Electronic Supplementary Material (ESI) for Molecular BioSystems.

Changes in the cardiac metabolome caused by perhexiline treatment in a mouse model of hypertrophic cardiomyopathy

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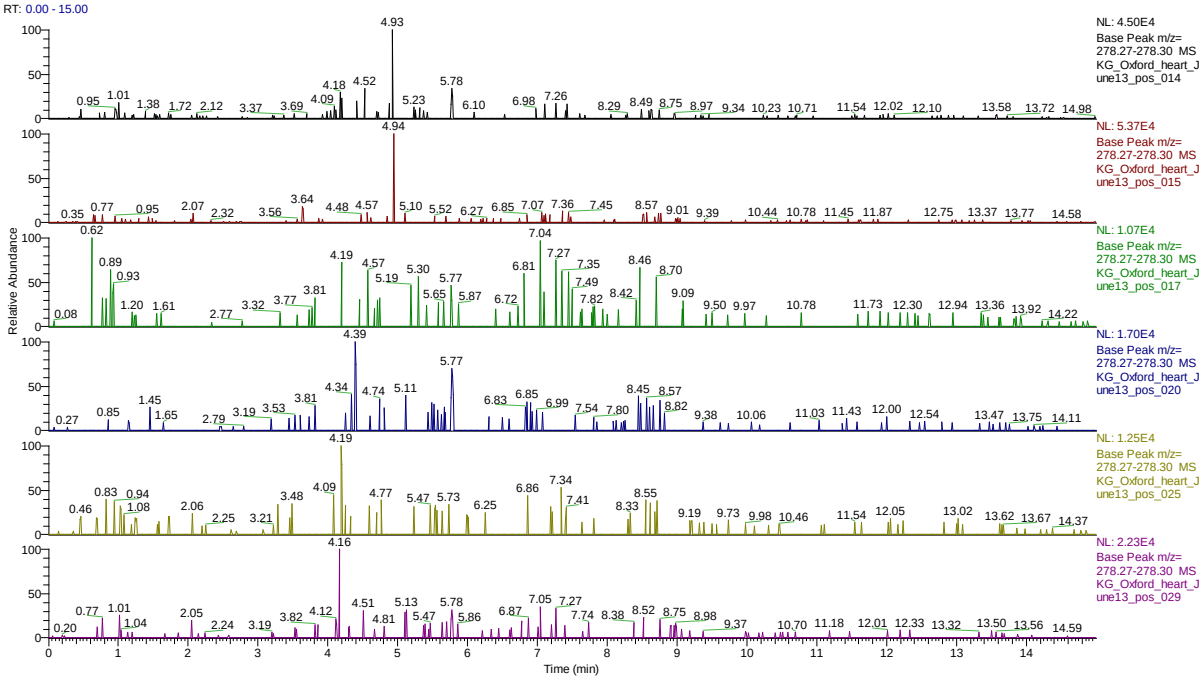
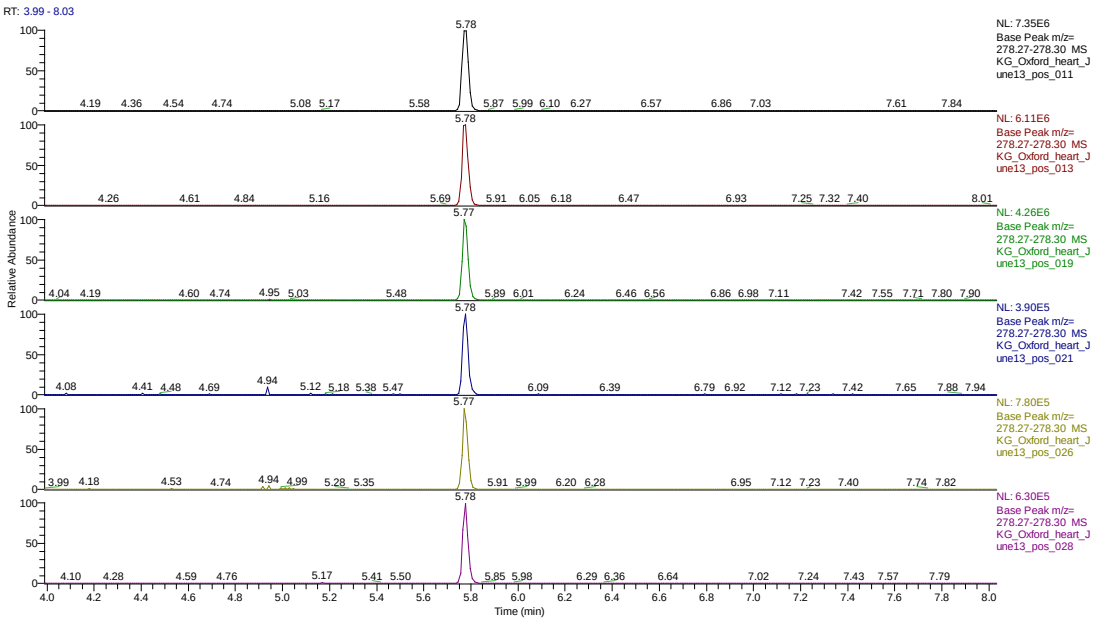
Supplementary Information:

Figure S1. Extracted Ion Chromatograms (EICs) for (a) perhexiline not detected in cardiac tissue of placebo-treated animals and (b) perhexiline detected in perhexiline-treated animals. Hydroxyperhexiline was not detected in perhexiline-treated or placebo animals.

(a)

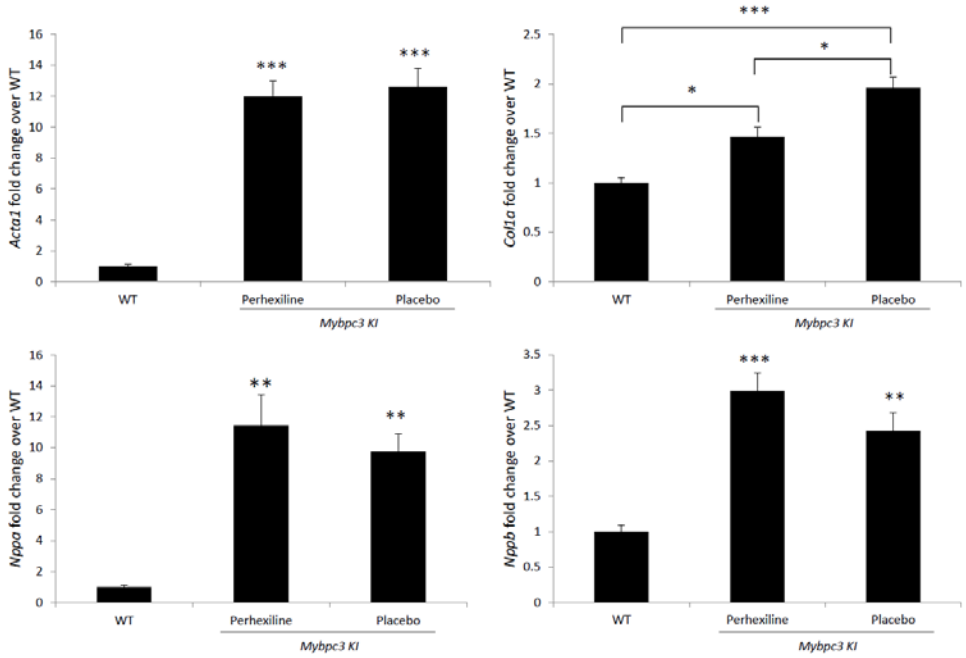


(b)



Figure

S2. Molecular parameters of hypertrophy (*Acta1, Nppa* and *Nppb*) in perhexiline- and placebo-treated *Mybpc3* KI mice compared to WT, assessed by qPCR. Expression was normalised to endogenous control *Gapdh* and the average of WT group set to 1.
*** p < 0.001, ** p < 0.01 compared to WT.



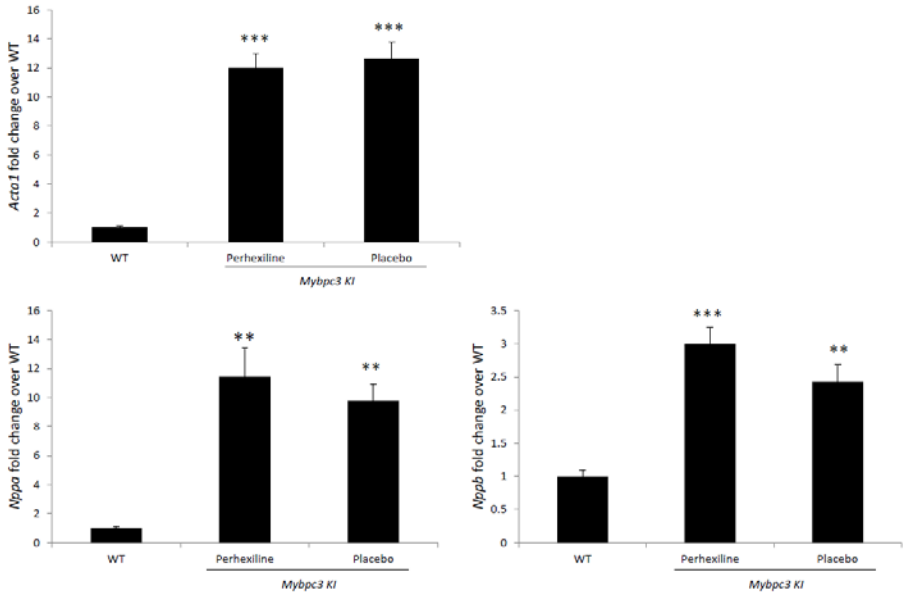


Figure S3. Molecular parameters of fibrosis in perhexiline- and placebo-treated *Mybpc3* KI mice compared to WT, assessed by qPCR: *Col1a* – collagen 1 alpha, *Col3a* – collagen 3 alpha, *Mmp2* – matrix metallopeptidase 2, *Mmp9* – matrix metallopeptidase 9. Expression was normalised to endogenous control *Gapdh* and the average of WT group set to 1. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared to WT, unless otherwise indicated.

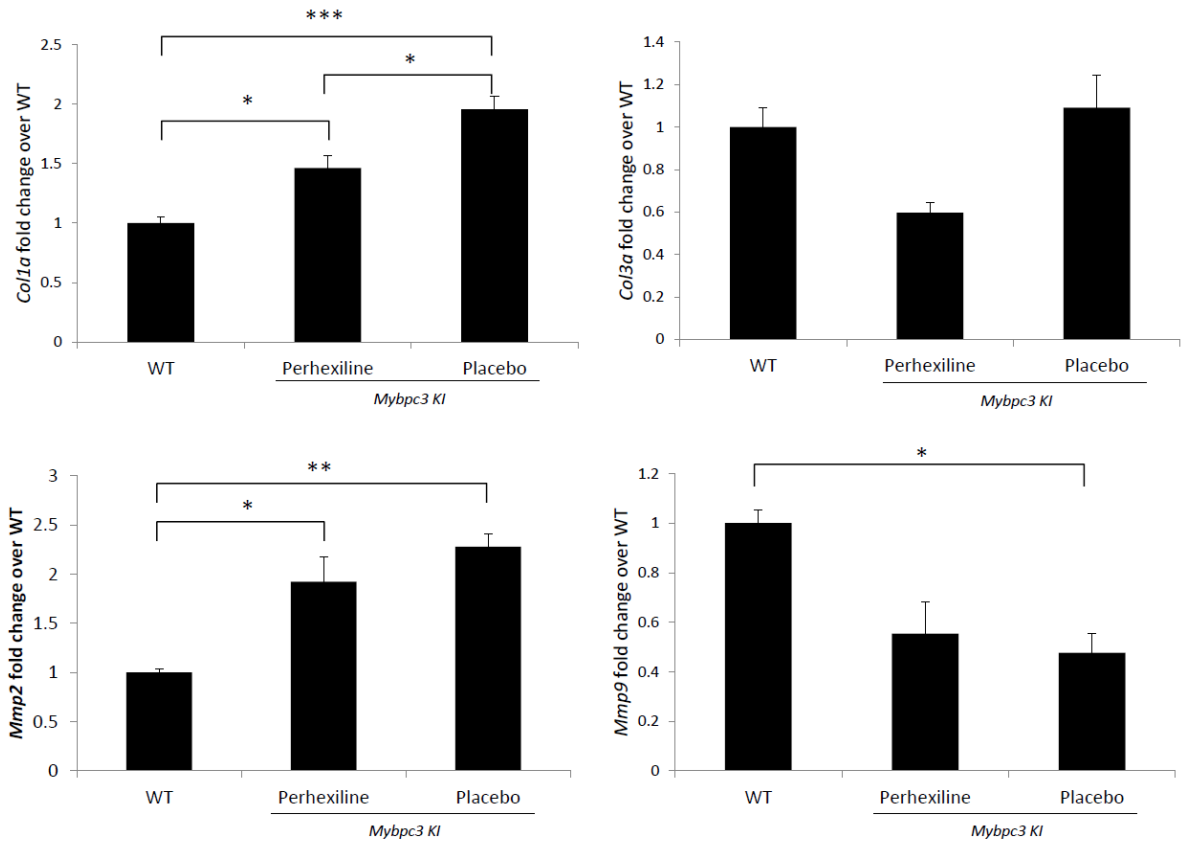


Figure S4. Collagen 1 protein levels in perhexiline- and placebo-treated *Mybpc3* KI mice compared to WT, assessed by Western blotting (A). For quantification (B), protein levels were normalised to endogenous protein calsequestrin (CSQ) and the average of WT group set to 100 %. Please note that this method detects soluble, freshly synthesised collagen.
(C) Total collagen content determined by hydroxyproline measurement in perhexiline- and placebo-treated *Mybpc3* KI mice compared to WT.

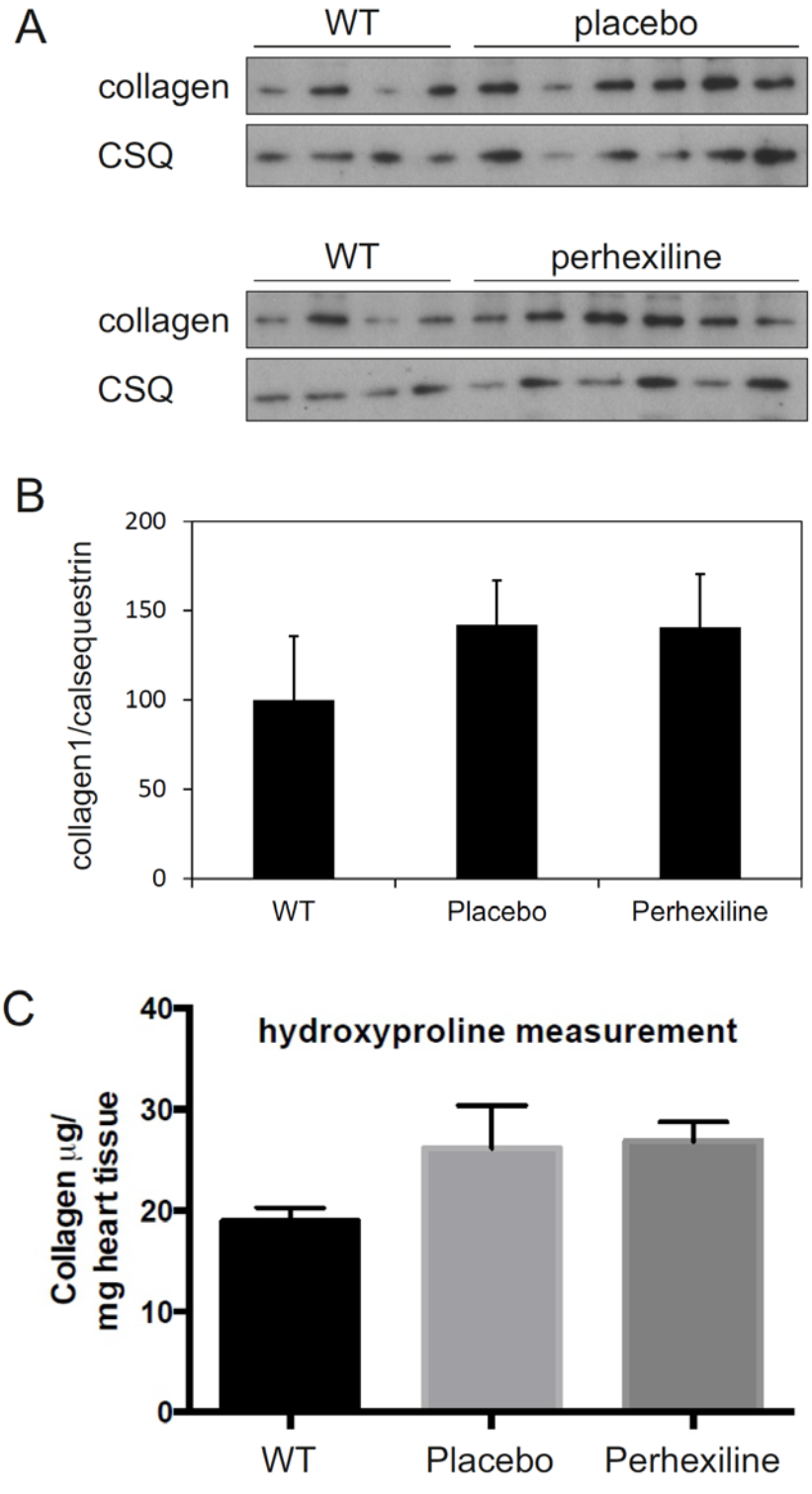


Table S1. Morphometric data on perhexiline- and placebo-treated *Mybpc3* KI mice compared to WT.

	Mybpc3 KI Perhexiline-treated	***Mybpc3 KI Placebo-treated***	***WT***
n	6	6	8
HW / mg	201 ± 7 ***	199 ± 8 ***	124.6 ± 1.3
BW / g	24.8 ± 0.8 **	24.9 ± 0.9 **	27.0 ± 0.3
TL / mm	16.76 ± 0.07	16.64 ± 0.18	16.67 ± 0.04
HW/BW / 10^{-3}	8.2 ± 0.4 ***	8.0 ± 0.4 ***	4.6 ± 0.1
HW/TL / mg/mm	12.0 ± 0.5 ***	11.9 ± 0.4 ***	7.5 ± 0.1
BW/TL / g/mm	1.48 ± 0.04 *	1.49 ± 0.04 *	1.62 ± 0.02

Abbreviations used are: KI,knock-in; HW,whole heart weight; BW, body weight; TL, tibia length. Data are presented as average ± SEM. *** $p < 0.001$ versus WT, ** $p < 0.01$ versus WT. * $p < 0.05$ versus WT. All other differences between groups are not significant.

Table S2. Left ventricular haemodynamic measurements on perhexiline- and placebo-treated *Mybpc3* KI mice compared to WT. For technically reasons, only 5 placebo-treated *Mybpc3* KI mice could be assessed. Significantly changed parameters between WT and *Mybpc3* KI mice are shown in red.
LVEDP - left ventricular end-diastolic pressure, LVSP - left ventricular systolic pressure, +dP/dt - maximal rate of left ventricular contraction, -dP/dt - maximal rate of left ventricular relaxation, SAP - systolic arterial pressure, DAP - diastolic arterial pressure, MAP - mean arterial pressure, Tau= time constant of left ventricular isovolumic relaxation

Included on extra page, page 8 (Table S2)

Table S3. List of metabolites highlighted as biologically important. Metabolites are grouped in to classes based on metabolic function or structural similiarity. Results for Wilcoxon–Mann–Whitney test are shown with the relative fold-change (median placebo-treated/median perhexiline-treated) and associated 95% confidence limits.

Included on extra page, pages 9-14 (Table S3)

Supplementary Table S4 – XCMS parameters

Matched Filter algorithm

```
library(Hmisc)

library(xcms)

library(CAMERA)

xset <- xcmsSet(step=0.02,snthresh=3,mzdiff = 0.05)

#grp <- group(xset,bw=10,mzwid =0.05,minfrac=0.25)

grp <- group(xset,bw=10,mzwid =0.05)

xsa <- annotate(grp, cor_eic_th=0)

peaklist<-getPeaklist(xsa)

write.csv(peaklist,'data_POS.csv')
```

Table S2 Left ventricular haemodynamic measurements on perhexiline- and placebo-treated *Mybpc3* KI mice compared to WT

baseline

		LVSP (mmHg)	LVEDP (mmHg)	Heart Rate (BPM)	+dP/dt (mmHg/s)	-dP/dt (mmHg/s)	Tau (s)
Mybpc3 KI perhexiline	average	87	10.9	483	6079	-3294	0.032
	SEM	4	1.2	35	1054	446	0.006
	n	6	6	6	6	6	6
Mybpc3 KI placebo	average	88	7.3	475	6841	-3522	0.027
	SEM	4	1.9	14	622	243	0.002
	n	5	5	5	5	5	5
WT	average	99	7.6	438	6066	-5890	0.022
	SEM	2	1.7	62	939	1193	0.007
	n	8	8	8	8	8	8
p	perhexiline versus placebo	ns	ns	ns	ns	ns	ns
	WT versus placebo	ns	ns	ns	ns	ns	ns
	WT versus perhexiline	< 0.05	ns	ns	ns	ns	ns

dobutamine 4 ng/g BW /min

		LVSP (mmHg)	LVEDP (mmHg)	Heart Rate (BPM)	+dP/dt (mmHg/s)	-dP/dt (mmHg/s)	Tau (s)
Mybpc3 KI perhexiline	average	87	10.7	507	6186	-3393	0.031
	SEM	4	1.4	42	1212	500	0.007
	n	6	6	6	6	6	6
Mybpc3 KI placebo	average	91	7.9	519	7558	-3812	0.025
	SEM	4	2.3	24	614	258	0.003
	n	5	5	5	5	5	5
WT	average	100	7.6	507	7201	-6113	0.016
	SEM	2	1.6	15	482	539	0.002
	n	8	8	8	8	8	8
p	perhexiline versus placebo	ns	ns	ns	ns	ns	ns
	WT versus placebo	ns	ns	ns	ns	< 0.02	ns
	WT versus perhexiline	< 0.05	ns	ns	ns	< 0.01	< 0.05

dobutamine 16 ng/g BW /min

		LVSP (mmHg)	LVEDP (mmHg)	Heart Rate (BPM)	+dP/dt (mmHg/s)	-dP/dt (mmHg/s)	Tau (s)
Mybpc3 KI perhexiline	average	87	8.4	534	6781	-3767	0.027
	SEM	4	1.4	43	1358	557	0.006
	n	6	6	6	6	6	6
Mybpc3 KI placebo	average	91	6.3	550	8372	-4221	0.020
	SEM	4	2.2	34	823	310	0.001
	n	5	5	5	5	5	5
WT	average	101	6.7	539	8673	-6945	0.019
	SEM	2	1.3	17	787	339	0.004
	n	8	8	8	8	8	8
p	perhexiline versus placebo	ns	ns	ns	ns	ns	ns
	WT versus placebo	ns	ns	ns	ns	< 0.001	ns
	WT versus perhexiline	< 0.05	ns	ns	ns	< 0.001	ns

aterial pressures

		SAP (mm Hg)	DAP (mm Hg)	MAP (mm Hg)
Mybpc3 KI perhexiline	average	77.7	53	61
	SEM	1.5	3	3
	n	6	6	6
Mybpc3 KI placebo	average	76.0	49	58
	SEM	1.6	3	3
	n	5	5	5
WT	average	79.2	51	61
	SEM	2.7	3	3
	n	8	8	8
p	perhexiline versus placebo	ns	ns	ns
	WT versus placebo	ns	ns	ns
	WT versus perhexiline	ns	ns	ns

Table S3. List of metabolites highlighted as biologically important.		
Metabolite	Fold change	P-value
	(non-treated/treated)	
ACYL CARNITINES		
Butanoylcarnitine	0.34 (0.18,1.97)	0.0163
Tetradecenoylcarnitine	0.63 (0.43,1.08)	0.0285
Hexanoylcarnitine	0.74 (0.59,0.97)	0.0209
Decanoylcarnitine	0.75 (0.58,0.99)	0.0283
2-Methylbutyroylcarnitine and/or Isovalerylcarnitine	1.32 (1.08,1.59)	0.0163
Tetradecanoylcarnitine	1.37 (1.11,1.74)	0.0330
Octanoylcarnitine	1.52 (1.05,2.42)	0.0472
Acetylcarnitine	1.71 (1.12,2.52)	0.0163
AMINO ACIDS AND RELATED METABOLITES		
Tyrosine	0.55 (0.30,1.80)	0.0143
Tryptophan	0.75 (0.63,0.91)	0.0090
Indoleamine	1.22 (1.06,1.42)	0.0090
Hexanoylglycine AND/OR Isovalerylalanine AND/OR Isovalerylsarc	1.29 (1.10,1.50)	0.0104
N-Formyl-L-methionineS-Acetonylcysteine	1.31 (1.12,1.54)	0.0090
Hydroxykynurenine	1.35 (1.06,1.82)	0.0163
Hexanoylglycine AND/OR Isovalerylalanine	1.36 (1.11,1.67)	0.0090
N-Acetyl-tryptophan	1.42 (1.09,1.95)	0.0330
FATTY ACIDS AND RELATED METABOLITES		
nonenoic acid	0.29 (0.12,0.63)	0.0090
hydroxy-tetradecadienoate	0.30 (0.09,0.63)	0.0143
5,6-Dihydroxyprostaglandin F1a	0.41 (0.21,1.51)	0.0446
dioxo-decanoic acid AND/OR Decenedioic acid	0.46 (0.24,3.74)	0.0163
Hydroxy-oxo-heptanedioate	0.49 (0.29,1.61)	0.0106
hydroxyoctanoic acid	0.50 (0.39,0.63)	0.0090
Dodecenedioic acid AND/OR dioxo-dodecanoic acid	0.51 (0.33,0.75)	0.0176
hexadecenoic acid	0.51 (0.30,0.76)	0.0163
dioxo-decanoic acid AND/OR Decenedioic acid	0.58 (0.35,1.61)	0.0163
Dihydroxyoctadecanoic acid AND/OR methyl-hexadecanoic acid	0.59 (0.37,1.36)	0.0250
Epoxyoctadecanoic acid AND/OR Hydroxyoctadecenoic acid AND/C	0.59 (0.34,2.26)	0.0176
(+/-)-11-HEPE AND/OR (+/-)-15-HEPE AND/OR (+/-)-18-HEPE ANI	0.63 (0.42,1.20)	0.0250
10,11-dihydro-20-dihydroxy-LTB4 AND/OR 19(R)-hydroxy-PGE1 AN	0.63 (0.46,0.98)	0.0285
dihydroxy-pentadecanoic acid AND/OR hexadecatrienoic acid AND/	0.63 (0.42,1.23)	0.0250
Epoxyoctadecadienoic acid AND/OR oxo-octadecadienoic acid	0.63 (0.40,1.47)	0.0163
methyl-nonadecanoic acid AND/OR dimethyl-octadecanoic acid AN	0.63 (0.39,1.59)	0.0374
Hydroxystearate	0.65 (0.48,0.94)	0.0176
Hydroxyhexanoic acid	0.68 (0.56,0.86)	0.0090
methyl 10,13-dihydroxy-9-oxo-11-octadecenoate AND/OR methyl 9,	0.68 (0.45,1.11)	0.0283
thio-octanoate	0.68 (0.34,1.03)	0.0090
Hydroxydodecanedioic acid	0.69 (0.50,0.93)	0.0330
decatrienoic acid	0.70 (0.52,0.91)	0.0106
methyl nonaoic acid AND/OR Decanoate	0.70 (0.52,1.05)	0.0163
10-Hydroxydecanoic acid	0.71 (0.58,0.88)	0.0090
hydroxy-tridecanoic acid	0.71 (0.52,1.12)	0.0250
oxo-decatetraenoic acid AND/OR Hexanoic acid	0.71 (0.60,0.86)	0.0090
hydroxy-Octadecadienetetraynoic acid	0.72 (0.49,1.31)	0.0250

Octadecatetraynoic acid	0.74 (0.56,0.98)	0.0283
11-methoxy-12,13-epoxy-9-octadecenoic acid AND/OR 8-methoxy-	0.75 (0.58,0.94)	0.0283
hydroxy-dodecenoic acid AND/OR oxo-dodecanoic acid	0.75 (0.63,0.89)	0.0104
Icosatetraenoic acid AND/OR eicosatetraenoic acid	0.75 (0.61,0.93)	0.0283
oxo-nonanoic acid AND/OR methyl-oxo-octanoic acid	0.75 (0.65,0.87)	0.0090
oxo-dihydroxyoctadecatrienoic acid	0.76 (0.62,0.94)	0.0283
Decatetraenedioic acid	0.78 (0.65,0.97)	0.0090
Docosahexaenoic acid ethyl ester AND/OR tetracosahexaenoic acid	0.79 (0.66,0.96)	0.0283
eicosadienoic acid AND/OR Icosatrienoic acid	0.80 (0.69,0.94)	0.0283
hydroxy-hexadecenoic acid AND/OR oxo-hexadecanoic acid	1.21 (1.06,1.39)	0.0163
eicosenoic acid	1.22 (1.04,1.44)	0.0250
octadecadienoic acid	1.22 (1.06,1.43)	0.0163
10,11-dihydro-20-trihydroxy-leukotriene B4	1.23 (1.04,1.48)	0.0374
oxo-heptadecatrienoic acid	1.23 (1.08,1.41)	0.0163
Hydroperoxyoctadecadienoic acid AND/OR dihydroxyoctadecadienc	1.24 (1.04,1.47)	0.0250
hydroxy-hexadecanoic acid	1.24 (1.02,1.55)	0.0163
oxo-docosanoic acid	1.25 (0.95,1.70)	0.0472
tridecadienoic acid	1.26 (1.05,1.50)	0.0163
Tetracosatetraenoic acid	1.27 (1.02,1.54)	0.0283
Octadecatrienoic acid	1.28 (1.08,1.52)	0.0163
docosadienoic acid	1.30 (0.97,1.70)	0.0472
methyl-pentadecanoic acid AND/OR dimethyl-tetradecanoic acid AN	1.31 (1.11,1.55)	0.0163
Dihydroxyoctadecenoic acid AND/OR hydroxy-epoxy-stearic acid AI	1.32 (1.04,1.73)	0.0250
Dodecatetraenedioic acid	1.32 (1.05,1.70)	0.0250
(5Z,13E)-11alpha-Hydroxy-9,15-dioxoprost-13-enoate AND/OR (5Z	1.33 (1.01,1.70)	0.0472
hydroxy-hexadecatetraenoate	1.33 (1.09,1.60)	0.0250
hydroxy-nonanoic acid	1.35 (1.12,1.63)	0.0104
Icosatetraenoic acid AND/OR eicosatetraenoic acid	1.35 (1.09,1.62)	0.0209
methyl-dodecanoic acid AND/OR Tridecanoic acid AND/OR	1.35 (1.13,1.59)	0.0209
Heneicosanedioic acid	1.37 (1.01,2.01)	0.0446
hydroxy-dodecanoic acid	1.40 (1.16,1.73)	0.0104
12-HETrE AND/OR 12R-HETrE AND/OR 15-oxo-11Z,13E-eicosadi	1.46 (1.03,2.30)	0.0250
methyl-heptadecanoic acid AND/OR dimethyl-hexadecanoic acid AI	1.60 (1.09,2.82)	0.0163
bromo-decanoic acid	1.74 (1.22,2.41)	0.0250
hydroxy-tetradecenoic acid AND/OR methyl-oxo-tridecanoic acid AN	1.84 (1.14,4.52)	0.0143
(+/-)-10-HDoHE AND/OR (+/-)-11-HDoHE AND/OR (+/-)-13-HDoHE	1.93 (1.24,2.99)	0.0283
Octadecenoic acid	2.24 (1.36,3.70)	0.0163
GLUTATHIONE AND RELATED METABOLITES		
S-Glutathionyl acetate	0.29 (0.14,8.24)	0.0283
Glutathione sulfinate	0.54 (0.36,1.02)	0.0275
Reduced glutathione	0.76 (0.62,0.93)	0.0250
S-(Formylmethyl)glutathione	1.39 (1.07,1.92)	0.0283
S-(2-Hydroxyethyl)glutathione	1.46 (1.00,2.46)	0.0472
GLYCEROL AND RELATED METABOLISM		
Glycerophosphocholine	0.65 (0.42,1.47)	0.0163
Glycerol-phosphate	1.27 (1.12,1.44)	0.0090
Glycerol	1.51 (1.17,1.91)	0.0039
Indoleglycerol phosphate	1.53 (1.23,2.01)	0.0209

HIGHLIGHTED METABOLITES		
Choline	0.63 (0.45,0.83)	0.0105
fructoselysine 3-phosphate AND/OR Fructoselysine 6-phosphate	0.66 (0.42,1.46)	0.0374
Malate	0.69 (0.54,0.94)	0.0062
Taurine	0.7 (0.62,0.79)	0.0062
Creatinine	0.72 (0.61,0.88)	0.0090
Citrate AND/OR Isocitrate	0.75 (0.65,0.86)	0.0090
Citrulline	0.78 (0.60,1.10)	0.0090
Succinate	1.33 (1.09,1.70)	0.0039
Fumarate AND/OR Maleic acid	1.43 (1.14,1.79)	0.0039
Oxoproline	1.47 (1.25,1.71)	0.0062
Orthophosphate	1.62 (1.29,2.13)	0.0090
PANTETHEINE METABOLISM		
Pantoate	0.21 (0.07,0.48)	0.0090
S-Acetylphosphopantetheine	0.42 (0.26,0.96)	0.0433
Pantothenic acid	0.69 (0.57,0.86)	0.0163
D-4'-Phosphopantothenate	0.76 (0.61,0.97)	0.0176
Pantetheine 4'-phosphate	0.78 (0.60,0.99)	0.0283
Pantetheine	1.45 (1.19,1.72)	0.0163
PEPTIDES		
gamma-L-Glutamyl-L-cysteinyl-beta-alanine	0.51 (0.29,1.74)	0.0250
Glycyl-leucine AND/OR Acetyllysine	0.54 (0.35,1.05)	0.0143
L-isoleucyl-L-proline AND/OR L-leucyl-L-proline	0.56 (0.41,0.73)	0.0105
lysyl-proline	0.59 (0.41,1.04)	0.0190
Leucyl-leucine	0.61 (0.43,1.04)	0.0143
L-gamma-glutamyl-L-isoleucine AND/OR L-gamma-glutamyl-L-leuc	0.71 (0.60,0.87)	0.0283
Polyproline	0.76 (0.58,0.97)	0.0283
L-gamma-glutamyl-L-isoleucine AND/OR L-gamma-glutamyl-L-leuc	0.77 (0.65,0.92)	0.0250
AND/OR Glutamylphenylalanine	1.32 (1.07,1.62)	0.0472
lipoyllysine	1.34 (1.03,1.74)	0.0472
PURINE, PYRIMIDINE AND RELATED METABOLISM		
Arabinosylhypoxanthine AND/OR Inosine	0.39 (0.19,1.52)	0.0209
3'-UMP AND/OR Pseudouridine 5'-phosphate AND/OR UMP AND/C	0.46 (0.25,2.06)	0.0250
6,8-Dihydroxypurine AND/OR Alloxanthine AND/OR Oxypurinol	0.58 (0.37,1.23)	0.0065
N4-Acetylcytidine	0.58 (0.39,0.92)	0.0285
3'-Azido-3'-Deoxythymidine-5'-Diphosphate AND/OR Adenosine 2',5	0.64 (0.40,1.06)	0.0472
Aminoadenosine	0.65 (0.41,1.61)	0.0446
Bromopurine	0.66 (0.54,0.81)	0.0090
UDP AND/OR 2'-deoxy-5-hydroxymethylcytidine-5'-phosphate AND/	0.68 (0.51,0.96)	0.0472
2-AminoAMP AND/OR 3'-Amino-3'-deoxy-AMP AND/OR Adenosine	0.69 (0.49,1.14)	0.0283
1-(5'-Phosphoribosyl)-5-formamido-4-imidazolecarboxamide AND/C	0.72 (0.56,0.93)	0.0285
8-Azaadenosine	0.72 (0.53,0.99)	0.0283
6-Mercaptopurine AND/OR Thiopurine	0.75 (0.69,0.82)	0.0090
dIDP	0.76 (0.59,0.94)	0.0472
Deoxyguanosine-5'-Monophosphate AND/OR 3'-AMP AND/OR 3'-A	0.77 (0.61,1.03)	0.0283
Pseudouridine AND/OR Uridine	0.78 (0.63,1.00)	0.0143
3'-CMP AND/OR CMP AND/OR Cytidine 2'-phosphate AND/OR cyti	0.80 (0.66,1.00)	0.0250
Hypoxanthine	1.22 (1.08,1.39)	0.0065
3-Deoxyguanosine AND/OR 9-Riburonosyladenine AND/OR Adeno	1.23 (1.11,1.36)	0.0209

5-Methyl-2'-deoxycytidine AND/OR 5-Methyldeoxycytidine	1.25 (1.07,1.47)	0.0163
9-Deazainosine-2',3'-O-ethylidenephosphonate	1.25 (1.05,1.49)	0.0472
3-Deazacytidine AND/OR 5-Methyl-2'-Deoxypseudouridine AND/OR	1.26 (1.11,1.43)	0.0143
5-Amino-6-(5'-phosphoribitylamino)uracil	1.29 (0.96,1.75)	0.0472
1-(5-Phosphoribosyl)imidazole-4-acetate AND/OR 5-Hydroxymethyl	1.33 (1.00,1.90)	0.0472
3'-Deoxy 3'-Amino Adenosine-5'-Diphosphate AND/OR	1.34 (0.98,1.78)	0.0472
Adenine	1.35 (1.14,1.61)	0.0065
Methylthioadenosine	1.35 (1.04,1.81)	0.0472
8-Hydroxy-2'-Deoxyguanosine AND/OR 8-Hydroxy-deoxyguanosine	1.37 (1.08,1.79)	0.0163
5'-Methylthioadenosine	1.45 (1.09,2.00)	0.0283
8-Oxo-2'-Deoxy-Guanosine-5'-Monophosphate AND/OR GMP AND	1.45 (1.03,2.35)	0.0283
Arabinosylhypoxanthine AND/OR Inosine	1.46 (1.09,2.16)	0.0163
2-Aminoadenosine AND/OR 3'-Oxo-Adenosine AND/OR 5'-Dehydro	2.24 (1.29,4.51)	0.0163
2'-Deoxyinosine 5'-phosphate AND/OR 7-Alpha-D-Ribofuranosyl-Pu	2.37 (1.07,8.56)	0.0472
SHORT CHAIN FATTY ACIDS		
4-Oxosebacic Acid	0.28 (0.11,0.55)	0.0106
4-Hydroxy-2-oxoglutarate	0.31 (0.11,0.54))	0.0090
5-Hydroxyferulate	0.58 (0.35,1.68)	0.0104
Amino-Methylpentanedioic Acid AND/OR Methylglutamate AND/OR	0.59 (0.36,1.68)	0.0143
2,6-Dioxo-6-phenylhexanoate	0.60 (0.37,1.53)	0.0106
Azelaic acid AND/OR diaminoheptane AND/OR Octenoic acid	0.72 (0.60,0.88)	0.0090
2-Amino-5-phosphopentanoic acid AND/OR	1.26 (1.04,1.53)	0.0250
Allantoate	1.43 (1.16,1.84)	0.0104
(E)-Glutaconate AND/OR Dioxopentanoate AND/OR 2-Methylmalea	1.45 (1.20,1.74)	0.0062
Dihydroxy-methylbutanoate	1.49 (1.24,1.80)	0.0065
STEROLS AND STEROIDS		
21-Hydroxy-5b-pregnane-3,11,20-trione AND/OR 11alpha,15alpha-I	0.59 (0.36,1.58)	0.0374
1alpha-hydroxy-24,25,26,27-tetranorvitamin D3 23-carboxylic acid A	0.69 (0.52,0.98)	0.0330
11beta,17,21-Trihydroxy-2alpha-methylpregn-4-ene-3,20-dione 21-a	0.72 (0.56,0.96)	0.0472
11b,21-Dihydroxy-5b-pregnane-3,20-dione AND/OR 11beta,21-Dihy	0.75 (0.58,1.06)	0.0374
3beta-Hydroxy-16-phosphonopregn-5-en-20-one monoethyl ester	1.34 (0.99,1.95)	0.0472
11-Keto-testosterone AND/OR 16alpha-Hydroxyandrost-4-ene-3,17	1.35 (0.95,1.83)	0.0472
2alpha-(Hydroxymethyl)-17-methyl-5alpha-androstane-3beta,17beta	1.38 (1.05,1.90)	0.0472
SUGARS AND RELATED METABOLITES		
3-Phospho-D-erythronate AND/OR 4-Phospho-D-erythronate AND/(	0.37 (0.13,0.64)	0.0143
alpha-D-Ribose 1-phosphate AND/OR alpha-D-Ribose 5-phosphate	0.45 (0.25,2.19)	0.0105
D-Ribose 1,5-bisphosphate AND/OR 1-Phospho-alpha-D-galacturor	0.48 (0.20,0.86)	0.0283
Acetyl-maltose	0.65 (0.43,0.98)	0.0472
1D-1-Guanidino-3-amino-1,3-dideoxy-scyllo-inositol 4-phosphate AN	0.67 (0.44,1.35)	0.0250
N-acetyl-alpha-D-galactosamine 1-phosphate AND/OR N-Acetyl-alp	0.68 (0.44,1.06)	0.0250
N-Acetyl-D-glucosamine 1,6-bisphosphate	0.68 (0.48,0.94)	0.0389
1D-myo-Inositol 1,4-bisphosphate AND/OR Alpha-D-Glucose 1,6-Bi	0.73 (0.59,0.91)	0.0283
D-Glycerate	0.73 (0.60,0.90)	0.0065
1-Phosphatidyl-D-myo-inositol AND/OR 5-hydroxy-6-methoxyindole	0.77 (0.57,1.05)	0.0446
(3S,4R)-Ketose 1-phosphate AND/OR 1D-myo-Inositol 1-phosphate	0.79 (0.70,0.91)	0.0163
1-O-Methyl-myo-inositol AND/OR 1-O-methyl-scyllo-inositol AND/OI	1.21 (1.07,1.38)	0.0163
2-Dehydro-D-xylonate	1.23 (1.07,1.44)	0.0090
Erythronic acid AND/OR Threonic acid AND/OR	1.23 (1.09,1.39)	0.0065
2,3-Bisphospho-D-glycerate AND/OR 2,3-Disphospho-D-glycerate A	1.33 (1.07,1.70)	0.0250
D-Ribitol 5-phosphate AND/OR Xylitol 5-phosphate AND/OR D-Altro	1.33 (1.06,1.76)	0.0163

Acetylgalactosamine-4-Sulfate AND/OR Acetyl-D-glucosamine 6-su	1.34 (1.02,1.92)	0.0330
Glyceraldehyde 3-phosphate AND/OR Dihydroxyacetone phosphate	1.34 (1.05,1.82)	0.0163
Sorbitol AND/OR Galactitol AND/OR L-Iditol AND/OR L-Sorbitol AN	1.41 (1.15,1.79)	0.0090
2-Deoxy-D-gluconate AND/OR 2-deoxygluconate AND/OR 3-Deoxy	1.42 (1.18,1.69)	0.0104
D-Mannitol 1-phosphate AND/OR Galactitol 1-phosphate AND/OR M	1.62 (0.74,2.60)	0.0250
3-deoxy-D-galactose AND/OR L-Rhamnitol AND/OR	1.73 (1.15,2.45)	0.0090
N-Acetylmannosaminitol	1.81 (1.20,3.19)	0.0163
2-Deoxyribonic acid AND/OR alpha-D-Lyxose AND/OR alpha-D-Rib	2.35 (1.62,3.49)	0.0090
OTHERS AND MIXED CLASSES		
S-Carboxymethyl-L-cysteine	0.26 (0.11,0.49)	0.0090
Canavanine AND/OR Nor-N-Omega-Hydroxy-L-Arginine AND/OR F	0.32 (0.17,2.20)	0.0090
Arabinonic acid AND/OR D-Xylonate AND/OR L-Arabinonate AND/(	0.33 (0.12,0.59)	0.0090
Allopurinol riboside AND/OR Xanthosine AND/OR 1-Deoxy-D-altro-I	0.35 (0.10,0.86)	0.0283
5-Hydroxy-2-polyprenylphenol	0.41 (0.21,7.80)	0.0163
Indoxyl sulfate	0.43 (0.23,2.00)	0.0090
N-Methylphenylethanolamine AND/OR N-Methyltyramine	0.44 (0.23,3.53)	0.0104
alpha-D-Glutamyl phosphate AND/OR 6-Hydroxykynurenate AND/C	0.46 (0.26,1.49)	0.0143
3-Phosphonooxypyruvate	0.47 (0.26,1.87)	0.0176
ubiquinone-2 AND/OR Ubiquinone-6	0.47 (0.28,1.20)	0.0283
DOPA sulfate	0.50 (0.28,2.26)	0.0163
Anandamide AND/OR N-(7Z,10Z,13Z,16Z-docosatetraenoyl)-ethan	0.53 (0.32,1.49)	0.0374
alpha-D-Glutamyl phosphate AND/OR alpha-L-glutamyl phosphate A	0.54 (0.35,1.12)	0.0104
N-Acetyl-D-galactosaminyl-(N-acetylneuraminyl)-D-galactosyl-D- AN	0.54 (0.32,1.73)	0.0163
N-Benzoyl-4-methoxyanthranilate AND/OR trans-2-(4-Methoxyphen	0.56 (0.37,1.05)	0.0163
Diethylphosphoric acid	0.59 (0.37,1.47)	0.0104
N2-Acetyl-L-ornithine AND/OR N5-Ethyl-L-glutamine AND/OR N-Ac	0.59 (0.41,0.99)	0.0163
N-Acetyl-aspartyl-glutamate AND/OR 2-Aminoadenosine AND/OR (	0.59 (0.37,1.32)	0.0250
O-ureidohomoserine AND/OR N-Formimino-L-aspartate	0.59 (0.45,0.79)	0.0090
mercaptoheptanoylthreonine	0.60 (0.37,1.62)	0.0374
Histidine AND/OR Nicotinamide N-oxide AND/OR Urocanate	0.61 (0.39,1.40)	0.0065
Hydroxyanthraquinone	0.62 (0.41,1.23)	0.0104
N-stearoyl taurine	0.62 (0.38,1.58)	0.0374
4-(beta-D-ribofuranosyl)aminobenzene-5'-phosphate	0.65 (0.40,1.73)	0.0209
Pantoate AND/OR Dihydroxy-methylpentanoate AND/OR Dihydroxy	0.65 (0.50,0.88)	0.0065
Quinolinecarboxylic acid AND/OR indole-3-glyoxal AND/OR	0.65 (0.47,1.04)	0.0163
delta(L-2-Aminoadipyl)-L-cysteinyl-D-valine	0.66 (0.42,1.48)	0.0446
Streptamine phosphate AND/OR N'-Formylkynurenine AND/OR De	0.66 (0.43,1.34)	0.0250
trans-3-Hydroxycotinine glucuronide	0.66 (0.41,1.50)	0.0374
PC(3:1)	0.67 (0.53,0.89)	0.0374
6-(Pentylthio)purine AND/OR Hydroxysuberic acid	0.68 (0.53,0.86)	0.0163
Decylubiquinone	0.68 (0.47,1.17)	0.0250
2',3'-Cyclic UMP AND/OR hydroxy-Decadienediynoic acid	0.69 (0.52,1.01)	0.0209
N-Acetyl-aspartyl-glutamate AND/OR (1-Ribosylimidazole)-4-acetat	0.69 (0.56,0.87)	0.0283
Nicotinamide AND/OR Picolinamide AND/OR allylthiourea	0.70 (0.54,0.92)	0.0090
Amino-oxohexanoate AND/OR Isobutyrylglycine AND/OR N-Butyryl	0.71 (0.63,0.82)	0.0090
Benzylcysteine	0.74 (0.61,0.94)	0.0090
N-Acetylmethionine	0.75 (0.62,0.92)	0.0163
Nitropropanoate AND/OR Aminomalonate	0.76 (0.65,0.89)	0.0090
3-Hydroxy-2-methylpyridine-4,5-dicarboxylate	0.77 (0.67,0.89)	0.0090
adrenochrome AND/OR Hippurate	0.77 (0.67,0.90)	0.0090
5'-Oxoinosine AND/OR 9-Riburonosylhypoxanthine AND/OR Prolylh	0.78 (0.64,0.95)	0.0163
Methylhippuric acid AND/OR Phenylacetylglycine	0.79 (0.72,0.88)	0.0090
Hexadecasphinganine	0.80 (0.66,0.98)	0.0283
Homovanillic acid sulfate AND/OR Benzylthiouracil	0.80 (0.68,0.94)	0.0163

Taurocyamine	0.80 (0.71,0.89)	0.0090
2-Dehydro-D-gluconate AND/OR 3-Dehydro-L-gulonate AND/OR 3-	1.22 (1.11,1.35)	0.0106
Threonolactone	1.22 (1.07,1.39)	0.0163
3-Ureidoisobutyrate AND/OR Alanylglycine AND/OR Glutamine AND	1.24 (1.09,1.40)	0.0065
phosphomalate AND/OR Phosphoenolpyruvate	1.24 (1.00,1.63)	0.0090
Aminohippuric acid AND/OR N-carbamoyl-D-phenylglycine	1.25 (1.11,1.40)	0.0143
Pentaporphyrin I AND/OR Porphyrin	1.25 (1.05,1.49)	0.0250
2-phenylethanol glucuronide	1.26 (1.01,1.66)	0.0250
cis-Aconitate AND/OR Dehydroascorbic acid AND/OR trans-Aconita	1.28 (1.11,1.49)	0.0090
Methylmalate AND/OR 2-dehydro-3-deoxy-D-pentonate AND/OR 2-	1.28 (1.08,1.49)	0.0065
Phenylsulfate AND/OR allylthiourea	1.29 (1.08,1.52)	0.0090
methylenediphosphonate	1.31 (1.10,1.54)	0.0106
3-Hydroxy-19-norpregna-1,3,5(10)-trien-20-one AND/OR 3-Methoxy	1.32 (1.09,1.63)	0.0275
Dimethylglutaric acid AND/OR Methyladipic acid AND/OR Amino He	1.35 (1.15,1.58)	0.0090
(5-L-Glutamyl)-L-glutamate AND/OR (R)-4-Dehydropantoate AND/O	1.36 (1.16,1.59)	0.0065
L-Tyrosine methyl ester 4-sulfate	1.36 (1.09,1.70)	0.0176
3,4-Dihydroxymandelate	1.38 (1.09,1.85)	0.0090
4-(glutamylamino) butanoate AND/OR Succinyl-L-ornithine	1.38 (1.05,1.92)	0.0163
N-Acetyl-aspartyl-glutamate AND/OR Threonolactone AND/OR	1.39 (1.02,2.00)	0.0374
Nicotine glucuronide AND/OR Galactosylhydroxylysine	1.39 (1.12,1.83)	0.0339
N-Succinyl-2-L-amino-6-oxoheptanedioate AND/OR N-Acetyl-D-gala	1.4 (1.02,2.06)	0.0283
1-Amino-1-deoxy-scyllo-inositol AND/OR 4-Deoxy-4-Amino-Beta-D-	1.44 (1.05,2.07)	0.0163
4-Sulfobenzyl alcohol AND/OR Thiopurine S-methylether	1.44 (1.14,1.87)	0.0090
Nicotinurate	1.44 (1.14,1.85)	0.0104
S-Dioxymethionine AND/OR Homocysteine AND/OR Methylcysteine	1.45 (1.10,2.07)	0.0090
6-Hydroxy-7,8-Dihydro Purine Nucleoside AND/OR 6-Hydroxyl-1,6-d	1.47 (1.06,1.99)	0.0283
2-Amino-3-oxoadipate AND/OR N-Acetyl-L-aspartate AND/OR N-Fo	1.49 (1.14,2.02)	0.0090
2-Polyprenylphenol	1.53 (1.05,2.06)	0.0163
Dihydroxy-dioxohexanoate AND/OR Anhydro-fructose AND/OR Deh	1.53 (1.17,2.09)	0.0090
Methylhistamine	1.54 (1.13,2.25)	0.0090
Shikimate-phosphate	1.57 (1.13,2.37)	0.0283
p-Cresol glucuronide	1.6 (1.09,2.64)	0.0163
Polyprenyl phosphate	1.65 (1.14,2.56)	0.0163
(5s)-5-Iododihydro-2,4(1h,3h)-Pyrimidinedione	1.67 (1.19,2.33)	0.0250
D-Altronate AND/OR D-Gluconic acid AND/OR Galactonic acid AND	1.67 (1.19,2.40)	0.0106
2,8-Dihydroxyquinoline-beta-D-glucuronide	1.73 (1.01,2.70)	0.0275
Methylcitric acid AND/OR Methylisocitric acid AND/OR Lipoic acid	1.77 (1.14,3.80)	0.0090
Dihydroxyheptenedioate AND/OR Ornithine	1.87 (1.24,3.28)	0.0163