Electronic Supplementary Material (ESI) for Analytical Methods.

Table S1. Instrumental parameters of the applied timed-PRM method including normalized collision energy (NCE%) for each metabolite and its isotope labelled internal standard. The underlined product ion was used as the quantifying ion.

ID	Analytes	Abbreviation	Precursor ion (m/z) $[M+H]^+$	RT (min)	Analysis Range (min)	NCE (%)	Product ions (m/z)
1	Cystathionine	CT	223.07470	0.85	0.2-1.4	30	134.02721
	Cystathionine-[$^2H_4$]	CT-[$^2H_4$]	227.0998	0.86	0.2-1.4	30	138.05232
2	Taurine	Tau	126.02194	0.88	0-2.0	20	108.01168
	Cystathionine-[$^2H_4$]	CT-[$^2H_4$]	227.0998	0.86	0.2-1.4	30	138.05232
3	Thiamine	B1	265.11176	1.00	0.3-1.5	20	122.07130
	Thiamine-[$^{13}C_3$]	B1-[$^{13}C_3$]	269.1296	1.00	0.3-1.5	20	147.05794
4	Nicotinamide	NA	123.05529	1.47	0.75-2.25	85	80.04951
							96.04459
	Nicotinamide-[$^{13}C_6$]	NA-[$^{13}C_6$]	129.07542	1.47	0.75-2.25	85	85.06628
5	Picolinic acid	PA	124.03930	1.67	0.95-2.45	85	96.04449
							78.03385
	Picolinic acid-[$^2H_4$]	PA-[$^2H_4$]	128.06441	1.51	0.95-2.45	85	100.06974
6	Niacin	B3	124.03930	1.66	0.95-2.45	140	53.03890
							96.04462
	Nicotinamide-[$^{13}C_6$]	NA-[$^{13}C_6$]	129.07542	1.47	0.75-2.25	85	85.06628
7	Neopterin	NP	254.08838	1.52	0.75-2.25	37	206.06708
							133.95898
	Neopterin-[$^{13}C_5$]	NP-[$^{13}C_5$]	259.10515	1.52	0.75-2.25	37	210.08041
8	Hypoxanthine	HX	137.04579	2.14	1.5-2.7	80	110.03500
	Theobromine	The	181.07200	5.87	4.0-6.0	40	138.06619
							110.07127
9	Dopamine	Dop	154.08626	2.16	1.5-2.7	35	137.05940
	Dopamine-[$^2H_4$]	Dop-[$^2H_4$]	158.11082	2.13	1.5-2.7	35	141.08449
10	Quinolinic acid	QA	168.02913	2.15	1.6-2.8	60	96.04451
							124.03922
	Quinolinic acid-[$^{13}C_4.^{15}N$]	QA-[$^{13}C_4.^{15}N$]	173.07834	2.13	1.6-2.8	60	99.04860
11	Pyridoxal 5′-phosphate	PLP	248.03185	2.31	1.6-2.8	40	150.05495
	Pyridoxine-[$^2H_3$]	B6-[$^2H_3$]	173.09945	2.47	1.9-3.1	40	155.08949
12	Pyridoxine	B6	170.08117	2.48	1.9-3.1	40	152.07066
							134.06003
	Pyridoxine-[$^2H_3$]	B6-[$^2H_3$]	173.09945	2.47	1.9-3.1	40	155.08949
13	3-Hydroxykynurenine	3HK	225.08698	2.81	2.3-3.5	15	208.06007
							110.06012
	3-Hydroxykynurenine-[$^{13}C_6$]	3HK-[$^{13}C_6$]	231.17676	2.81	2.3-3.5	15	214.08353
14	Tyrosine	Tyr	182.08117	3.34	3.0-3.8	30	136.07563
							165.05466
	Tyrosine-[$^2H_7$]	Tyr-[$^2H_7$]	189.12511	3.27	3.0-3.8	30	143.11952
15	Serotonin	Ser	177.10224	4.16	3.0-4.5	10	160.07578
	Serotonin-[$^2H_4$]	Ser-[$^2H_4$]	181.12735	4.12	3.0-4.5	10	164.10089
16	Kynurenine	Kyn	209.09207	4.74	4.05-5.55	20	192.06512
							94.065450
	Kynurenine-[$^{13}C_6$]	Kyn-[$^{13}C_6$]	215.17736	4.74	4.05-5.55	20	198.08536
17	3-Hydroxyanthranilic acid	3HAA	154.04987	4.85	4.15-5.65	20	136.03903
							108.04455
	3-Hydroxyanthranilic acid-[$^{13}C_6$]	3HAA-[$^{13}C_6$]	160.07000	4.85	4.15-5.65	20	142.05916
18	5-hydroxytryptophan	5HT	221.09207	4.94	4.5-5.5	30	204.06561
							162.05498
	Tryptophan-[$^{13}C_{11}.^{15}N_2$]	Trp-[$^{13}C_{11}.^{15}N_2$]	218.12813	6.20	5.8-6.6	10	200.10481
19	Phenylalanine	PhA	166.08626	5.42	5.0-6.0	55	120.08096
							90.94795

	Phenylalanine-[$^{2}H_5$]	PhA-[$^{2}H_5$]	171.11764	5.33	5.0-6.0	55	125.11234
20	Pantothenic acid	B5	220.11795	5.70	5.2-5.7	10	90.05515
	Theobromine	The	181.07200	5.87	4.0-6.0	40	138.06619
							110.07127
21	Tryptophan	Trp	205.09715	6.22	5.8-6.6	10	188.07034
							146.06006
	Tryptophan-[$^{13}C_{11}.^{15}N_2$]	Trp-[$^{13}C_{11}.^{15}N_2$]	218.12813	6.20	5.8-6.6	10	200.10432
22	Cyanocobalamin	B12	678.29098	6.20	5.8-6.6	32	147.09154
	Cyanocobalamin-[$^{13}C_7$]	B12-[$^{13}C_7$]	681.80272		5.8-6.6	17	366.12585
23	Folate	B9	442.14696	6.26	5.8-6.6	25	295.09427
							367.28220
	Folate-[$^{13}C_5.^{15}N$]	B9-[$^{13}C_5.^{15}N$]	448.35728	6.26	5.8-6.6	25	313.10452
24	Xanthurenic acid	XA	206.04478	6.53	6.1-6.9	55	178.04951
							132.04419
	Xanthurenic acid-[$^{13}C_6$]	XA-[$^{13}C_6$]	212.06490	6.54	6.1-6.9	55	184.06964
25	Anthranilic acid	AA	138.05496	6.53	6.1-6.9	10	120.04446
							92.04964
	Anthranilic acid-[$^{13}C_6$]	AA-[$^{13}C_6$]	144.0751	6.54	6.1-6.9	10	126.06459
26	Kynurenic acid	KA	190.04987	6.56	6.2-7.0	55	162.05472
	Kynurenic acid-[$^{2}H_5$]	KA-[$^{2}H_5$]	195.08125	6.54	6.2-7.0	55	167.08607
27	Biotin	B7	245.09544	6.58	6.2-7.0	35	227.08540
	Biotin-[$^{2}H_2$]	B7-[$^{2}H_2$]	247.10745	6.58	6.2-7.0	35	229.09794
28	Riboflavin	B2	377.14556	6.64	6.2-7.0	40	243.08792
	Biotin- [$^{2}H_2$]	B7-[$^{2}H_2$]	247.10745	6.58	6.2-7.0	35	229.09794
29	Melatonin	Mel	233.12845	7.00	6.5-7.5	40	174.09148
							102.12820
	Melatonin-[$^{2}H_4$]	Mel-[$^{2}H_4$]	237.15356	7.00	5.1-6.0	40	178.11659

Table S2. Calibration and quality control (QC) standards were prepared from independent stocks. Stock A contained the lowest concentration of analytes, while Stock B with an intermediate and Stock C with the highest concentration of analytes.

Calibration and QC stock A (prepared in duplicate)		
Analyte	*Source stock* (mg/mL)	*Volume (µL)*
Melatonin	0.01	50
Cyanocobalamin	0.01	200
Biotin, Folate	1	10
Picolinic acid, Anthranilic acid, Xanthurenic acid, Kynurenic acid, Neopterin	1	20
Dopamine	1	40
Nicotinamide, Thiamine, Riboflavin, Pyridoxine, Pyridoxal 5'-phosphate	1	50
Quinolinic acid, 3-Hydroxykynurenine, 3-Hydroxyanthranilic acid	1	100
Cystathionine, Serotonin, Pantothenic acid,	1	200
Hypoxanthine	1	1000
NA	MeOH	1440

Total volume		4000

Calibration and QC stock B (prepared in duplicate)

Analyte	*Source stock* (mg/mL)	*Volume (µL)*
Kynurenine	1	100
Niacin	1	50
Stock A	Stock A	400
NA	MeOH	1450
Total volume		2000

Calibration and QC stock C (prepared in duplicate)

Stock B	Stock B mg/mL	1000
Tryptophan	1	500
5-hydroxytryptophan	1	500
Phenylalanine	1	500
Tyrosine	1	500
Taurine	1	500
NA	MeOH	21500
Total volume		25000

Table S3. Preparation of working calibration and QC dilutions in MeOH. The calibration solutions were prepared at eight levels and QC's at four levels.

Calibration working solution preparation	**Solution to dilute**	**Volume of solution (µL)**	**Volume of MeOH (µL)**
Calibration level 8 (ULOQ)	Stock C	NA	NA
Calibration level 7	Calibration level 8 (ULOQ)	2000	2000
Calibration level 6	Calibration level 7	2000	2000
Calibration level 5	Calibration level 6	2400	1600
Calibration level 4	Calibration level 5	2688	1344
Calibration level 3	Calibration level 4	1568	2352
Calibration level 2	Calibration level 3	1920	1920
Calibration level 1 (LLOQ)	Calibration level 2	1920	1920
QC working solution preparation	**Solution to dilute**	**Volume of solution (µL)**	**Volume of MeOH (µL)**
QC level 7 (QCH)	Calibration level 8 (ULOQ)	2000	2000
QC level 6	Calibration level 7	2000	2000
QC level 5	Calibration level 6	2400	1600
QC level 4 (QCM)	Calibration level 5	2688	1344
QC level 3	Calibration level 4	1568	2352
QC level 2 (QCL)	Calibration level 3	1920	1920
QC level 1 (LLOQ)	Calibration level 2	1920	1920

Table S4. The concentration of calibration, QC's series and LLOQ values

Calibration series preparation - analyte concentration (ng/mL)

	Melatonin	Cyanocobalamin	Folate, Biotin	PA, AA, XA, KA, Neopterin	Dopamine	Nicotinamide, Thiamine, Riboflavin Pyridoxine, PLP	QA, 3-HK, 3-HAA	Cystathionine, Serotonin, Pantothenic acid	Niacin	Kyn, HX	Phenylalanine	5-hydroxytryptophan, Tyrosine, Taurine	Tryptophan
Calibration level 8 (ULOQ)	1	4	20	40	80	100	200	400	1000	2000	10000	20000	60000
Calibration level 7	0.5	2	10	20	40	50	100	200	500	1000	5000	10000	30000
Calibration level 6	0.25	1	5	10	20	25	50	100	250	500	2500	5000	15000
Calibration level 5	0.15	0.6	3	8	12	15	30	60	150	300	1500	3000	9000
Calibration level 4	0.1	0.4	2	6	8	10	20	40	100	200	1000	2000	6000
Calibration level 3	0.04	0.16	0.8	4	3.2	4	8	16	40	80	400	800	2400
Calibration level 2	0.02	0.08	0.4	1	1.6	2	4	8	20	40	200	400	1200
Calibration level 1	0.01	0.04	0.2	0.4	0.8	1	2	4	10	20	100	200	600

QC series preparation – analyte concentration (ng/mL)

	Cyanocobalamin Folate, Biotin Melatonin	PA, AA, XA, KA, Neopterin	Dopamine	Nicotinamide, Thiamine, Riboflavin, Pyridoxine, PLP	QA, 3-HK, 3-HAA	Cystathionine, Serotonin, Pantothenic acid, Hypoxanthine	Niacin Kyn	5-hydroxytryptophan, Tyrosine, Tryptophan, Phenylalanine
QC level 7 (QCH)	10	20	40	50	100	200	1000	10000
QC level 4 (QCM)	2	6	8	10	20	40	200	2000
QC level 2 (QCL)	0.4	1	1.6	2	4	8	40	400
QC level 1 (LLOQ)	0.2	0.4	0.8	1	2	4	20	200

Table S5. Preparation of the internal standard working solution used in the assay

Analytes	**Source stock (mg/mL)**	**Volume (µL)**	**Concentration (ng/mL)**
Picolinic acid-[$^{2}H_4$], 3-Hydroxykynurenine-[$^{13}C_6$], 3-Hydroxyanthranilic acid-[$^{13}C_6$], Anthranilic acid-[$^{13}C_6$], Xanthurenic acid-[$^{13}C_6$], Kynurenic acid-d5, Nicotinamide-[$^{13}C_6$]	0.1	25	100
Thiamine-[$^{13}C_3$], Pyridoxine-[$^{2}H_3$]	0.1	62.5	250
Kynurenine-[$^{13}C_6$],	0.1	125	500
Quinolinic acid-[$^{13}C_4.^{15}N$], Tryptophan-[$^{13}C_{11}.^{15}N_2$], Phenylalanine-[$^{2}H_5$], Tyrosine-[$^{2}H_7$], Serotonin-[$^{2}H_4$], Theobromine,	0.1	250	1000
Neopterin-[$^{13}C_5$], Cystathionine-[$^{2}H_4$], Dopamine-[$^{2}H_4$]	0.01	250	100
Cyanocobalamin-[$^{13}C_7$]	0.001	125	5
Melatonin-[$^{2}H_4$]	0.001	250	10
Biotin-[$^{2}H_2$], Folate-[$^{13}C_5.^{15}N$],	0.001	1250	50
Total volume	25000 µL		

Table S6. Instrumental parameters of the applied timed-PRM method including normalized collision energy (NCE%) for each metabolite of the semi-quantification analysis and its isotope labelled internal standard. The underlined product ion was used as the quantifying ion.

ID	Analytes	Abbreviation	Precursor ion (m/z) $[M+H]^+$	RT (min)	Analysis Range (min)	NCE (%)	Product ions (m/z)	Internal standard
1	Citrulline	Cit	176.10297	1.0	0.00-2.00	30	<u>159.07642</u> 113.07094	Thiamine-[$^{13}C_3$]
2	Acetylcholine	ACh	146.11756	1.1	0.00-2.00	50	<u>87.04406</u> 60.08078	Thiamine-[$^{13}C_3$]
3	Asymmetric dimethylarginine	ADMA	203.15025	1.2	0.00-2.00	30	<u>116.07078</u>	Thiamine-[$^{13}C_3$]
4	Methionine	Met	150.05833	1.9	1.00-3.00	50	<u>104.05285</u> 133.03178	Quinolinic acid-[$^{13}C_4.^{15}N$]
5	5-hydroxyindoleacetic acid	HIAA	192.06552	4.7	4.00-6.00	50	<u>146.06004</u>	Kynurenine-[$^{13}C_6$]
6	3-indoleacetic acid	IAA	176.07061	7.0	6.00-8.00	50	<u>130.06513</u>	Melatonin-[$^{2}H_4$]

Table S7. Metabolites excluded from method validation or failed to meet the validation criteria

Name	Reason for exclusion
Picolinic acid and Niacin	Isomers, could not be separated chromatographically
Anthranilic acid	Linearity issue and carry over
Melatonin, Neopterin and Pyridoxal phosphate	Lack of method sensitivity and unsatisfactory results for precision and accuracy
Folate, Cyanocobalamin and Dopamine	Linearity issue might be due to instability and possible degradation in presence of light, heat and acidic conditions
Taurine	Unsatisfactory results

Table S8. All metabolites that used an internal standard or a surrogate internal standard had a correlation coefficient R2 of > 0.990 except the ones marked with red.

Analytes	Calibration - repeat 1	Calibration - repeat 2	Calibration - repeat 3	Calibration - repeat 4	Calibration - repeat 5	Average
3-Hydroxyanthranilic acid	0.995	0.999	0.997	0.993	0.981	0.993
3-Hydroxykynurenine	0.989	0.995	0.993	0.995	0.961	0.987
5-hydroxytryptophan	0.991	0.996	0.992	0.991	0.938	0.982
Anthranilic acid	0.978	0.971	0.926	0.98	0.967	0.964
Biotin	0.998	0.995	0.982	0.99	0.982	0.989
Cystathionine	0.994	0.991	0.978	0.984	0.986	0.987
Hypoxanthine	0.986	0.956	0.969	0.978	0.961	0.97
Kynurenic acid	0.983	0.976	0.971	0.976	0.964	0.974
Kynurenine	0.996	0.998	0.995	0.998	0.985	0.994
Melatonin	0.994	0.99	0.986	0.996	0.991	0.992
Neopterin	0.972	0.95	0.999	0.978	0.823	0.944
Nicotinamide	0.983	0.992	0.9	0.99	0.986	0.97
Pantothenic acid	0.999	0.99	0.994	0.993	0.999	0.995
Phenylalanine	0.984	0.999	0.995	0.998	0.997	0.995
Pyridoxal 5'-phosphate	0.985	0.978	0.94	0.971	0.941	0.963
Pyridoxine	0.998	0.999	0.998	0.996	0.997	0.998
Quinolinic acid	0.997	0.995	0.983	0.993	0.983	0.99
Riboflavin	0.991	0.993	0.996	0.986	0.993	0.992
Serotonin	0.996	0.997	0.999	0.997	0.994	0.997
Taurine	0.994	0.991	0.999	0.993	0.984	0.992
Tryptophan	0.998	0.999	0.998	0.999	0.999	0.999
Tyrosine	0.997	0.999	0.999	0.997	0.972	0.993
Xanthurenic acid	0.978	0.96	0.961	0.983	0.955	0.967

Table S9. Precision and accuracy of 22 metabolites in human plasma reported at four QC's levels that prepared in methanol: low limit of quantification (LLOQ), low (L), medium (M), high (H)

			Intra				Inter			
ID	Level	mean.spec. amount (ng/mL)	mean.calc. amount (ng/mL)	n	CV	mean.bias	mean.calc. amount (ng/mL)	n	CV	mean.bias
Anthranilic.acid	LLOQ	0.4	0.7	3	5.4	77.4	0.5	NA	NA	NA
	L	1	0.9	3	18.3	-5.6	0.7	4	10.6	-3.6
	M	6	4.3	3	8.8	-28.2	4.5	2	NA	NA
	H	20	22.6	3	9.9	13.2	24.6	5	2.9	9.4
Biotin	LLOQ	0.2	NA	0	NA	NA	0.4	2	NA	NA
	L	0.4	0.6	2	13.9	38.4	0.5	4	7.9	-0.3
	M	2	2	3	3.2	1.8	2	12	6.8	-6.3
	H	10	9.6	3	13.6	-3.7	10.6	11	4.6	2.9
Cystathionine	LLOQ	4	3.7	1	NA	-6.8	4.5	8	10.4	4.6
	L	8	6.4	3	4.6	-20.3	6.8	7	3.8	-11.3
	M	40	37.8	3	5.6	-5.5	39.6	13	5.7	0.2
	H	200	232.6	3	0.9	16.3	229.8	6	3.3	11.2
Hydroxyanthranilic.acid	LLOQ	2	1.9	3	6.3	-4.9	2	12	4.8	-1.7
	L	4	3.6	3	4.6	-9.3	3.5	11	5.8	-7.4
	M	20	19.2	3	2.8	-3.8	18.6	13	4.3	-5.6
	H	100	104.1	3	5	4.1	99.1	12	6.4	-3.7
Hydroxykynurenine	LLOQ	2	1.1	3	22.9	-43.8	1.9	9	8.6	4.7
	L	4	3.6	3	19.2	-10.7	3.8	9	7.1	2.1
	M	20	19.5	3	4.8	-2.3	19.2	11	5.5	-5.4
	H	100	110.4	3	9	10.4	109.1	11	3.0	6.2
Hydroxytryptophan	LLOQ	200	201.7	3	5.8	0.8	205	14	4.6	2.5
	L	400	365.2	3	3	-8.7	359.8	12	2.9	-8.6
	M	2000	1849.4	3	6.4	-7.5	1717.3	9	5.8	-10.3
	H	10000	9084.7	3	5.7	-9.2	9021.6	12	8.1	-8.
Hypoxanthine	LLOQ	4	NA	0	NA	-193.7	14.2	1	NA	-193.4
	L	8	1.3	3	57.4	-84	1.3	5	66.4	-119.8
	M	40	40.2	3	4.1	0.4	37	9	6.1	-0.6
	H	200	216	3	3.6	8	196.9	12	8.6	0.0
Kynurenic.acid	LLOQ	0.4	0.4	3	9.1	1.3	0.6	14	51.6	38.4
	L	1	0.8	3	5.7	-15.3	1	12	7.1	-0.4
	M	6	4.7	3	2.7	-21.5	4.8	14	6.7	-20.3
	H	20	23.3	3	6.9	16.4	24.1	14	4.8	20.8
Kynurenine	LLOQ	20	19.1	3	8.2	-4.6	16	14	17	-19.8
	L	40	38.1	3	3.4	-4.8	35.2	14	9.6	-12.1
	M	200	199	3	3.1	-0.5	187.1	14	6.6	-6.5
	H	1000	1001.5	3	8.8	0.2	958.1	14	4.8	-4.2
Melatonin	LLOQ	0.2	0.3	3	9	32.5	0.3	14	5.4	29.8
	L	0.4	0.5	3	12.7	31.4	0.5	14	7.9	28.7
Neopterin	LLOQ	0.4	NA	0	NA	NA	0.2	3	43.2	-46
	L	1	NA	0	NA	NA	0.7	5	21.6	-29.4
	M	6	4.7	2	1.9	-22.1	4.9	12	16	-19.2

	H	20	22.5	3	8.1	12.4	22.8	14	16.7	14
Nicotinamide	LLOQ	1	0.9	3	117.3	-14.6	0.1	14	1651.9	-86.2
	L	2	0.7	3	20	-64.9	0.3	14	832.4	-83
	M	10	9.6	3	3	-4	8.7	10	6.7	-1.4
	H	50	52.9	3	2.9	5.8	54.5	14	13.2	8.9
Pantothenic.acid	LLOQ	4	3.1	3	5.7	-21.7	3.5	9	8.8	0.3
	L	8	6.9	3	2.7	-14.2	7	10	8.2	-5.5
	M	40	38.2	3	5.8	-4.4	36.9	11	6.6	-2.8
	H	200	215.6	3	6.3	7.8	209.9	10	2.9	1.4
Phenylalanine	LLOQ	200	199.8	3	7	-0.1	286	13	0.001	0.8
	L	400	402.3	3	2.8	0.6	403	14	5.4	0.8
	M	2000	2067.9	3	1.7	3.4	1948.7	14	5.2	-2.6
	H	10000	9587.4	3	3.6	-4.1	8873.4	14	7.3	-11.3
pyridoxal.phosphate	LLOQ	1	NA	0	NA	NA	2.5	4	121.5	148.8
	L	2	NA	0	NA	NA	5.4	6	61.2	170.1
	M	10	NA	0	NA	NA	16.3	10	27.5	62.9
	H	50	83.3	3	16.6	66.6	76.5	14	25.5	53
Pyridoxine	LLOQ	1	1.1	3	6.9	7	1.1	14	5.3	7
	L	2	2.1	3	5.7	4	2	14	5.4	1.8
	M	10	10.4	3	5.8	4.2	10	14	6	-0.2
	H	50	50.7	3	3.8	1.4	51	14	2.5	1.9
Quinolinic.acid	LLOQ	2	NA	0	NA	NA	2.1	10	39	5.9
	L	4	5.2	3	11	28.9	3.9	6	10.6	-2.7
	M	20	18.2	3	11.6	-9	17.2	9	5.8	-7.4
	H	100	101.3	3	5.8	1.3	99.2	14	5.8	-0.8
Riboflavin	LLOQ	1	0.6	2	14.4	-41.9	1.1	13	30.7	8.6
	L	2	2.2	3	26	8.7	2.2	14	14.9	9.5
	M	10	11.4	3	4.7	14.4	10.4	14	10.3	4.4
	H	50	58.9	3	5.9	17.7	57.4	14	7.9	14.7
Serotonin	LLOQ	4	5	3	4.3	26.2	5	12	5.1	25.7
	L	8	9.5	3	6.2	18.9	8.9	14	13.6	11.5
	M	40	50.7	3	4.7	26.8	48.1	14	6.9	20.3
	H	200	241.1	3	5.1	20.5	245.7	14	3.2	22.9
Tryptophan	LLOQ	200	202.4	3	7	1.2	215.9	14	18	8
	L	400	392.8	3	2.6	-1.8	393.6	14	4.5	-1.6
	M	2000	2058.6	3	6.8	2.9	1968.4	14	7.7	-1.6
	H	10000	10112.5	3	5.6	1.1	9852.7	14	4.5	-1.5
Tyrosine	LLOQ	200	200.5	3	1.7	0.2	339.6	12	5.7	8.1
	L	400	398.9	3	2.1	-0.3	401.2	14	6.4	0.3
	M	2000	2096.3	3	7.2	4.8	1882.6	10	5.3	-3.2
	H	10000	10246.1	3	7.5	2.5	10018.4	14	6.3	0.2
Xanthurenic.acid	LLOQ	0.4	1	3	9.5	159.8	0.8	14	22.6	102.6
	L	1	1.2	3	4	23.8	1.1	14	13.6	10.4
	M	6	4.9	3	3	-17.8	5.2	14	13.3	-13.9
	H	20	24	3	10	19.8	27.4	14	10.2	37

Note: The compounds highlighted with green passed the intraday and interday precision and accuracy criteria for 67% of QCs (50% at each level) were within 15% (LLOQ 20%). The ones highlighted with yellow passed the intraday and interday precision and accuracy at some QC's levels while the ones highlighted with red did not pass the validation parameters at any QC's level.

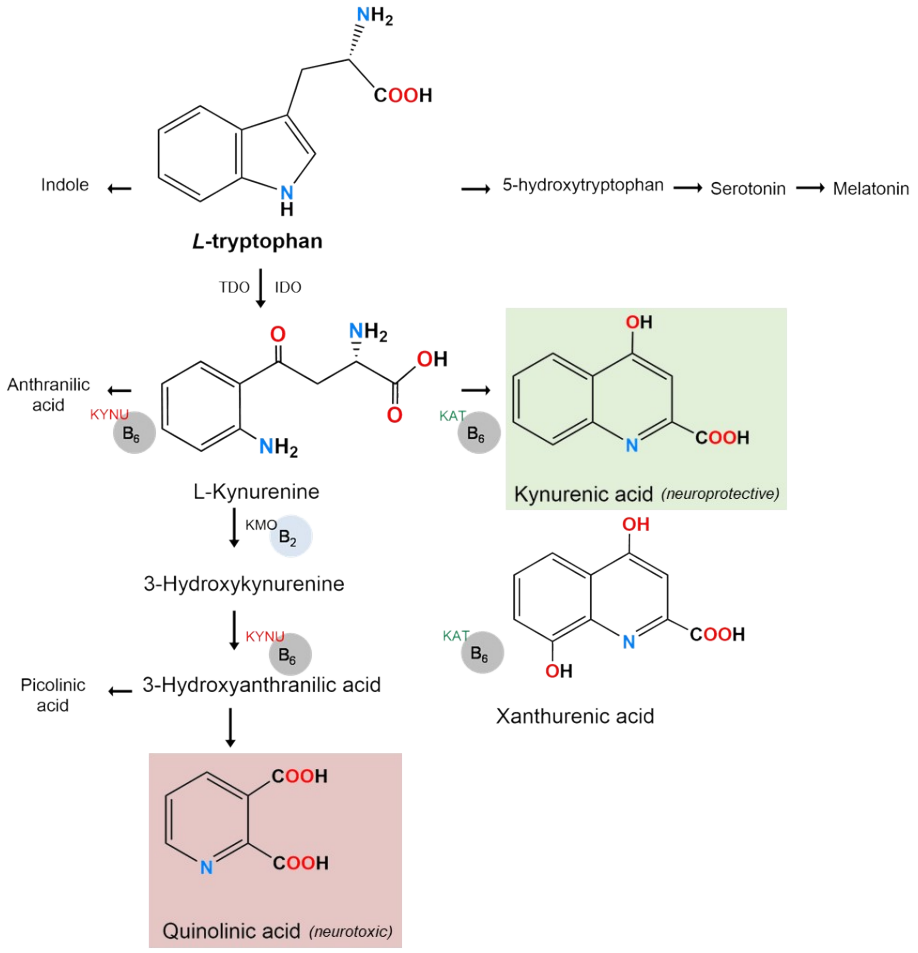
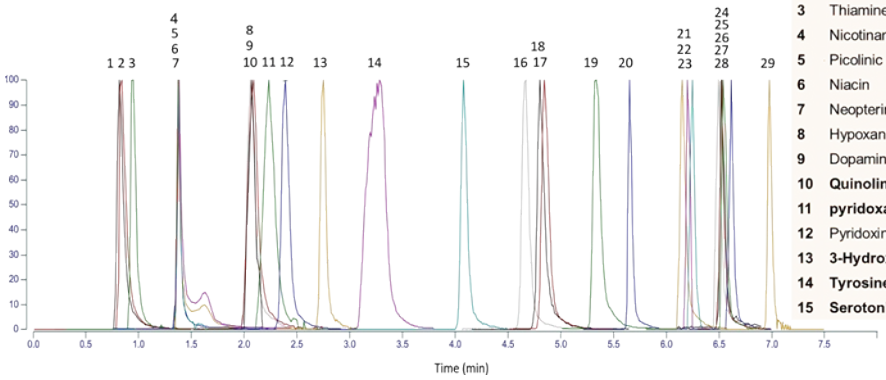


Figure S1. The major metabolites investigated in this study include tryptophan (Trp) degradation pathway, highlighting its key intermediates and major products. Trp is primarily metabolised via the kynurenine pathway (KP) by the enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). B vitamins, in particular vitamin B2 and B6, are crucial for the pathway: B2 is a cofactor for kynurenine 3-monooxygenase (KMO) and B6 is a cofactor for kynurenine aminotransferase (KAT) and kynureninase (KYNU). This metabolic pathway produces either kynurenic acid (KA), a neuroprotective agent, or quinolinic acid (QA), a neurotoxin.

A.



ID	Analyte	ID	Analyte
1	Cystathionine	16	**Kynurenine**
2	Taurine	17	**3-Hydroxyanthranilic acid**
3	Thiamine	18	5-hydroxytryptophan
4	Nicotinamide	19	**Phenylalanine**
5	Picolinic acid	20	**Pantothenic acid**
6	Niacin	21	**Tryptophan**
7	Neopterin	22	Cyanocobalamin
8	Hypoxanthine	23	Folate
9	Dopamine	24	**Xanthurenic acid**
10	**Quinolinic acid**	25	**Anthranilic acid**
11	**pyridoxal 5'-phosphate**	26	**Kynurenic acid**
12	Pyridoxine	27	**Biotin**
13	**3-Hydroxykynurenine**	28	**Riboflavin**
14	**Tyrosine**	29	Melatonin
15	**Serotonin**		

B.

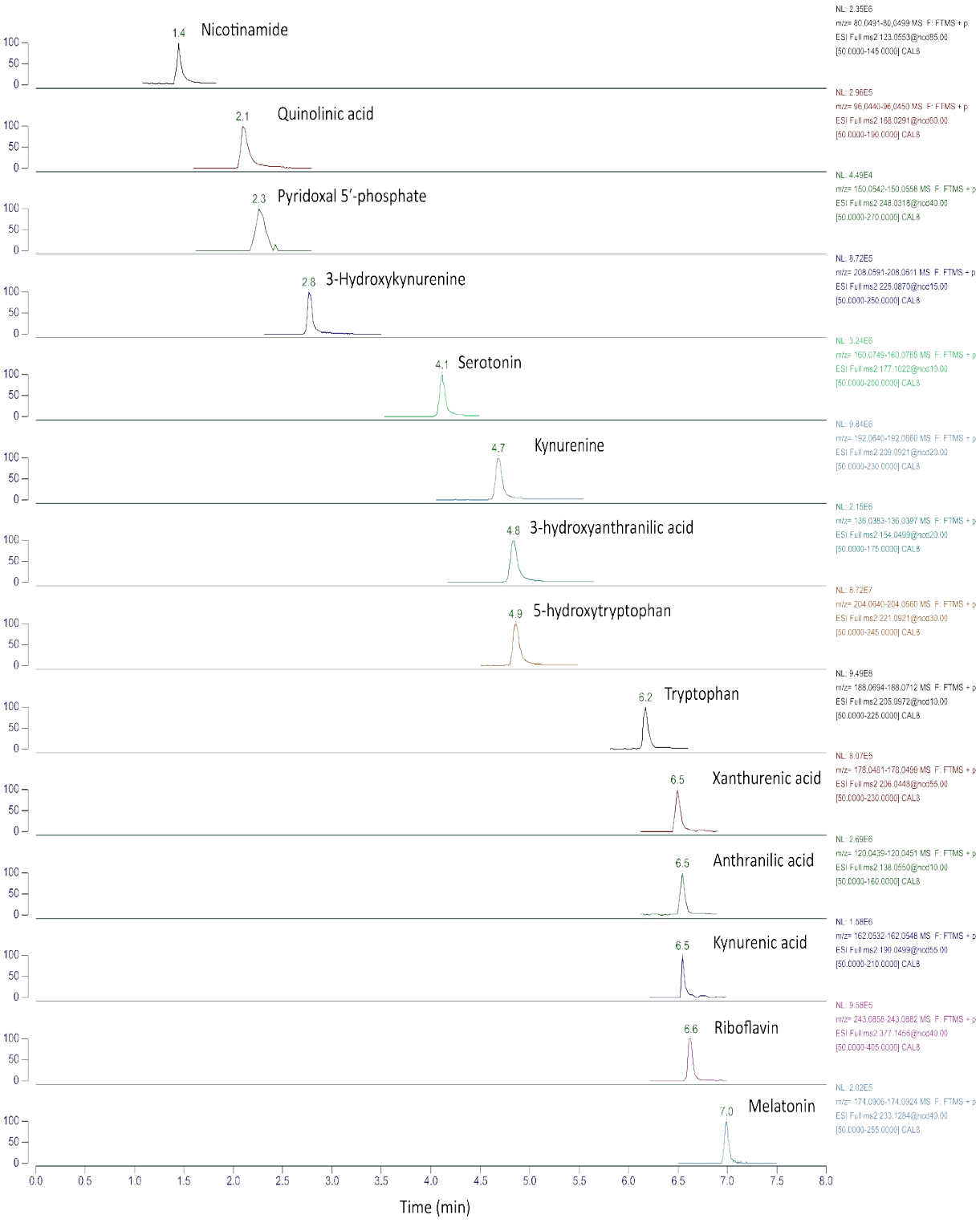


Figure S2. A shows an extracted ion chromatogram for 29 analytes using a reverse-phase chromatography method, ranging from cystathionine (the most polar) to melatonin (the most hydrophobic) in terms of retention time. The analytes numbered as (8-10, 17-18, 21-23, 24-28) could not be chromatographically separated, but instead were identified by mass spectrometry transitions. However, picolinic acid and niacin are isomers (peak number 5 and 6) could not be separated using this chromatography method, nor differentiated using their mass

transitions. In figure S2. B shows the chromatogram of tryptophan, 11 of its metabolites, as well as the cofactors pyridoxal 5'-phosphate (B6) and riboflavin (B2), which are essential for the enzymes involved in the tryptophan-kynurenine pathway.

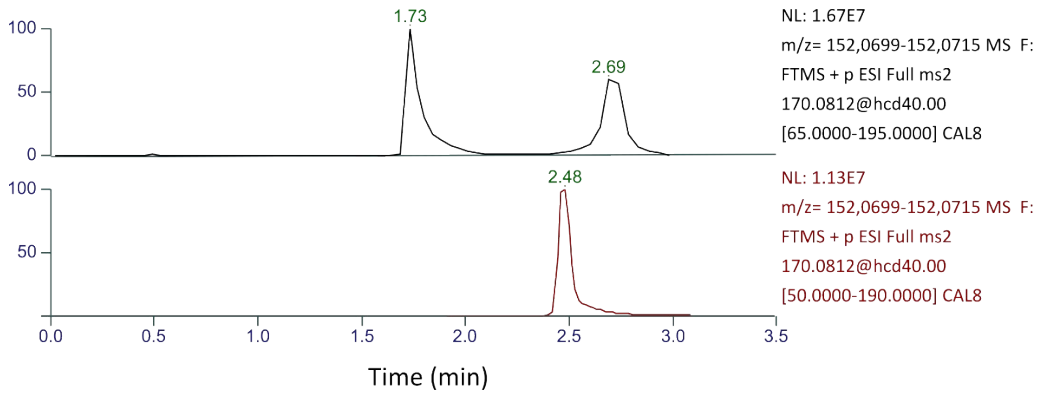


Figure S3. In the chromatogram above, pyridoxine shows two distinct peaks when 0.1% formic acid is used in both mobile phase A and B. However, this issue was resolved by increasing the formic acid concentration to 0.6% in both mobile phase A and B.

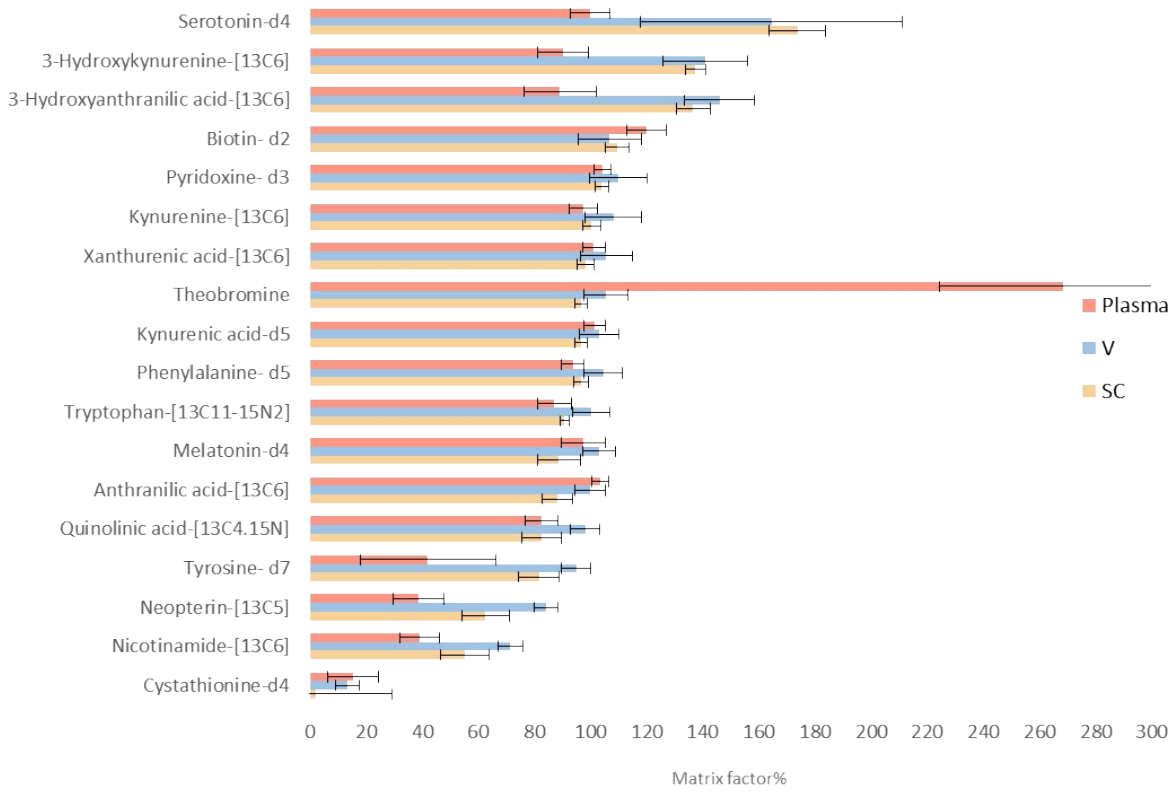


Figure S4. A bar plot displays the mean matrix factor, MF (%), for human plasma (6 individuals X 4 replicates) and murine visceral white adipose tissue (V), n=6 and murine subcutaneous white adipose tissue (SC), n=6, as evaluated by spiking isotope labeled internal standards (SILIS) to methanol (n=6) andand spiked to the samples. The MF (%) was calculated by dividing the peak area of the SILIS in each sample by the peak area in the

methanol. The Y-axis displays the SILIS used, and the X-axis shows the MF (%). The error bars represent the coefficient of variation CV (%) of the MF (%). Further details of SILIS can be found in Supplement Table S5.

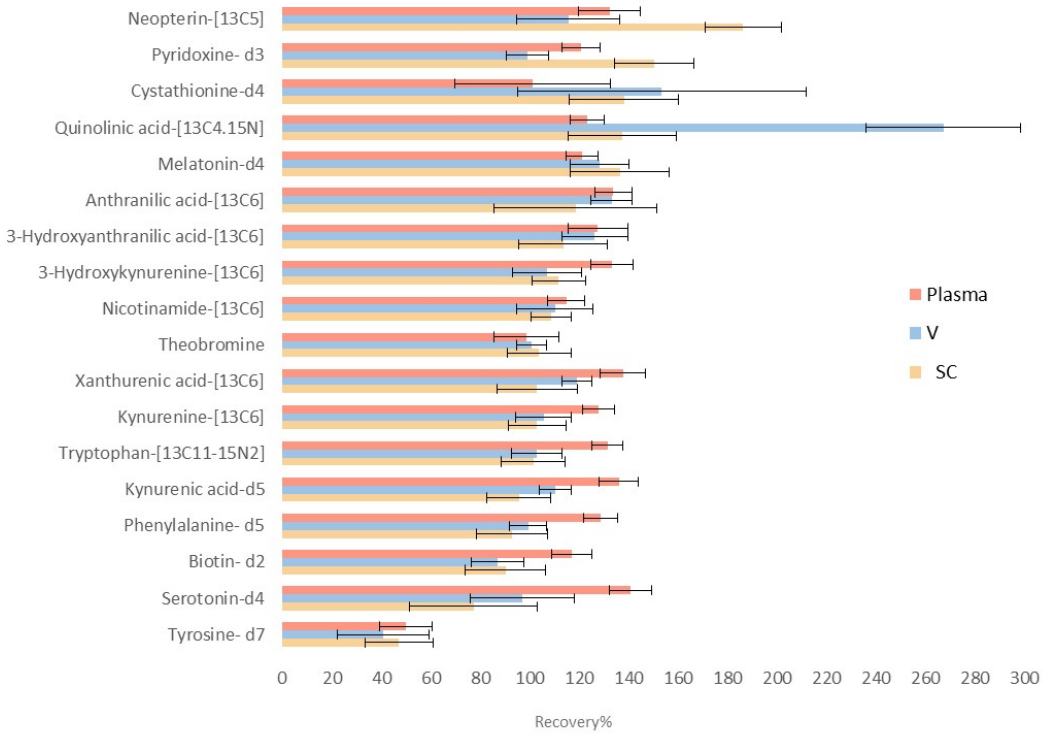


Figure S5. A barplot shows the mean recoveries of sample preparation methods using protein precipitation, evaluated by spiking stable isotope labeled internal standards (SILIS) to human plasma (6 individuals x 4 replicates) and murine visceral white adipose tissue (V), n=6 and murine subcutaneous white adipose tissue (SC), n=6, respectively. The x-axis of the chart displays the recovery%, which was calculated by dividing the peak area of SILIS spiked after sample preparation by the peak area of SILIS spiked before sample preparation. The y-axis shows the internal standard used, while the error bars represent the CV% of the recovery%. The concentration of SILIS is as given in Supporting Table S5.

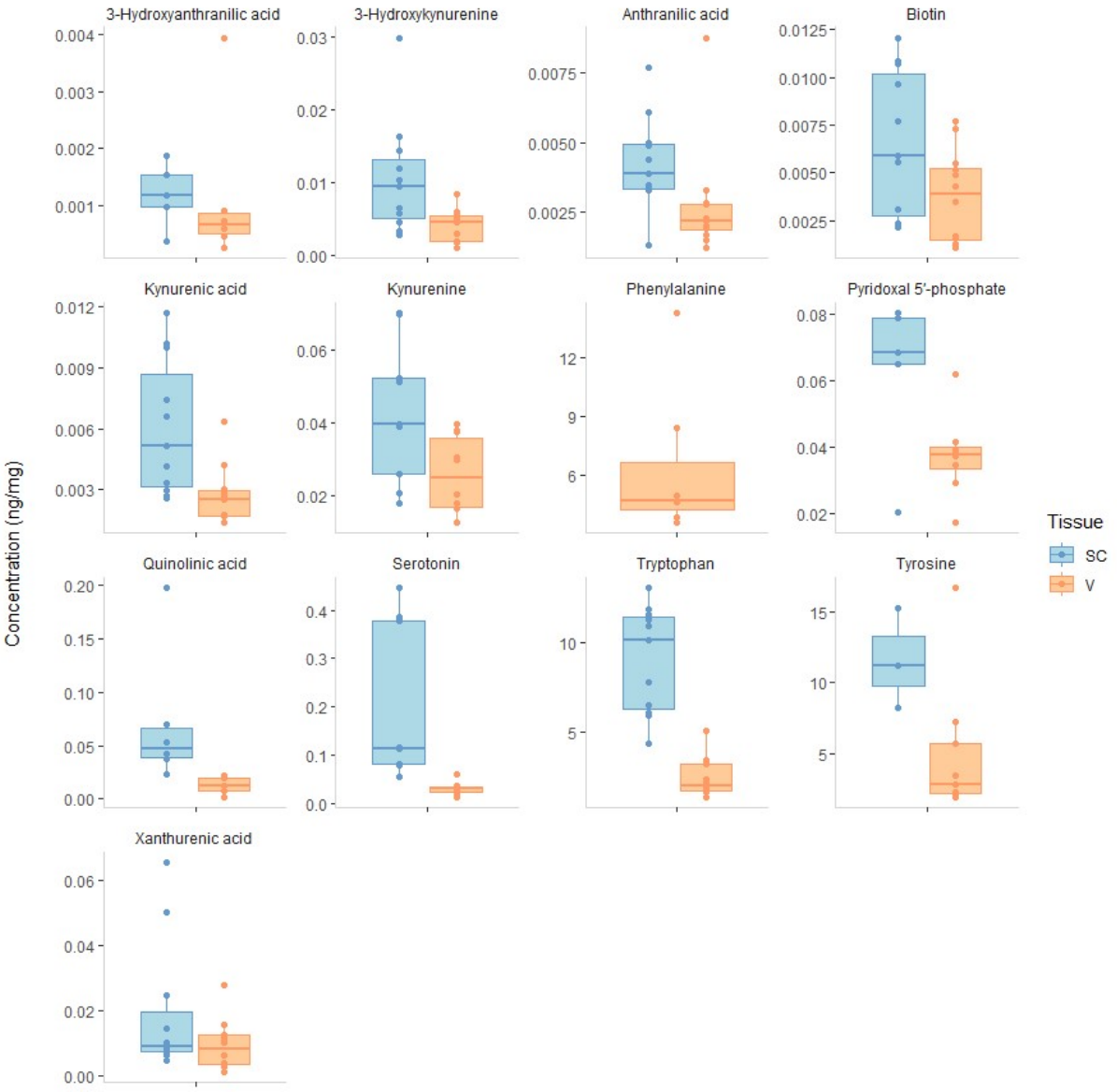


Figure S6. The median concentrations of the aromatic amino acids; tryptophan and its metabolites, tyrosine and phenylalanine. In addition to B vitamins (biotin and pyridoxal 5'-phosphate) were determined in ng/mg of frozen tissue in both subcutaneous (SC) and visceral (V) white adipose tissue, with 12 samples for each tissue. Comparing concentrations of compounds in SC and v tissue, the results showed that all compounds were higher in SC. The level of phenylalanine exceeded ULOQ and was thus excluded from the boxplot in SC.

Figure S7 To compare our findings, we calculated the median and mean values of tryptophan metabolites in human plasma from healthy control (n=24). Our values, referred to as Study P median and Study P mean, were compared to those found in previous studies (denoted as P1–P8). P, stands for plasma and S stands for serum. The plasma levels in studies P1 and P3 were given as median values via liquid chromatography coupled to triple quadrupole mass spectrometry (LC-TQMS) analysis, while P2 employed LC-QExactive. Mean values from studies P4 through P8 were obtained via LC-fluorescence for P6 and P8, and LC-TQMS for P4, P5, and P7. Lastly, the serum values for S1 through S5 were given as means using LC-TQMS. Except for S4, which was analysed using linear ion trap quadrupole (LITQ).

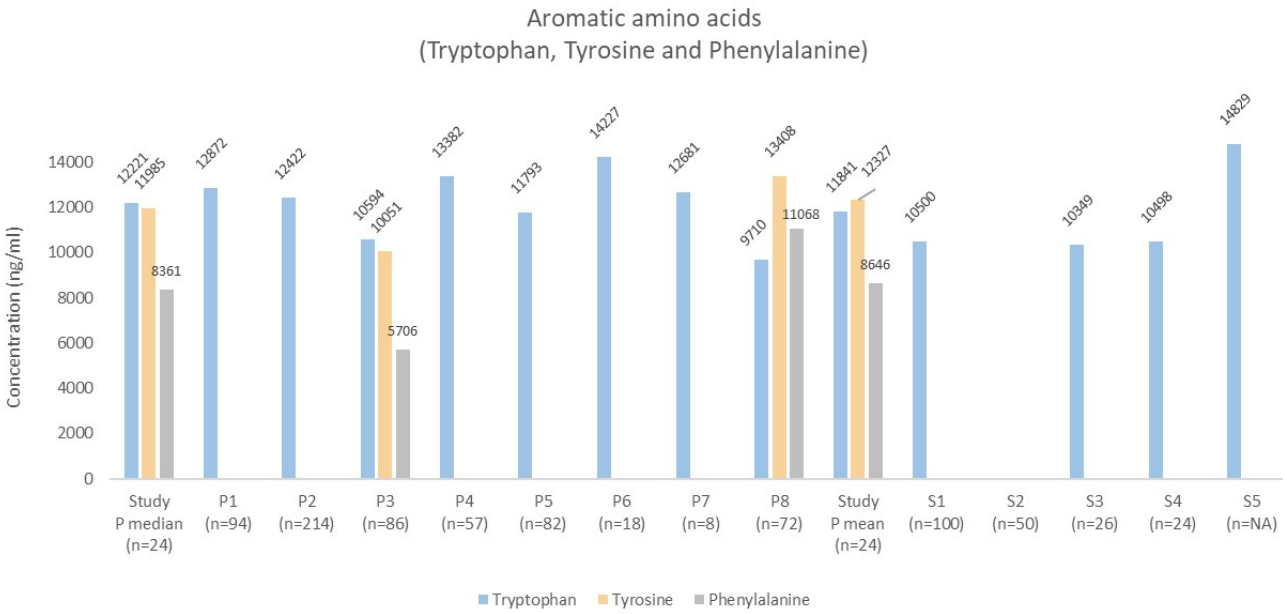


Figure S8 To compare our findings, we calculated the median and mean values of three aromatic amino acids: tryptophan, tyrosine, and phenylalanine present in human plasma from healthy control (n=24). Our values, referred to as Study P median and Study P mean, were compared to those found in previous studies (denoted as P1–P8). P, stands for plasma and S stands for serum. The plasma levels in studies P1 and P3 were given as median values via liquid chromatography coupled to triple quadrupole mass spectrometry (LC-TQMS) analysis, while P2 employed LC-QExactive. Mean values from studies P4 through P8 were obtained via LC-fluorescence for P6 and P8, and LC-TQMS for P4, P5, and P7. Lastly, the serum values for S1 through S5 were given as means using LC-TQMS. Except for S4, which was analysed using linear ion trap quadrupole (LITQ).

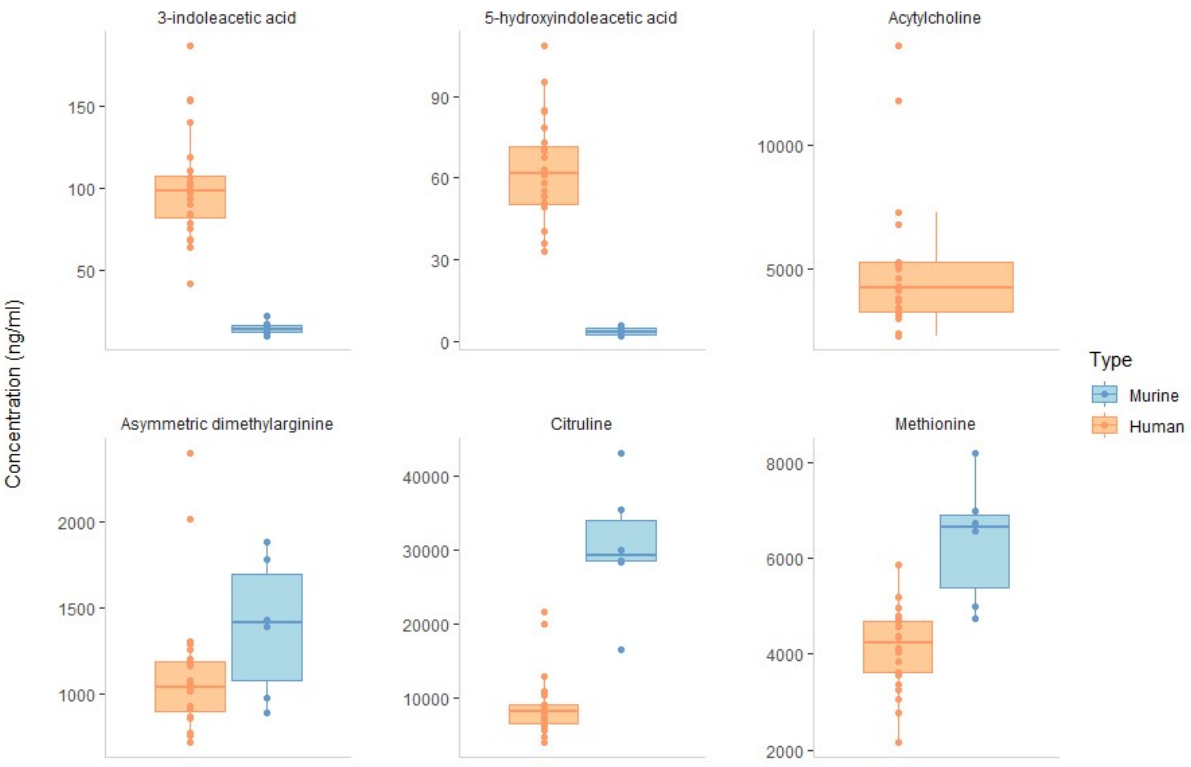


Figure S9. The median concentrations of 5-hydroxyindoleacetic acid, 3-indoleacetic acid, citruline, acytylcholine, asymmetric dimethylarginine, and methionine were determined in ng/mL in human (n=24) and murine (n=6) plasma from healthy controls using semi-quantitative analysis. Acetylcholine was not detected in murine plasma.