High-throughput fractionation coupled to mass spectrometry for improved quantitation in metabolomics.

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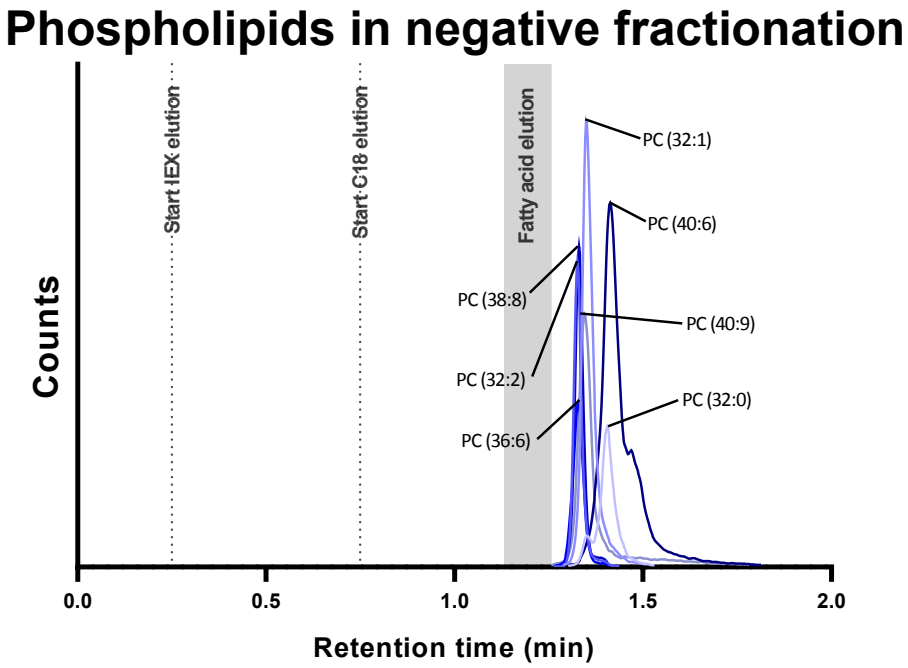

Figure S1. Extracted ion chromatogram of phospholipids in a pooled plasma sample measured by the negative fractionation method and positive MS polarity. The elution window of the fatty acids are indicated by the grey area.

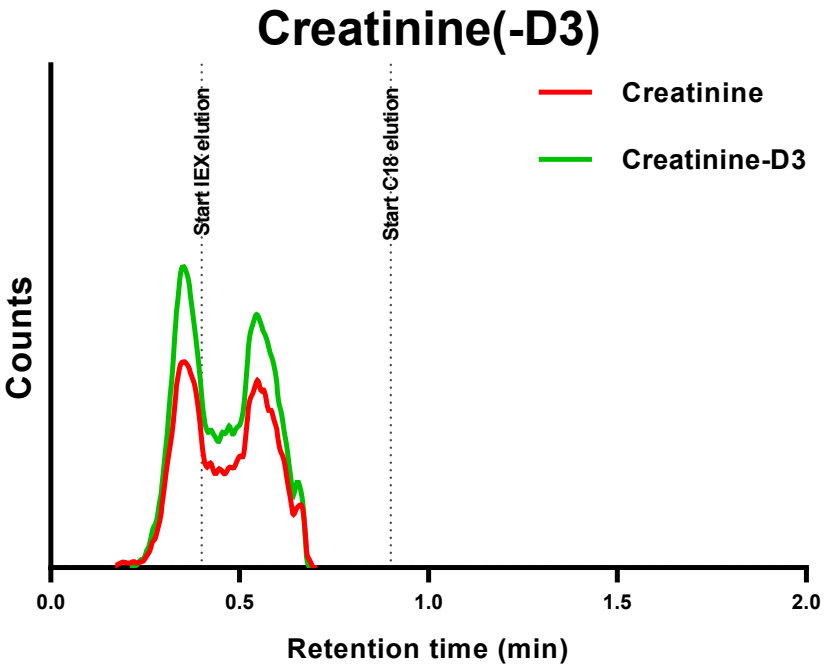

Figure S2. Extracted ion chromatogram of creatinine and creatinine-D3 measured by the fractionation method in a pooled plasma sample in positive mode. The peak shape and retention time are overlapping.

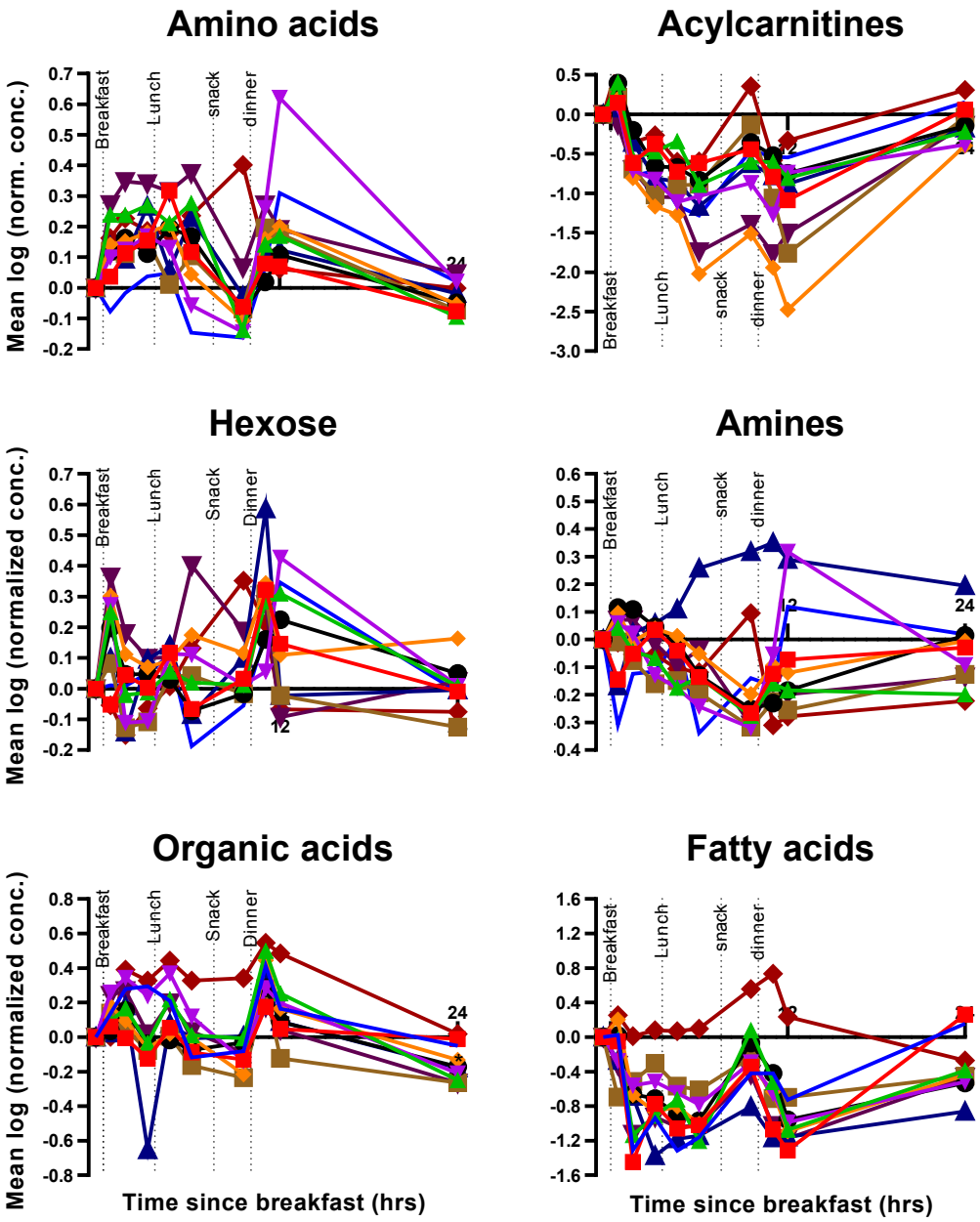

Figure S3. The individual mean natural logarithm of metabolite concentrations over time. Normalization was performed on the first time point. Within each compound class, metabolites were averaged per time point. The 10 volunteers are depicted in different colours. The time frame comprises four standardized feeding times and meals and one night rest. The time is presented with respect to the breakfast time.

Table S1. Calibration standards

Name	CheBi ID	Standard	Supplier	Solvent	C7 (µM)
1-methylhistidine	70958	1-methylhistidine	HMDB	Water	80
3-methylhistidine	70959	3-methylhistidine	HMDB	Water	40
Alanine	16977	L-alanine	Sigma-Aldrich	Water	1600
Arginine	16467	L-arginine hydrochloride	Sigma-Aldrich	Water	400
Betaine	17750	Betaine hydrochloride	Sigma-Aldrich	Water	300
Carnitine	16347	L-Carnitine hydrochloride	Sigma-Aldrich	Water	200
Citrulline	16349	Citrulline	HMDB	Water	100
Cystine	16283	L-cystine	Fluka	1M Hydrochloric acid	100
Glutamic acid	16015	L-glutamic acid	Sigma-Aldrich	Water	400
Glutamine	18050	L-glutamine	Fluka	Water	2000
Histidine	15971	L-histidine monohydrochloride monohydrate	Fluka	Water	600
Isoleucine	17191	L-Isoleucine	Fluka	Water	200
Leucine	15603	L-Leucine	Fluka	Water	200
Lysine	18019	L-lysine monohydrochloride	Fluka	Water	800
Methionine	16643	L-methionine	Fluka	Water	200
Ornithine	15729	L-Ornithine hydrochloride	Sigma-Aldrich	Water	400
Phenylalanine	17295	L-phenylalanine	Fluka	Water	300
Proline	17203	L-proline	Fluka	Water	800
Serine	17115	L-serine	Fluka	Water	600
Taurine	15891	Taurine	Fluka	Water	400
Threonine	16857	L-threonine	Fluka	Water	600
Tryptophan	16828	L-tryptophan	Sigma-Aldrich	0.5M hydrochloric acid	200
Tyrosine	17895	L-tyrosine	Fluka	1M hydrochloric acid	200
Valine	16414	L-valine	Fkuka	Water	800
TMAO	15724	Trimethylamine-N-oxide dihydrate	Sigma-Aldrich	Water	100
Choline	15354	Choline chloride	Sigma-Aldrich	Water	100
Creatinine	16737	Creatinine	Sigma-Aldrich	Water	400
Urea	16199	Urea	Sigma-Aldrich	Water	10000
Glucose	17634	D-(+)-glucose	Sigma-Aldrich	Water	10000
Guanine	16235	Guanine	HMDB	0.1M sodium hydroxide	8
Hypoxanthine	17368	Hypoxanthine	Sigma-Aldrich	1M sodium hydroxide	200
Uric acid	17775	Uric acid	Sigma-Aldrich	1M sodium hydroxide	2000
Octanoylcarnitine	18102	Octanoyl -L-carnitine HCl	VU medical center	Methanol	1
Decanoylcarnitine	68830	Decanoyl-L-carnitine HCl	VU medical center	Methanol	1
Palmitoylcarnitine	17490	Hexadecanoyl-L-carnitine HCL	VU medical center	Methanol	0,4
Stearoylcarnitine	84644	Octadecanoyl-L-carnitie HCl	VU medical center	Methanol	0,2
Aspartic acid	22660	DL-aspartic acid	Sigma-Aldrich	1M sodium hydroxide	160
Citric acid	30769	Citric acid	Sigma-Aldrich	Water	800
Lactic acid	422	lactic acid lithium salt	Acros organics	Water	3000
Isocitric acid	30887	Isocitric acid	HMDB	Water	40
Pyruvic acid	32816	sodium pyruvate	Sigma-Aldrich	Water	400
Aconitic acid	32805	Cis-aconitic acid	Sigma-Aldrich	Water	225
Alpha-ketoglutaric acid	30915	Alpha-ketoglutaric acid disodium salt hydrate	Fluka	Water	80
2-Hydroxybutyric acid	1148	2-Hydroxybutyric acid sodium salt (97%)	Sigma-Aldrich	Water	400
3-Hydroxybutyric acid	20067	3-hydroxybutyric acid	Sigma-Aldrich	Water	800
Malic acid	6650	DL-Malic acid	Sigma-Aldrich	Water	120
Indoxyl sulfate	43355	indoxyl slufate potassium salt	Sigma-Aldrich	Methanol	20
Alpha-linolenic acid	27432	Alpha-linolenic acid	Cayman chemical	Ethanol	10
Docosahexaenoic acid	28125	Docosahexaenoic acid	Cayman chemical	Ethanol	4
Docosapentaenoic acid	65136	Docosapentaenoic acid	Cayman chemical	Ethanol	2
Linoleic acid	17351	Linoleic acid	Sigma-Aldrich	Methanol	200
Arachidonic acid	15843	Arachidonic acid	Cayman chemical	Ethanol	30
Palmitic acid	15756	Palmitic acid	HMDB	Methanol	200
Oleic acid	16196	Oleic acid	Sigma-Aldrich	Ethanol	200
Stearic acid	28842	Stearic acid	Sigma-Aldrich	Isopropanol	100

Table S2. Internal standards. The C6 values are the concentrations (µM) which were present in the internal standard solution. During sample preparation, the same volume of internal standard solution and plasma is added.

Name	Standard	Supplier	Solvent	C6 (µM)
Phenyl alanine	DL-Phenyl-d5-alanine	CDN isotopes	Water	320
Trimethylamine-N-oxide	Trimethylamine N-oxide-D9	Cambridge Isotope	Water	160
Alanine	DL-ALANINE-2,2,3,3-d3, 99.8% D	CDN isotopes	Water	1600
Choline	Choline-D4 Chloride	CDN Isotopes	Water	60
Carnitine	L-carnitine-d3 HCl (methyl-d3)	CDN Isotopes	Water	180
Creatinine	Creatinine-D3	Cambridge Isotope	Water	360
Valine	L-VALINE (D8)	Cambridge isotopes	Water	800
Leucine	DL-Leucine-D3	CDN Isotopes	1M hydrochloric acid	240
Ornithine	L-Ornithine-D6 hydrochloride	CDN Isotopes	Water	280
Hypoxanthine	Hypoxanthine-D2	CDN Isotopes	1M sodium hydroxide	140
Glutamine	L-GLUTAMINE (2,3,3,4,4-D5)	Cambridge isotopes	Water	2000
Lysine	L-LYSINE 2 HCL (4,4,5,5-D4)	Cambridge isotopes	Water	720
Glutamic acid	L-GLUTAMIC ACID (1,2-13C2)	Cambridge isotopes	1M sodium hydroxide	400
Arginine	L-Arginine-15N2 hydrochloride	Cortecnet	Water	400
Tryptophan	L-TRYPTOPHAN (U-13C11, U15N2)	Cambridge isotopes	Water	200
Glucose	Glucose-13C6	Cortecnet	90% methanol	20000
Octanoylcarnitine	Octanoyl carnitine-D3 HCl	CDN Isotopes	Methanol	0,8
Pyruvic acid	Pyruvic acid-13C3	Omicron Biochemicals	Water	140
Lactic acid	Lactic acid-13C3	Biomedical Isotopes	Water	4000
Malic acid	Malic acid-13C4	Cambridge Isotope	Water	24
Citric acid	Citric acid-D4	Cambridge Isotope	Water	480
Palmitic acid	Palmitic acid-D2	Cambridge Isotope	Ethanol	480
Stearic acid	Stearic acid-D3	Cambridge Isotope	Ethanol	200

Table S3. Solid-phase extraction column information

Brand	Type	Phase	Functional group	Amount of sorbent (mg)	Particle size (µm)	pH stability	Loading buffer	Elution buffer
Hysphere	SCX	PS-DVB	Sulfonate	13	10	1-14	0.1% formic acid	100 mM ammonium acetate pH 10
Hysphere	SAX	PS-DVB	Quaternary amine	13	10	1-14	5 mM ammonium acetate	1% formic acid
Oasis	WCX	Oasis HLB	Carboxylate	10.4	30	0-14	5 mM ammonium acetate	1% formic acid
Oasis	WAX	Oasis HLB	tertiary/secondary amine	10.4	30	0-14	5 mM ammonium acetate	100 mM ammonium formate pH 10
Sepax	SCX	PS-DVB	Sulfonate	Not disclosed	3	2-12	0.1% formic acid	100 mM ammonium acetate pH 10
Sepax	SAX	PS-DVB	Quaternary amine	Not disclosed	1.7	2-12	5 mM ammonium acetate	1% formic acid
Sepax	WCX	PS-DVB	Carboxylate	Not disclosed	1.7	2-12	5 mM ammonium acetate	1% formic acid
Sepax	WAX	PS-DVB	Tertiary amine	Not disclosed	5	2-12	5 mM ammonium acetate	100 mM ammonium formate pH 10
Zirchrom	SCX	Zirconia dioxide	Ethylenediamine-N,N'-tetramethylphosphonic acid	150	3	1-10	0.1% formic acid	100 mM ammonium acetate pH 10
Zirchrom	SAX	Zirconia dioxide	Polyethyleneimine	150	3	1-12	5 mM ammonium acetate	1% formic acid
Zirchrom	WCX	Zirconia dioxide	Phosphate	150	3	1-10	5 mM ammonium acetate	1% formic acid
Zirchrom	WAX	Zirconia dioxide	Polyethyleneimine	150	3	3-9	5 mM ammonium acetate	100 mM ammonium formate pH 9

Table S4. Fractionation LC and valve parameters for positive mode

Time (min)	Flow rate (ml/min)	%A	%B	IEX mobile phase	WAX and SCX columns
0.00	0.8	100	0	Out	In
0.40	0.8	100	0	In	In
1.00	0.8	100	0	Out	In
1.01	0.8	0	100	Out	Out
2.10	0.8	0	100	Out	Out
2.11	0.8	100	0	Out	Out
2.35	0.8	100	0	Out	In
3.00	0.8	100	0	Out	In

Table S5. Fractionation LC and valve parameters for negative mode

Time (min)	Flow rate (mL/min)	%A	%B	IEX mobile phase	WAX column
0.00	0.5	100	0	Out	In
0.25	0.5	100	0	In	In
0.30	0.5	100	0	In	In
0.31	0.8	100	0	In	In
0.75	0.8	100	0	Out	Out
0.76	0.8	20	80	Out	Out
0.95	0.8	15	85	Out	Out
0.96	0.8	0	100	Out	Out
2.00	0.8	0	100	Out	Out
2.01	0.8	100	0	Out	Out
2.25	0.8	100	0	Out	In
3.00	0.8	100	0	Out	In

Table S6. Mass spectrometry parameters

Platform	Polarity	Gas 1 (psi)	Gas 2 (psi)	Curtain gas (psi)	Temp (°C)	Spray voltage (V)	Declustering Potential (V)	Collision energy (eV)	Mass range (*m/z*)
Fractionation, RP and HILIC	Positive	40	60	40	650	5500	80	5	50-880
	Negative	40	60	40	650	-4500	-60	-5	50-880
FIA	Positive	40	40	30	550	5500	80	5	50-500
	Negative	40	40	30	550	-4500	-80	-5	50-500

Table S7. Overview of the different fractions

Compounds	pH at loading	Method	Net charge during loading	Fraction	Compound class
Valine/Betaine	2.5	Positive	+/neutral	Flow-through	Amino acids/Amines
Leucine/Isoleucine	2.5	Positive	+/neutral	Flow-through	Amino acids
Tryptophan	2.5	Positive	+/neutral	SCX	Amino acids
Trimethylamine-N-oxide	2.5	Positive	Neutral	Flow-through	Amines
Ornithine	2.5	Positive	+/++	SCX	Amino acids
Carnitine	2.5	Positive	+/neutral	Flow-through	Amines
1/3-methylhistidine	2.5	Positive	+/++	SCX	Amino acids
Phenylalanine	2.5	Positive	+/neutral	Flow-through	Amino acids
Serine	2.5	Positive	+/neutral	Flow-through	Amino acids
Citrulline	2.5	Positive	+/neutral	Flow-through	Amino acids
Methionine	2.5	Positive	+/neutral	Flow-through	Amino acids
Arginine	2.5	Positive	+/++	SCX	Amino acids
Alanine	2.5	Positive	+/neutral	Flow-through	Amino acids
Cystine	2.5	Positive	+/++/neutral	SCX	Amino acids
Aspartic acid	2.5	Positive	+/neutral	Flow-through	Amino acids
Glutamic acid	2.5	Positive	+/neutral	Flow-through	Amino acids
Histidine	2.5	Positive	+/++	SCX	Amino acids
Lysine	2.5	Positive	+/++	SCX	Amino acids
Proline	2.5	Positive	+/neutral	Flow-through	Amino acids
Threonine	2.5	Positive	+/neutral	Flow-through	Amino acids
Urea	2.5	Positive	Neutral	Flow-through	Amines
Glutamine	2.5	Positive	+/neutral	Flow-through	Amino acids
Glucose	2.5	Positive	Neutral	Flow-through	Hexose
Tyrosine	2.5	Positive	+/neutral	Flow-through	Amino acids
Choline	2.5	Positive	+	Flow-through	Amines
Hypoxanthine	2.5	Positive	+/neutral	Flow-through	Purines
Guanine	2.5	Positive	+/neutral	Flow-through	Purines
Uric acid	2.5	Positive	Neutral	Flow-through	Amines
Creatinine	2.5	Positive	+	Flow-through/SCX	Amines
Taurine	2.5	Positive	Neutral	Flow-through	Amino acids
Palmitoylcarnitine	2.5	Positive	+/neutral	C18	Acylcarnitines
Stearoylcarnitine	2.5	Positive	+/neutral	C18	Acylcarnitines
Decanoylcarnitine	2.5	Positive	+/neutral	C18	Acylcarnitines
Octanoylcarnitine	2.5	Positive	+/neutral	C18	Acylcarnitines
(Iso)Citric acid	7	Negative	-3	WAX	Organic acids
Malic acid	7	Negative	-2	WAX	Organic acids
Lactic acid	7	Negative	-	Flow-through	Organic acids
Pyruvic acid	7	Negative	-	Flow-through	Organic acids
Aconitic acid	7	Negative	-3	WAX	Organic acids
Alpha-ketoglutaric acid	7	Negative	-2	WAX	Organic acids
2/3-Hydroxybutyric acid	7	Negative	-	Flow-through	Organic acids
Indoxylsulfate	7	Negative	-(sulfate)	WAX	Organic acids
Palmitic acid	7	Negative	-	C18	Fatty acids
Stearic acid	7	Negative	-	C18	Fatty acids
Alpha-linolenic acid	7	Negative	-	C18	Fatty acids
Docosahexaenoic acid	7	Negative	-	C18	Fatty acids
Docosapentaenoic acid	7	Negative	-	C18	Fatty acids
Linoleic acid	7	Negative	-	C18	Fatty acids
Arachidonic acid	7	Negative	-	C18	Fatty acids
Oleic acid	7	Negative	-	C18	Fatty acids

Table S8. Method validation parameters (CO = carryover and ME = matrix effect)

Name	Labeled ISTD	Repeatability			Intermediate precision			CO (1^{st})	CO (2^{nd})	R^2	ME (%)	LOD (µM)	LLOQ (µM)
		C0 RSD (%)	C2 RSD (%)	C4 RSD (%)	C0 RSD (%)	C2 RSD (%)	C4 RSD (%)						
Val/Bet	Val	1.6	2.0	1.4	3.4	2.8	2.3	0.0	0.0	0.992	71	0.4	1
Leu/Ile	Leu	2.9	1.1	1.7	2.5	3.1	2.9	0.0	0.0	0.993	65	0.5	1
Trp	Tryp	5.8	0.9	2.0	4.7	1.8	2.4	0.0	0.0	0.997	336	0.3	1
TMAO	TMAO	18.1	3.6	1.0	14.8	7.5	3.1	0.0	0.0	0.999	54	0.4	1
Orn	Lys	13.9	3.4	1.4	11.1	6.5	3.8	0.0	0.0	0.994	137	3	10
Carnitine	Car	3.8	0.1	2.0	3.0	1.4	1.7	0.0	0.0	1.000	54	0.01	0.03
1/3-Mhis	Arg	10.5	0.8	1.4	8.8	1.7	2.0	0.0	0.0	0.996			
Phe	Phe	6.6	1.6	3.6	7.2	5.9	4.9	0.0	0.0	0.993	36	1	4
Ser	Val	6.3	2.4	1.9	4.9	4.6	4.2	0.0	0.0	0.994			
Cit	Val	3.9	2.1	2.6	6.0	4.7	5.8	0.0	0.0	0.997			
Met	Val	5.4	1.8	1.6	5.1	3.8	3.5	5.3	1.5	0.999			
Arg	Arg	3.3	1.2	0.8	3.6	1.8	2.3	2.0	0.0	0.999	89	0.2	0.5
Ala	Glu	4.7	1.4	3.8	5.2	2.3	3.8	0.0	0.0	0.997	68	8	27
Cys-Cys	Lys	6.0	2.2	3.7	7.9	3.3	5.0	0.0	0.0	0.991			
Asp	Val	4.8	3.7	4.3	5.9	6.3	4.2	0.0	0.0	0.995			
Glu	Leu	3.2	0.7	1.4	3.2	3.5	2.4	0.0	0.0	0.996	91	0.9	1
His	Arg	12.1	1.6	0.9	10.0	4.6	2.9	0.5	0.1	0.995			
Lys	Lys	4.3	2.7	3.3	3.6	3.0	3.1	0.0	0.0	0.995	130	1	4
Pro	Val	2.3	1.9	2.4	1.8	2.1	1.6	0.0	0.0	0.998			
Thr	glu	5.7	1.8	3.2	4.2	4.7	2.4	0.0	0.0	0.995			
Urea	Phe	4.2	3.9	3.4	4.7	3.1	2.4	0.0	0.0	0.993			
Gln	glu	3.6	0.7	2.0	2.7	1.9	1.8	0.0	0.0	0.999	69	1	4
Glucose	Glucose	7.2	1.2	7.9	5.3	2.7	5.1	0.0	0.0	0.998	77	3	10
Tyr	Glu	2.2	6.4	3.7	4.4	7.6	4.1	0.0	0.0	0.999			
Choline	Choline	3.4	1.4	1.2	2.7	1.5	1.6	0.0	0.0	0.999	81	0.04	0.1
Hyp	Phe	6.6	4.2	1.2	12.1	4.9	2.7	0.0	0.0	0.998			
Gua	Leu	18.9	8.2	6.5	15.4	8.8	6.7	0.0	0.0	0.991			
Urate	Glu	1.6	2.2	1.3	2.3	3.7	4.3	0.0	0.0	0.991			
Creat	Creat	4.2	0.4	1.5	3.4	1.3	1.3	0.0	0.0	0.999	49	0.09	0.3
Tau	Glu	3.2	2.1	2.6	3.7	4.4	4.4	0.0	0.0	0.997			
C16 carnitine	C8 carnitine	3.8	4.0	1.4	5.5	9.0	10.0	1.8	0.0	0.982			
C18 carnitine	C8 carnitine	11.6	6.0	3.3	11.2	11.7	6.9	0.0	0.0	0.987			
C10 carnitine	C8 carnitine	4.1	1.4	5.4	9.1	6.0	8.6	2.4	1.3	0.990			
C8 carnitine	C8 carnitine	4.6	3.1	5.0	5.8	3.8	4.1	0.0	0.0	0.994	98	0.002	0.003
Citrate	Citrate	1.5	1.3	3.5	2.0	1.8	3.3	0.3	0.2	0.999	562	0.7	1
Malate	Malate	10.6	3.2	0.6	14.7	5.1	3.9	0.4	0.0	0.993	75	0.4	0.9
Lactate	Lactate	1.5	0.7	4.1	1.9	3.0	2.7	0.0	0.0	0.991	34	5	13
Pyruvate	Lactate	4.0	4.8	2.9	4.9	4.9	5.0	0.0	0.0	0.993	41	9	19
Aconate	Citrate	5.8	1.9	3.2	8.5	4.7	3.6	0.9	0.0	0.998			
α-keto-glutarate	Citrate	8.5	7.3	4.1	11.4	6.8	6.6	2.0	0.1	0.996			
2/3-hydroxy-butyrate	Lactate	8.3	0.6	0.7	7.0	4.0	3.2	0.0	0.0	0.999			
Indoxyl-sulfate	Citrate	3.1	3.7	7.3	9.6	6.7	8.0	0.0	0.0	0.995			
FA(16:0)*	FA(16:0)	8.9	10.1	10.9	13.5	8.6	11.0	0.8	0.0	0.996	94	0.6	0.7
FA(18:0)*	FA(16:0)	9.2	6.6	5.0	14.4	8.4	9.7	0.0	0.0	0.996	98	0.2	0.6
FA(18:3)	FA(16:0)	3.6	3.7	0.9	13.6	14.3	6.9	1.9	0.8	0.996			
FA(22:6)	FA(16:0)	7.6	2.3	0.3	6.9	4.5	6.2	0.4	0.0	0.998			
FA(22:5)	FA(18:0)	6.3	12.6	2.5	7.9	10.7	6.8	1.6	0.3	0.974			
FA(18:2)	FA(18:0)	5.0	3.3	2.2	11.6	5.6	2.7	1.2	0.3	0.998			
FA(20:4)	FA(18:0)	4.9	3.0	1.9	7.9	3.8	4.5	2.0	0.6	0.998			
FA(18:1)	FA(18:0)	3.7	6.0	2.1	4.7	6.4	5.2	0.9	0.1	0.996	562		

**high background signals experienced. Background subtraction was used to account for this.*

Table S9. The matrix effect and fraction of 22 internal standards in fractionation, liquid-liquid extraction coupled to flow injection analysis (LLE-FIA) and flow injection analysis (FIA). An ion suppression of 0% indicates a compound that is either enhanced or not suppressed. An ion suppression of 100% means that the compound is not detected

Compounds	Fractionation		LLE-FIA		FIA
	Matrix effect (%)	Fraction	Matrix effect (%)	fraction	Matrix effect (%)
Valine-d8	71	Flow-through	5	Polar	1
Leucine-d3	65	Flow-through	4	Polar	0
Tryptophan-13C11, 15N2	336	SCX	4	Polar	0
TMAO-d9	54	Flow-through	9	Polar	4
Ornithine d6	137	SCX	2	Polar	0
Carnitine-d3	54	Flow-through	14	Polar	6
Phenylalanine-d5	36	Flow-through	4	Polar	1
Arginine-15N2	89	SCX	7	Polar	3
Alanine-d3	68	Flow-through	0	Polar	0
Glutamic acid-13C2	91	Flow-through	0	Polar	0
Lysine-d4	130	SCX	5	Polar	2
Glutamine-d5	69	Flow-through	1	Polar	0
Glucose-13C6	77	Flow-through	25	Polar	17
Choline-d4	81	Flow-through/SCX	24	Polar	14
Creatinine-d3	49	Flow-through	20	Polar	6
Octanoyl carnitine-d3	98	C18	32	Apolar	8
Citric acid-D4	562	WAX	86	Polar	677
Malic acid-13C4	75	WAX	19	Polar	24
Lactic acid-13C3	34	Flow-through	14	Polar	14
Pyruvic acid-13C3	41	Flow-through	11	Polar	15
Palmitic acid-D2	94	C18	76	Apolar	12
Stearic-D3	98	C18	70	Apolar	10

Table S10. The LLOQ of literature LC-MS methods. The LLOQ determination, MS mode, derivatization, analysis time and reference are also indicated in the table.

	LLOQ (μM)	LLOQ determination	MS mode	Derivatization	Analysis time (min)	Reference
Valine	10	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Leucine	10	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Tryptophan	5	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
TMAO	0.1	S/N	Tandem	No	10	Liu *et al.*[2]
Ornitine	5	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Carnitine	0.06	S/N	Tandem	No	10	Liu *et al.*[2]
Phenyl alalnine	5	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Arginine	5	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Alanine	20	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Glutamic acid	5	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Lysine	10	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Glutamine	20	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
Glucose	11	S/N	Tandem	No	4	Matsunami *et al.*[3]
Choline	0.1	S/N	Tandem	No	10	Liu *et al.*[2]
Creatinine	10	Unknown	Tandem	Yes	7	Trabado *et al.*[1]
C8 carnitine	0.006	S/N	Tandem	Yes	22	Giesbertz *et al.*[4]
Citrate	0.2	S/N	Tandem	No	Unknown	Kadhi *et al.*[5]
Malate	0.2	S/N	Tandem	No	Unknown	Kadhi *et al.*[5]
Lactate	3	S/N	Tandem	No	Unknown	Kadhi *et al.*[5]
Pyruvate	1	Unknown	Tandem	No	3.5	Chuang *et al.*[6]
FA(16:0)	0.8	Calibration curve	High-resolution	No	15	Takahashi *et al.*[7]
FA(18:0)	0.07	calibration curve	High-resolution	No	15	Takahashi *et al.*[7]

References Table S10

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Table S11A. The reversed phase (RP) gradient performed on an UPLC HSS T3 (1.7 µm, 2.1 x 100 mm). Mobile phase A and B consisted of 0.1% formic acid in water and acetonitrile, respectively. The column oven was set at 30 °C. The sample preparation, injection volume and MS parameters were similar to the fractionation method.

Time (min)	Mobile phase B (%)	Flow rate (mL/min)
0.0	0	0.4
7.0	15	0.4
10	55	0.4
10.5	100	0.4
12.5	100	0.4
12.6	0	0.4
15.0	0	0.4

Table S11B. The hydrophilic interaction liquid chromatography (HILIC) gradient performed on a Sequant ZIC-HILIC (3 µm, 2.1 x 100 mm). Mobile phase A consisted of 10 mM ammonium formate and 0.075% formic acid in 90/10 (v/v) acetonitrile/water and mobile phase B of 10 mM ammonium formate and 0.075% formic acid in 10/90 (v/v) acetonitrile/water. The column oven was set at 30 °C. The sample preparation, injection volume and MS parameters were similar to the fractionation method.

Time (min)	Mobile phase B (%)	Flow rate (mL/min)
0.0	0	0.5
1.2	0	0.5
9.16	75	0.5
14.0	75	0.5
14.2	0	0.5
18.0	0	0.5

Table S11C. The number of detectable features in the fractionation, RP and HILIC method as determined by the 'Non-targeted Peaks' function in Analytics of Sciex OS 1.6. The minimum retention time was determined by the dead volume of the platform and the maximum retention time was determined by the start of the column equilibration. The peak detection sensitivity was set at exhaustive, the Area Ratio Threshold (Unknown/Control) was set at 0 and the 'Group peaks by adduct or charge' function was checked. The number of features was corrected for the presence of multiple adducts. Feature m/z values were rounded to two decimal points. Features that were identified in both positive and negative mode were indicated as one feature. All individual features are reported in a separated excel file, called: 'detected_features.xslx'.

Platform	Unique retention time and m/z features	Unique m/z features
Fractionation	2289	2089
RP	3475	2465
HILIC	3529	2325

Table S12. P-values and FDR adjusted p-values of several compound classes on different time points in comparison to baseline levels

Compound class	Time after breakfast (hours)	P-value	FDR adjusted p-value
Amino acids	0.5	0.006	0.063
	1.5	0.004	0.046
	3	0.002	0.032
	4.5	0.002	0.032
	6	0.037	0.296
	9.5	0.16	0.942
	11	0.002	0.032
	12	0.002	0.032
	24	0.131	0.829
Amines	0.5	0.846	1
	1.5	0.557	1
	3	0.232	1
	4.5	0.105	0.686
	6	0.064	0.498
	9.5	0.084	0.576
	11	0.084	0.576
	12	0.557	1
	24	0.16	0.942
Acylcarnitines	0.5	0.014	0.13
	1.5	0.002	0.032
	3	0.002	0.032
	4.5	0.002	0.032
	6	0.002	0.032
	9.5	0.006	0.063
	11	0.002	0.032
	12	0.002	0.032
	24	0.232	1
Hexose	0.5	0.02	0.166
	1.5	0.77	1
	3	0.922	1
	4.5	0.002	0.032
	6	0.557	1
	9.5	0.16	0.942
	11	0.002	0.032
	12	0.084	0.576
	24	1	1
Organic acids	0.5	0.002	0.034
	1.5	0.004	0.046
	3	0.846	1
	4.5	0.006	0.058
	6	0.846	1
	9.5	0.105	0.686
	11	0.002	0.034
	12	0.02	0.166
	24	0.006	0.058
Fatty acids	0.5	0.193	1
	1.5	0.004	0.046
	3	0.004	0.046
	4.5	0.004	0.046
	6	0.004	0.046
	9.5	0.105	0.686
	11	0.037	0.296
	12	0.004	0.046
	24	0.01	0.097