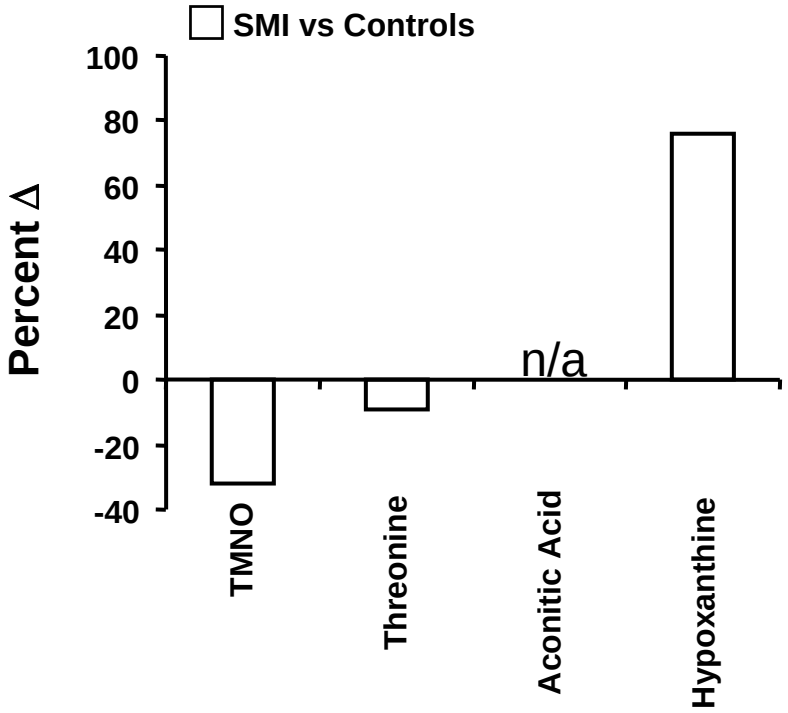

I
Taurine
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Mass Spec Intensity (a.u.)
X
0.001
0.01
0.1
1
10
100
Concentration (μg/ml)

Figure 3


SMI vs Controls
Percent Δ
100
80
60
40
20
0
-20
-40
n/a
TMNO
Threonine
Aconitic Acid
Hypoxanthine