

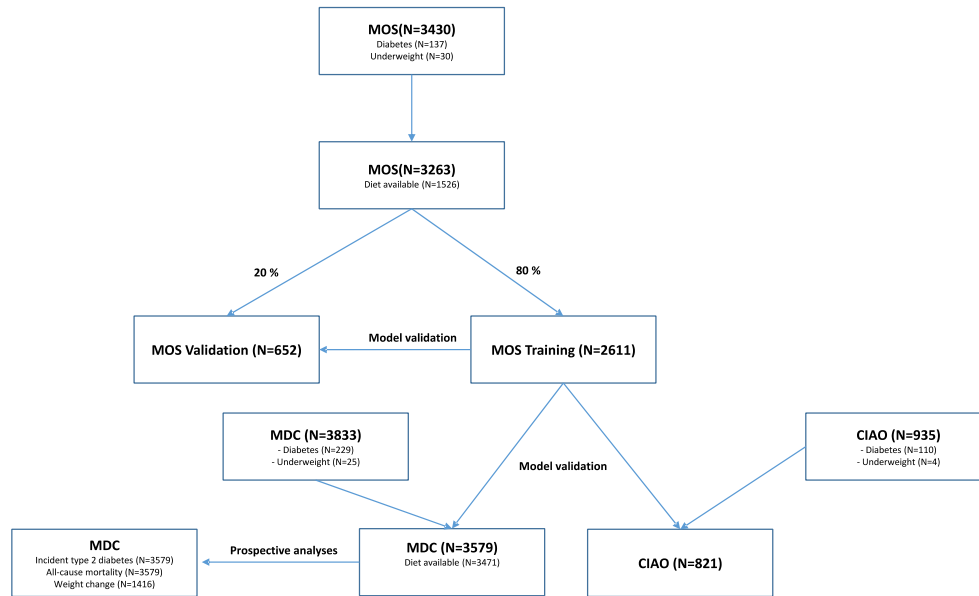


Figure S1. Study design flow chart, including exclusion criteria for all three studies: Malmö Offspring Study (MOS), Malmö Diet and Cancer Study (MDC) and Cilento on Ageing Outcomes Study (CIAO).

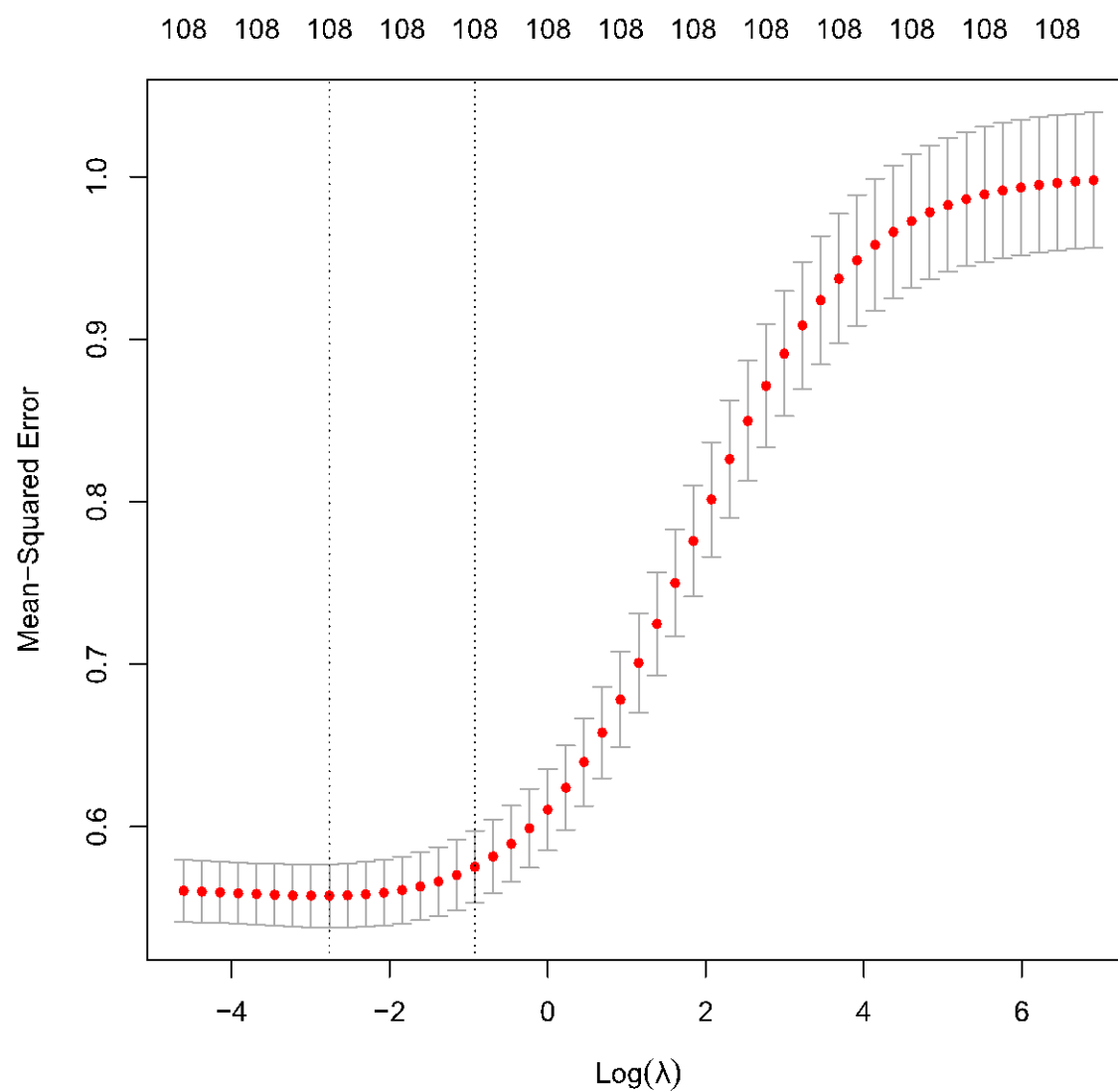


Figure S2. Optimization of λ in cross-validated Ridge-regression models.

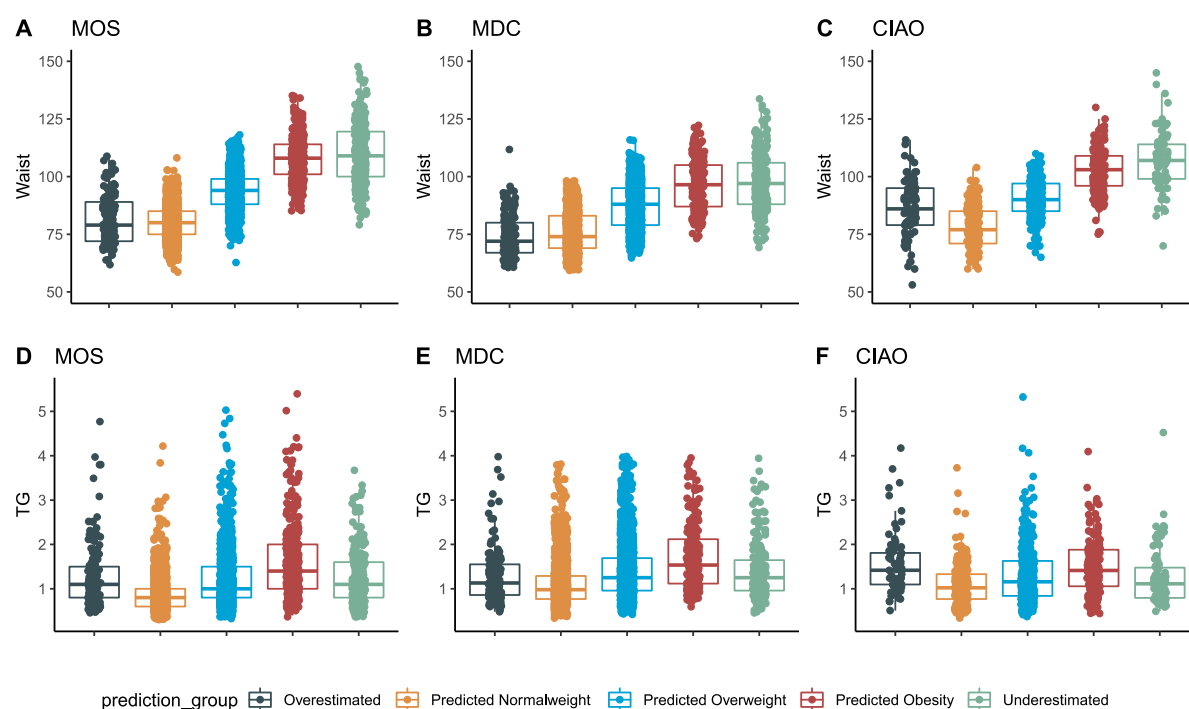


Figure S3. Differences in waist circumference (cm) (A-C) and fasting plasma triglyceride levels (TG) (mmol/L) (D-F) between metabolic BMI outlier groups. Results from the Malmö Offspring Study (N=3263) are shown in (A) and (D), the Malmö Diet and Cancer Study (N=3579) in (B) and (E) and the Cilentano in Aging Outcomes study (N=821) in (C and F).

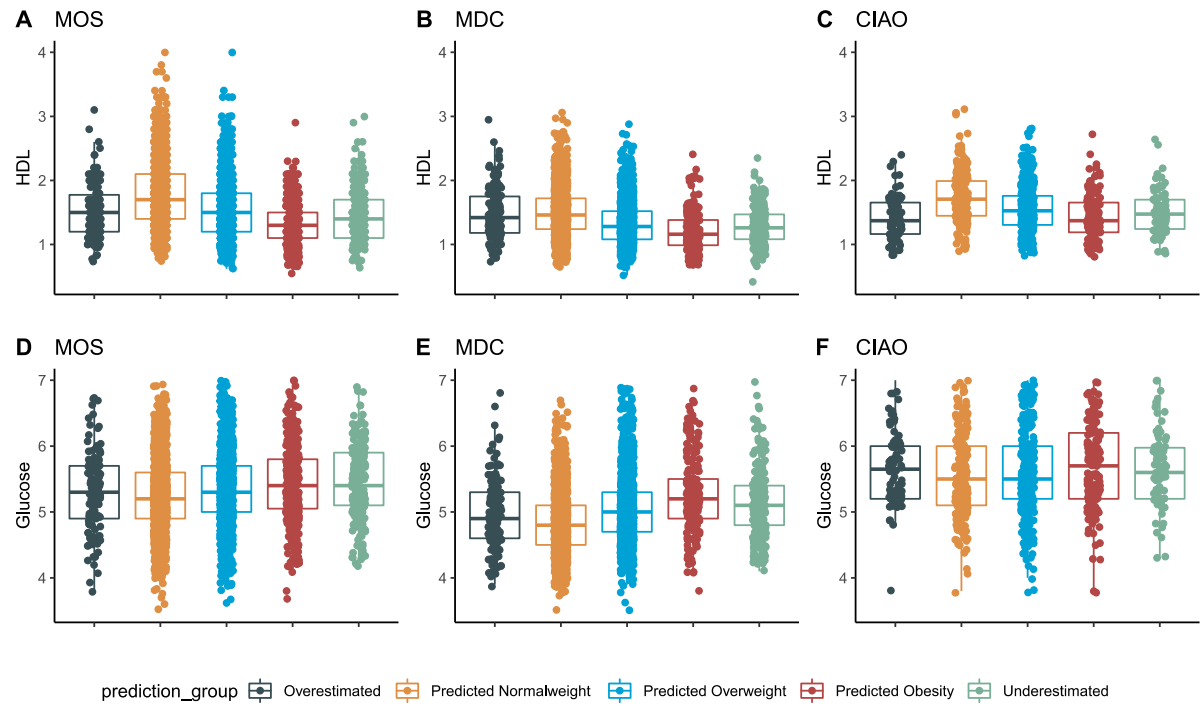


Figure S4. Differences in fasting plasma HDL cholesterol levels (mmol/L) (A-C) and fasting plasma glucose levels (mmol/L) (D-F) between metabolic BMI outlier groups. Results from the Malmö Offspring Study (N=3263) are shown in (A) and (D), the Malmö Diet and Cancer Study (N=3579) in (B) and (E) and the Cilento in Aging Outcomes study (N=821) in (C and F).

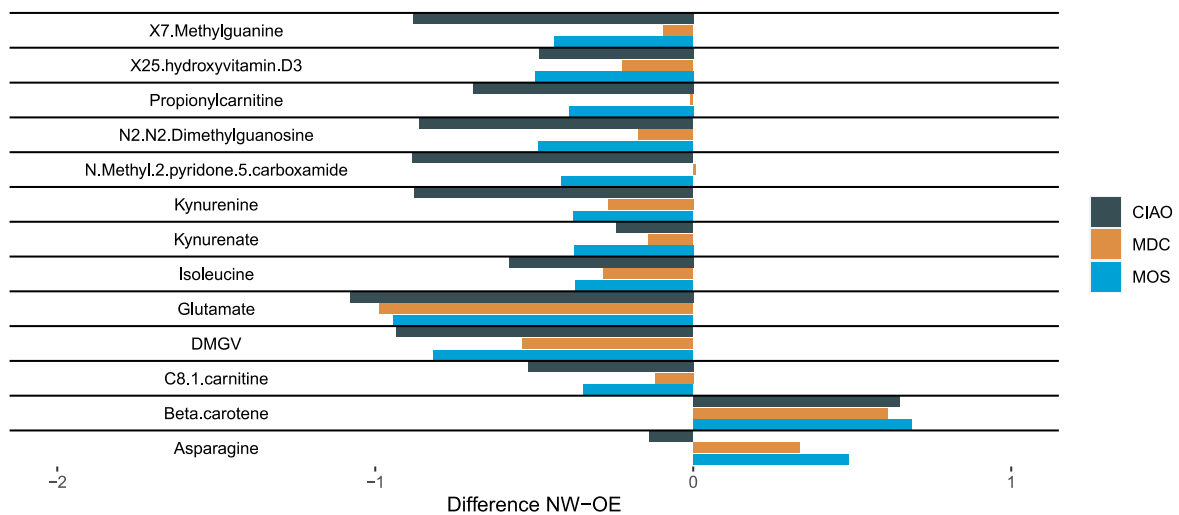


Figure S5. Differences plasma metabolite levels between correctly predicted normal weight (NW) and overestimated (OE). Results are from the Malmö Offspring Study (MOS) (N=3263) (D), the Malmö Diet and Cancer Study (N=3579) and the Cilento in Aging Outcomes study (N=821). The 13 displayed metabolites had significantly different ($p < 4.6e-4$) levels between NW and OE in MOS, as calculated by ANOVA.

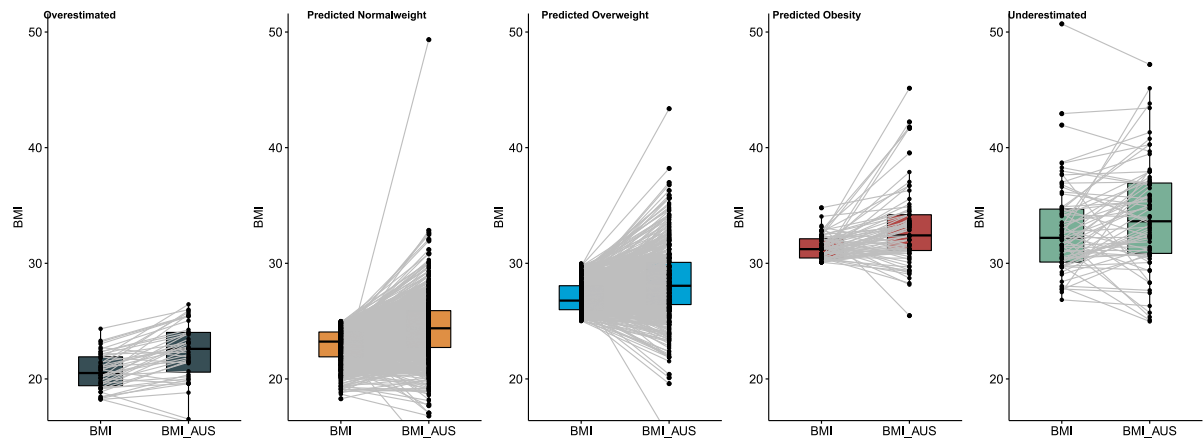


Figure S6. BMI (kg/m²) at baseline and follow-up examination in the Malmö Diet and Cancer Study (N=1416), for metabolic BMI outlier groups. Lines indicate change in BMI from baseline to follow-up examination (mean follow up time 15.6 years).

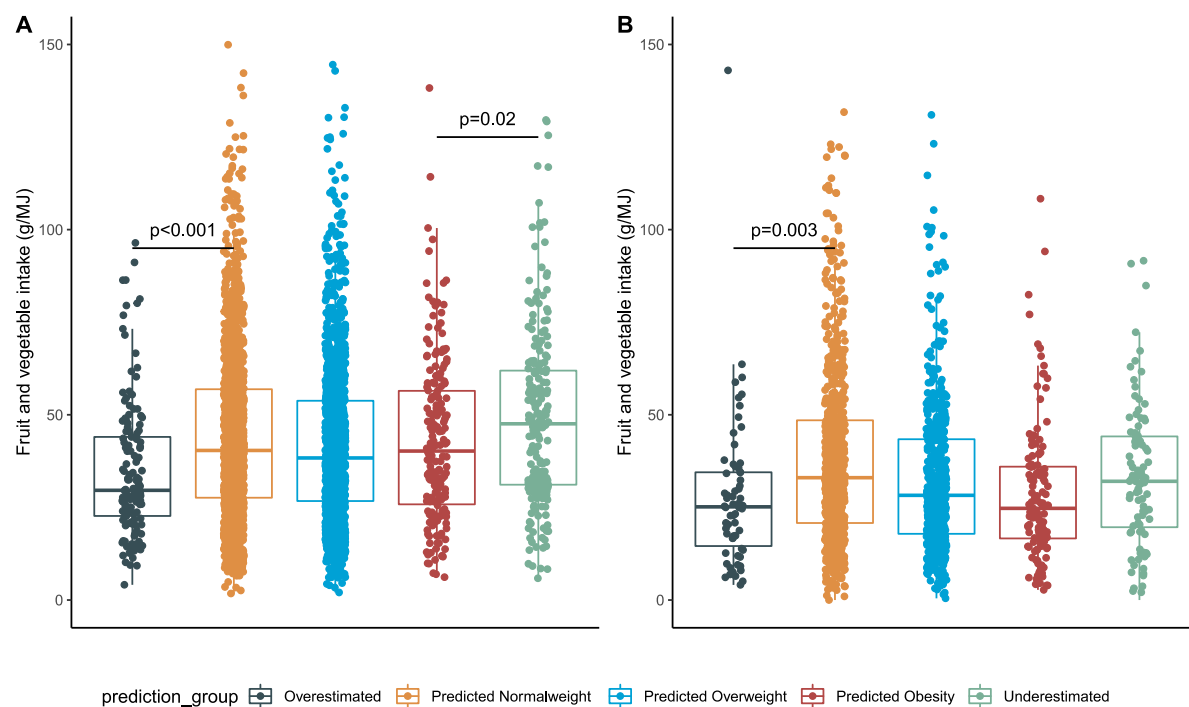


Figure S7. Differences in dietary intake of fruit and vegetables (g/MJ) between metabolic BMI outlier groups. Results from The Malmö Diet and Cancer Study (N=3471) in (A) and The Malmö Offspring Study (N=1526) are shown in (B).

Material S1

Biochemical measurements

Measurements of fasting total cholesterol, HDL cholesterol, triglycerides, and glucose were made according to standard procedures at the Department of Clinical Chemistry at Malmö University Hospital. LDL cholesterol was estimated with the Friedewald equation.

Assessment of dietary intake, physical activity and smoking

The dietary intakes for participants from MOS were performed using a 4-day web-based food record, Riksmaten2010, which was developed by the Swedish National Food Agency¹. In MDC, dietary intakes were assessed with a method combining a 7-day menu book, a food frequency questionnaire and a 45 minute interview². Seven dietary factors related to the Swedish dietary guidelines from the Swedish Food Agency³ were examined: polyunsaturated fat (PUFA) (% of energy intake), saturated fat (SFA) (% of energy intake), fruits and vegetables (g/MJ), fish and shellfish (g/MJ), red meat (g/MJ), added sugar (% of energy intake) and whole-grain (MOS: g/day and MDC: portions of whole-grain meals/day). SFA and PUFA were used as markers of foods included in the guidelines that are rich in those fats, such as high-fat dairy products, high-fat meats, plant oils and nuts. In MOS, leisure-time physical activities were assessed using a questionnaire where participants were categorized as followed: 1: light exercise < 2 hours per week, 2: light exercise > 2 hours per week, 3: moderate exercise 1-2 hours per week and 4: regular exercise > 3 times per week. In MDC, the leisure-time physical activity was assessed using a questionnaire including 17 activities, which was adapted from the Minnesota Leisure Time Physical Activity Questionnaire⁴. Cigarette smoking was elicited by a self-administered questionnaire, with current cigarette smoking defined as any use within the past year.

Metabolite normalization and annotation

Samples were analysed in batches of 180 samples, where quality control samples were run in the beginning of each batch and every eight analytical sample, in order to condition the column and to capture analytical drift, respectively. Metabolite peak areas were integrated using Agilent Profinder B.06.00 (Agilent Technologies, Santa Clara, CA, USA). Metabolites were normalized using metabolite measurements in the quality control samples. First, a low-order nonlinear locally estimated smoothing function was fitted to the metabolite signals in the quality control samples as a function of the injection order. The α -parameter, reflecting the proportion of samples to be used when constructing the correction curve, was set to 2/3. Using this function, a correction curve for the analytical samples was interpolated, to which the metabolite measurements in the analytical samples were normalized⁵. The normalization was performed in R 3.6.1. Metabolites were annotated using synthetic standards or by matching MS/MS fragmentation with The Human Metabolome Database (HMDB)⁶ and METLIN⁷ or by matching fragment ions to putative molecule fragments. In total, 108 Identified and putatively annotated metabolites were measured. A list of reported metabolites can be found below, including corresponding m/z, chromatographic retention time, HMDB identifier, coefficient of variation (CV) in quality control samples and annotation confidence⁸.

Metabolites	m/z	rt	HMDB ID	CV	Annotation Level	Imputed (%)
Urea	61.0393	1.98	HMDB00294	7.1	1	0.1
Trimethylamine-N-Oxide	76.0753	4.28	HMDB00925	6.4	1	3.0
Alanine	90.0553	7.5	HMDB00161	10	1	0.2
Dimethylglycine	104.0703	6.59	HMDB00092	20	1	0.4
2-aminoisobutyrate	104.0713	6.9	HMDB01906	11.1	1	0.1
Choline	104.1074	4.01	HMDB00097	6.9	1	0.1

Serine	106.0503	8.56	HMDB00187	6.1	1	0.1
Creatinine	114.0663	4.55	HMDB00562	4.7	1	0.1
Proline	116.0703	6.64	HMDB00162	6.9	1	0.1
Guanidineacetate	118.0613	7.56	HMDB00128	5.5	1	0.0
Betaine	118.0864	6.14	HMDB00043	7.5	1	0.1
Valine	118.0864	6.57	HMDB00883	14	1	0.0
Threonine	120.0653	7.85	HMDB00167	10.9	1	0.1
Nicotinamide	123.0553	1.36	HMDB01406	19.2	1	0.3
Taurine	126.0223	6.62	HMDB00251	7.2	1	1.1
Pyroglutamate	130.0503	2.56	HMDB00267	19.8	2	0.4
N-methylproline	130.0863	6.25	HMDB94696	10.6	1	3.0
Pipecolate	130.0863	6.84	HMDB00716	14.2	1	0.5
Creatine	132.0773	7.19	HMDB00064	5.4	1	0.1
Isoleucine	132.1023	6.09	HMDB00172	6.7	1	0.3
Leucine	132.1023	5.92	HMDB00687	6.8	1	0.1
Asparagine	133.0603	8.7	HMDB00168	7.2	1	0.1
Ornithine	133.0973	11.5	HMDB00214	6.7	1	0.0
		8				
Hypoxanthine	137.0463	3.8	HMDB00157	7.3	1	0.1
Trigonelline	137.0474	6.38	HMDB00875	7.3	1	0.8
Methylnicotinamide	137.0704	4.96	HMDB03152	8.5	1	0.2
Urocanate	139.0493	2.38	HMDB00301	18.2	1	1.8
Proline.betaine	144.1023	6.21	HMDB04827	4.3	1	0.2
4-trimethylammoniobutanoate	146.1174	4.58	HMDB01161	8.7	1	0.1
Glutamine	147.0763	8.48	HMDB00641	5.6	1	0.1
Lysine	147.1133	11.4	HMDB00182	6.3	1	0.0
Glutamate	148.0603	8.14	HMDB00148	6.2	1	0.1
Methionine	150.0583	6.32	HMDB00696	9.7	1	0.2
N-Methyl-4-pyridone-3-carboxamide	153.0653	1.92	HMDB04194	14.1	1	0.2
Homostachydrine	158.1173	6.02	HMDB33433	7.2	1	0.9
Methyllysine	161.1283	10.6	HMDB02038	4.5	1	0.0
		6				
Carnitine	162.1124	6.19	HMDB00062	7.9	1	0.2
Methionine-S-oxide	166.0533	8.23	HMDB02005	10.5	1	2.6
7-methylguanine	166.0723	4.77	HMDB00897	10.5	1	0.1
Phenylalanine	166.0863	5.88	HMDB00159	5.8	1	0.1
3-methylhistidine	170.0923	10.8	HMDB00479	5.5	1	0.1
		2				
1-methylhistidine	170.0923	9.72	HMDB00001	6.7	1	0.1
Acetylornithine	175.1093	7.86	HMDB03357	4.9	1	0.3
Arginine	175.1193	10.9	HMDB00517	12.5	1	0.1
		2				
Citrulline	176.1023	9.04	HMDB00904	5.2	1	0.1
Dimethyllysine	176.1513	10.1		6.9	1	0.0
		7				

Cotinine	177.1023	1.43	HMDB0001046	-	1	61.9
Hippurate	180.0653	1.14	HMDB00714	6.1	1	1.4
Paraxanthine	181.0723	1.33	HMDB01860	6	1	0.9
Tyrosine	182.0813	6.64	HMDB00158	13	1	2.4
Acisoga	185.1283	1.63	HMDB61384	7.1	1	0.1
Homoarginine	189.1343	10.5	HMDB00670	5.4	1	0.0
Histidine	189.1343	10.5	HMDB00177	9.4	1	0.1
NMMA	189.1343	10.2	HMDB29416	12.9	1	2.4
Trimethyllysine	189.1594	10.1	HMDB01325	5.5	1	0.4
Kynurenate	190.0503	5.06	HMDB00715	13.7	1	0.1
Homocitrulline	190.1183	8.86	HMDB00679	14.7	1	1.3
Hydroxycotinine	194.1044	2.06	HMDB0001390	-	1	51.9
Caffeine	195.0873	1	HMDB01847	10.8	1	10.4
AAMU	199.0823	4.61	HMDB04400	7.4	1	16.5
DMGV	202.1184	6.01	HMDB0240212	10.3	1	0.3
ADMA	203.1503	9.52	HMDB01539	15.6	1	0.4
SDMA	203.1503	9.51	HMDB03334	17.6	1	0.9
Acylcarnitine C2:0	204.1234	4.45	HMDB00201	6.5	1	0.1
Tryptophan	205.0973	5.93	HMDB00929	9	1	0.1
3-hydroxytrimethyllysine	205.1544	10.8	HMDB01422	6.9	1	0.3
Kynurenine	209.0923	6	HMDB00684	6	1	0.1
Acetylarginine	217.1293	7.08	HMDB04620	7.7	1	0.0
Acylcarnitine C3:0	218.1384	4.06	HMDB00824	6.8	1	0.0
Pantothenate	220.1183	1.63	HMDB00210	11.7	1	0.3
Ergothioneine	230.0953	7.35	HMDB03045	8.5	1	0.3
Acylcarnitine C4:0	232.1534	3.63	HMDB02013	6.3	1	0.2
Cystine	241.0313	13.0	HMDB00192	9.4	1	0.1
Tiglylcarnitine	243.1464	3.46	HMDB02366	11.4	2	2.3
Acylcarnitine C5:0	245.1624	3.23	HMDB00688	7.7	1	0.2
Acylcarnitine C4:0-OH	248.1486	4.84	0	5.6	2	7.6
Glycerophosphocholine	259.1173	8.71	HMDB00086	29.2	2	0.1
Acylcarnitine C6:0	260.1854	2.88	HMDB00756	8.5	2	0.3
Phenylacetylglutamine	265.1173	1.98	HMDB06344	13.4	1	0.1
1-methyladenosine	282.1203	6.46	HMDB03331	10	2	0.1
Acylcarnitine C8:1	286.2014	2.73	HMDB13324	7.1	2	0.1
Piperine	287.151	0.83	HMDB002937	6.2	1	0.4
Acylcarnitine C8:0	288.2164	2.49	HMDB00791	3.9	1	0.3
Acylcarnitine C9:0	302.2324	2.23	HMDB13288	7.2	2	0.1
Acylcarnitine C8:0-OH	304.2114	4.06	0	12.5	2	0.2
Acylcarnitine C10:3	310.2014	2.42	0	7	2	0.2

N2-N2-dimethylguanosine	312.1303	4.74	HMDB04824	14.2	1	0.4
Acylcarnitine C10:2	312.2164	2.44	0	7.7	2	0.1
Acylcarnitine C10:1	314.2314	2.34	HMDB13205	8.7	2	0.2
Acylcarnitine C10:0	316.2484	2.18	HMDB00651	7.6	1	0.2
Acylcarnitine C11:1	328.2474	2.28	0	7	2	0.1
Acylcarnitine C11:0	330.2624	2.14	HMDB13321	9.4	2	0.2
Acylcarnitine C10:0-OH	332.2434	3.52	0	12.7	2	0.3
Acylcarnitine C12:2	340.2494	2.23	0	10.6	2	0.5
Acylcarnitine C12:1	342.2624	2.16	HMDB13326	8.9	2	0.1
Acylcarnitine C12:0	344.2784	2.13	HMDB02250	7.6	2	0.1
Acylcarnitine C13:1	356.2784	2.1	0	10.2	2	0.1
Acylcarnitine C13:0	358.2944	2.03	0	7.9	2	0.2
Acylcarnitine C14:2	368.2794	2.08	HMDB13331	8.3	2	0.1
Acylcarnitine C14:1	370.2934	2.03	HMDB024058	7.9	2	0.1
			8			
Acylcarnitine C14:0	372.3104	1.94	HMDB05066	7.8	1	0.2
Acylcarnitine C16:1	398.3254	1.93	HMDB13207	7.7	2	0.2
Acylcarnitine C16:0	400.3424	1.85	HMDB00222	5	1	0.1
25-hydroxyvitamin-D3	401.3413	0.81	HMDB03550	18.9	1	0.1
Acylcarnitine C18:2	424.3454	1.87	0	7.4	2	0.1
Acylcarnitine C18:1	426.3564	1.86	0	8	2	0.1
Acylcarnitine C18:0	428.3714	1.84	HMDB00848	9.7	2	0.1
Beta-carotene	537.4453	0.83	HMDB00561	8.4	1	0.1

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