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“A metabolomics and lipidomics profile of ischemic stroke”

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Summary

Ischemic stroke is one of the leading causes of mortality and adult disability worldwide. Although there has been extensive research and great progress over the last years, treatment options are still limited to thrombolysis and thrombectomy. Therefore, a deeper understanding of the underlying metabolic alterations and processes related to stroke is necessary. Metabolomics and lipidomics approaches offer great opportunities for fundamental and clinical research, focusing on the detection of as many compounds as possible. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the method of choice when it comes to the analysis of lipids and metabolites within an organism. However, high-confidence identification of compounds and thorough method development are essential.

This thesis describes the generation of an MS² library, comprising fragmentation spectra for 506 biologically relevant metabolites. Additionally, an LC library to describe the chromatographic behavior of 294 metabolites was built. Based on these databases, a metabolomics screen was developed. Applying the metabolomics method and an established lipidomics screen, human serum samples of 112 ischemic stroke patients were analyzed, resulting in the detection of 188 lipid species out of 12 lipid classes and 76 metabolites. Compared to control patients, numerous compounds were altered in the serum of ischemic stroke patients. Serum levels of several lipids and metabolites were increased or decreased over three consecutive days after the ischemic attack. Particularly noteworthy are the changes in relation to the phospholipid metabolism. Interestingly, alterations could also be observed regarding the outcome of patients. Based on the modified Rankin Scale after 90 days (mRS90), a decreased phosphatidylcholine (PC) to phosphatidylethanolamine (PE) ratio was observed in patients with a bad outcome. Therefore, an activation of the phosphatidylethanolamine N-methyltransferase (PEMT) pathway, metabolizing mainly long-chain, polyunsaturated PEs to PCs, is hypothesized to be beneficial for the outcome of patients. Furthermore, 2 hours and 24 hours after permanent distal cerebral artery occlusion (pMCAO) in a mouse model of ischemic stroke, plasma, as well as liver and brain tissue, were collected and analyzed. The metabolomics and lipidomics analysis complemented and partly confirmed the results from the human study. Among others, metabolites of the transmethylation and transsulfuration pathways, which are directly connected to PEMT, were upregulated on the affected, ipsilateral side of the brain. Metabolites of the arginine and nitric oxide (NO) pathway were altered as well. Concentrations of several lipids and metabolites in plasma, liver and brain were significantly correlated with the infarct size.

Summary

In summary, our comprehensive data enables the investigation of several pathways and mechanisms in relation to ischemic stroke. Particularly, a possible activation of the PEMT pathway after an ischemic attack and the involvement in the recovery of patients is discussed. Further studies are necessary to confirm these results and to potentially facilitate the development of new treatment options for ischemic stroke patients.

Zusammenfassung

Der ischämische Schlaganfall ist weltweit eine der häufigsten Todesursachen und führt bei betroffenen Patienten häufig zu anhaltender Behinderung. Behandlungsoptionen sind limitiert und umfassen hauptsächlich thrombolytische Ansätze. Trotz intensiver Forschung und vielversprechender Erkenntnisse ist daher ein noch tieferes Verständnis der dem Schlaganfall zugrundeliegenden molekularen Mechanismen nötig. Die Metabolomics- und Lipidomics-basierte Analytik bietet zahlreiche Möglichkeiten im Rahmen der Grundlagenforschung und klinischen Forschung, da sie auf die Messung möglichst vieler Substanzen ausgerichtet ist. Flüssigchromatographie kombiniert mit Tandem-Massenspektrometrie (*engl.: liquid chromatography coupled to tandem mass spectrometry, LC-MS/MS*) ist die bevorzugte Methode zur Bestimmung von Metaboliten und Lipiden innerhalb eines Organismus. Eine eindeutige Identifizierung von Substanzen und eine fundierte Methodenentwicklung sind hierbei essentiell.

In dieser Arbeit wird die Erstellung einer MS²-Datenbank beschrieben, welche Fragmentspektren von 506 biologisch relevanten Metaboliten enthält. Zusätzlich wurde eine LC-Datenbank zum chromatographischen Verhalten von 294 Metaboliten generiert. Basierend auf diesen Datenbanken wurde anschließend eine Metabolomics-Methode entwickelt. Die entwickelte Metabolomics- und eine etablierte Lipidomics-Methode wurden zur Untersuchung humaner Serumproben von 112 Schlaganfallpatienten angewendet. Die Messungen resultierten in der Detektion von 188 Lipiden aus 12 Lipidklassen und 76 Metaboliten. Verglichen mit Kontrollpatienten zeigten Schlaganfallpatienten veränderte Serumkonzentrationen für zahlreiche Verbindungen. An drei aufeinanderfolgenden Tagen waren viele Lipide und Metabolite hoch- oder runterreguliert. Besonders hervorzuheben sind die Veränderungen in Bezug auf den Phospholipid-Stoffwechsel. Interessanterweise konnten auch in Abhängigkeit von der funktionellen Besserung der Patienten Unterschiede festgestellt werden. Basierend auf der modifizierten Rankin-Skala nach 90 Tagen (*engl.: modified Rankin Scale, mRS90*), wurde ein niedrigeres Verhältnis von Phosphatidylcholin (PC) zu Phosphatidylethanolamin (PE) in Patienten mit geringer funktioneller Besserung festgestellt. Eine Aktivierung der Phosphatidylethanolamin N-Methyltransferase (PEMT), welche vorrangig für die Umwandlung langkettiger, ungesättigter PEs zu PCs zuständig ist, könnte daher eine funktionelle Besserung der Patienten begünstigen. Des Weiteren wurden 2 Stunden und 24 Stunden nach permanenter Okklusion der *Arteria cerebri media* im Mausmodell des ischämischen Schlaganfalls (*engl.: permanent distal cerebral artery occlusion, pMCAO*) Plasma, sowie Leber- und Hirngewebe entnommen. Die Untersuchung mithilfe der Metabolomics- und Lipidomics-Methoden konnte die Ergebnisse der Humanstudie ergänzen

und teilweise bestätigen. In der betroffenen, ipsilateralen Hirnhälfte waren zahlreiche Metabolite hochreguliert, die zum Teil aus der Transmethylierung und der Transsulfurierung stammen, welche in direkter Verbindung zu PEMT stehen. Metabolite des Arginin- und Stickstoffmonooxid (NO)-Stoffwechsels waren ebenfalls verändert. Metabolit- und Lipidkonzentrationen in Plasma, Leber und Gehirn korrelierten zum Teil signifikant mit der Infarktgröße.

Die Ergebnisse dieser Arbeit bieten die Möglichkeit, verschiedene Stoffwechselwege und Mechanismen in Bezug auf den ischämischen Schlaganfall zu untersuchen und zu diskutieren. Im Speziellen wird die Hypothese aufgestellt, dass der PEMT Stoffwechsel nach einem ischämischen Schlaganfall aktiviert wird und einen Einfluss auf die funktionelle Besserung der Patienten hat. Weiterführende Studien und Untersuchungen zur Bestätigung der Hypothese werden benötigt, um letztendlich neue Behandlungsmöglichkeiten für Schlaganfallpatienten zu ermöglichen.

Abbreviations

2-HB	2-Hydroxybutyrate
5'-MTA	5'-Methylthioadenosine
AcCa	Acylcarnitines
ADMA	Asymmetric dimethylarginine
AGC	Automatic gain control
AIF	All ion fragmentation
AldDH	Aldehyde dehydrogenase
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionization
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
BMI	Body mass index
BSA	Bovine serum albumin
CBS	Cystathionine β -synthase
CDP	Cytidine diphosphate
CE	Cholesterylester
Cer	Ceramides
CerG	Cerebrosides
CerPE	Ceramide phosphoethanolamine
CID	Collision-induced dissociation
CSF	Cerebrospinal fluid
CV %	Coefficient of variance
DAG	Diacylglycerol
DDAH	Dimethylarginine dimethylamino hydrolase
DM	Diabetes mellitus
EA	Ethanolamides
EDTA	Ethylenediaminetetraacetic acid
eNOS	Endothelial nitric oxide synthase
cNOS	Constitutive nitric oxide synthase
ESI	Electrospray ionization
FA	Fatty acid

Abbreviations

FDR	False discovery rate
FFT	Fast Fourier transform
GABA	Gamma-aminobutyric acid
GM1	Monosialotetrahexosylganglioside
GM3	Monosialodihexosylganglioside
GPx	Glutathione peroxidase
Gsulfo-Cer	Cerebroside sulfate
GWAS	Genome-wide association study
HCD	Higher-energy C-trap dissociation
HILIC	Hydrophilic interaction liquid chromatography
HMDB	Human Metabolome Database
HRMS	High-resolution accurate mass
i.p.	Intraperitoneal
IEM	Ion evaporation model
IL-6	Interleukin-6
IFN-γ	Interferon gamma
iNOS	Inducible nitric oxide synthase
IT	Injection time
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC	Liquid chromatography
log P	Partition coefficient
LPC	Lysophosphatidylcholines
LPE	Lysophosphatidylethanolamines
LPI	Lysophosphatidylinositol
m/z	Mass to charge ratio
MCAO	Middle cerebral artery occlusion
MCP-1	Monocyte chemoattractant protein-1
MRI	Magnetic resonance imaging
mRS90	Modified Rankin scale
MS	Mass spectrometry
MSM	Metabolite standard mix
MSMLS	Mass Spectrometry Metabolite Library of Standards
MSR	Methionine sulfoxide reductase
NAD	Nicotinamide adenine dinucleotide

NADPH	Nicotinamide adenine dinucleotide phosphate
NAFLD	Non-alcoholic fatty liver disease
NCE	Normalized collision energy
NIHSS	National Institutes of Health Stroke Scale
NKT	Natural killer T
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NVU	Neurovascular unit
OEA	Oleoylethanolamide
ORA	Over representation analysis
PC	Phosphatidylcholines
PE	Phosphatidylethanolamines
PEA	Palmitoylethanolamide
PEMT	Phosphatidylethanolamine N-methyltransferase
PFP	Pentafluorophenylpropyl
PG	Phosphatidylglycerol
PI	Phosphatidylinositols
PRM	Parallel Reaction Monitoring
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
QC	Quality control
R2	Coefficient of determination
RAI1	Retinoic Acid Induced 1
RASD1	Ras Related Dexamethasone Induced 1
RF	Radio frequency
ROC	Receiver operating characteristics
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RP	Reversed-phase
RT	Retention time
S/N	Signal-to-noise ratio
SAH	S-Adenosylhomocysteine
SAM	S-Adenosylmethionine
SAMDC	S-Adenosylmethioinine decarboxylase

Abbreviations

SDMA	Symmetric dimethylarginine
SM	Sphingomyelin
SMPDB	Small Molecule Pathway Database
SMS	Sphingomyelin synthase
SOD	Superoxide dismutase
SPE	Solid-phase extraction
sPLS-DA	Sparse partial least squares regression discriminant analysis
SVM	Support vector machine
TNF-α	Tumor necrosis factor alpha
TOF	Time of flight
TG	Triacylglycerol
tPA	Tissue-type plasminogen activator
t-SIM/dd-MS2	Targeted single ion monitoring followed by data-dependent fragmentation

1 Introduction

1.1 The metabolome and lipidome

In life sciences and medical research, “omics” are gaining more and more importance when it comes to the comprehensive understanding of a biological system [1]. “Omics” technologies include several disciplines and enable the analysis of an enormous number of biological molecules. While genomics, transcriptomics and proteomics investigate the genotype, RNA and protein levels, respectively, lipidomics and metabolomics represent the lipidome and metabolome. They are considered the final and dynamic output of genetic, epigenetic and non-genetic factors and display the actual state of an organism [2].

The metabolome comprises all small (< 1 - 2 kDa) molecules in a biological sample, like amino acids, carbohydrates and vitamins, but also exogenous compounds like drugs or xenobiotics (**Figure 1.1**) [3]. The great chemical and biological diversity makes the metabolome an interesting target for many research settings, including biomarker discovery or personalized medicine. The lipidome, as part of the metabolome, describes hydrophobic compounds, like glycerophospholipids, sphingolipids, glycerolipids and other lipid classes. Lipids are diverse and fulfil numerous functions in an organism. Next to energy storage and being the main component of structural elements like cell membranes, the signaling role of lipids becomes increasingly evident [4].

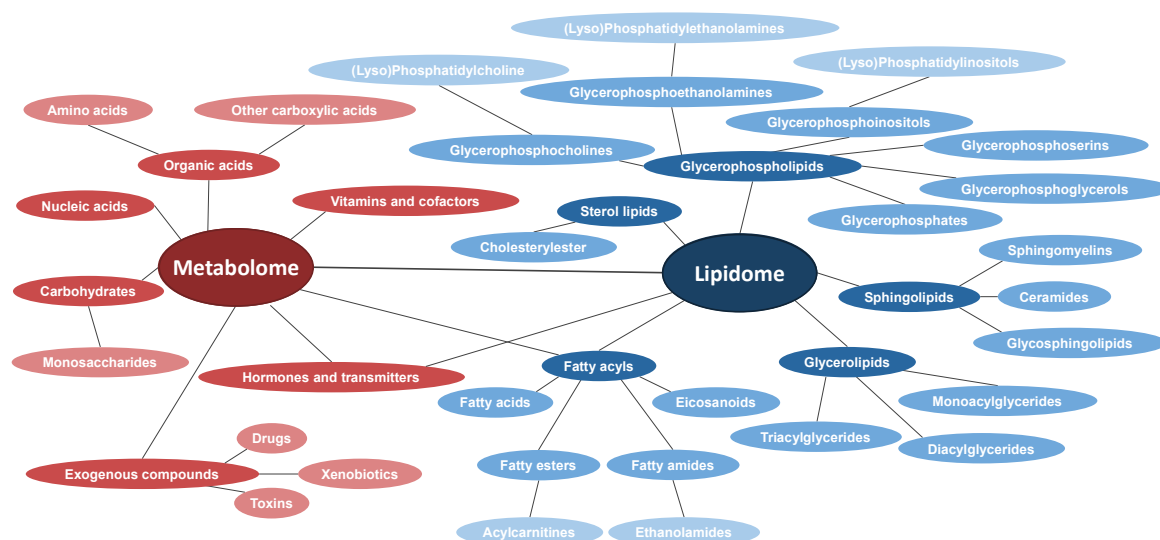


Figure 1.1 Compound classes of the metabolome and lipidome (incomplete overview). Metabolite and lipid classes according to Kyoto Encyclopedia of Genes and Genomes (KEGG) [5] and LIPID MAPS®[6].

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The total number of biologically relevant metabolites and lipids is hard to estimate [7]. The Human Metabolome Database (HMDB, Version 5.0) currently comprises over 200,000 compounds [8]. Next to nuclear magnetic resonance (NMR), mass spectrometry (MS) based approaches have been the method of choice for lipid and metabolite profiling as they offer excellent sensitivity and selectivity. However, until now, there is no single analytical platform to cover the whole metabolome or lipidome. Structural diversity and dramatically different concentration ranges make metabolomics and lipidomics challenging and restrict the insight to a more or less small fragment of the actual system.

1.2 Metabolomics and lipidomics with LC-MS/MS

This work describes metabolomics and lipidomics profiling with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Although the focus is on detecting chemically different sets of compounds, both metabolomics and lipidomics studies aim to generate new hypotheses. Strategies can be classified as targeted or untargeted, depending on the method setup. In contrast to targeted approaches, which are optimized for the analysis of predefined compounds and absolute quantification, untargeted approaches intend to detect as many compounds as possible, including unknowns [9]. Despite the aim to represent the whole lipidome or metabolome, analyses are never truly unbiased [7, 10, 11]. The selection of analytical conditions, especially the extraction protocol and LC method, automatically limits the coverage of a method. The capabilities of the mass analyzer confine the results as well, therefore high-resolution accurate mass (HRMS) platforms are essential for metabolomics and lipidomics analysis.

1.2.1 Electrospray ionization

In MS, compounds are detected based on their mass to charge ratio (m/z). Several techniques are available to ionize the analytes. For metabolomics and lipidomics, electrospray ionization (ESI) is the most common method [12, 13]. It is a soft ionization technique that avoids fragmentation, resulting in a reproducible ionization pattern. However, ionization efficiency is easily suppressed by the presence of salts, limiting the use of buffers to volatile compounds like ammonium acetate. Nevertheless, ion suppression can occur when measuring biological samples. ESI can be operated in both positive and negative mode, allowing the investigation of a broad range of compounds. For metabolites and lipids, primarily single-charged, protonated ($[M+H]^+$) and deprotonated ($[M-H]^-$) ions are generated in positive and negative mode, respectively. The m/z of the molecular ion allows the tentative identification of a signal without further calculations. However, other

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adducts can be generated, especially sodium adducts, decreasing the signal intensity of the actual precursor ion. Certain additives, like formic acid, can increase ionization efficiency, especially in the positive mode, by augmenting the formation of preferred, protonated ions.

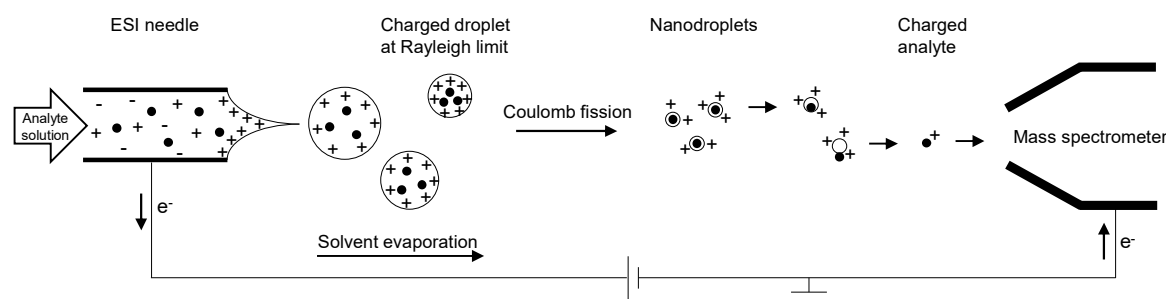


Figure 1.2 Schematic illustration of the electrospray ionization process. Operation in positive mode. Modified after Konermann et al., 2013 [14] and Banerjee et al., 2012 [15].

In the ESI source, a high voltage is applied at atmospheric pressure (**Figure 1.2**). The inserted liquid, containing solvents and analytes, forms a Taylor cone-jet that emits fine droplets. The stability of the spray depends on the liquid flow rate and the applied voltage [16]. Positively or negatively charged droplets are shrinking through solvent evaporation, often supported by heating. When the charge density equals the repulsion (Rayleigh limit), a Coulomb fission takes place, producing smaller droplets. Repetition of these events finally leads to so-called nanodroplets. For small molecules, like metabolites and lipids, the generation of intact gas-phase ions is described by the ion evaporation model (IEM). Briefly, it is postulated that solvated ions are ejected from the droplet surface by the electrical field originating from the highly charged nanodroplet [14]. Finally, the charged analyte enters the mass spectrometer. Present matrix components can compete with the analyte for access to the droplet surface for gas-phase emission, decreasing ionization efficiency and sensitivity. To correct for ion suppression effects, isotopically labeled internal standards should be used [17].

1.2.2 Tandem mass spectrometry

HRMS platforms are of great value for metabolomics and lipidomics. With increasing resolution and mass accuracy, the number of possible molecular formulas corresponding to an m/z can be reduced dramatically [7]. The Orbitrap mass spectrometer (Q Exactive, ThermoFisher Scientific) used in this study, offers a mass resolution of up to 140,000 at $m/z = 200$. The mass resolution is defined as the m/z value divided by the m/z deviation of two ions that can be separated [18]:

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$$R = m/z / \Delta(m/z) \rightarrow 140.000 = 200 / \Delta(m/z) \quad (A)$$

Consequently, a distinction of two compounds with a mass deviation of only 0.0014 Da can be achieved. Mass accuracy is promoted by the manufacturer to be < 3 ppm under defined conditions.

The Q Exactive hybrid quadrupole-Orbitrap mass spectrometer combines a quadrupole mass filter with the Orbitrap mass analyzer (**Figure 1.3**) [20]. The vacuum system consists of several stages of differential pumping, providing a pressure reduction from the atmospheric pressure of the ESI source to 10^{-10} mbar inside the Orbitrap. Ions entering the mass spectrometer from the ESI source are transferred through the S-lens and flatapole ion guides. In case of a full scan experiment, ions pass the quadrupole and are collected in the nitrogen-filled C-cell, a curved linear ion trap. Through collision with the bath gas, ions are slowed down and accumulated in ion packages. This procedure is necessary to couple the continuous ESI source with the Orbitrap mass analyzer, which works in a pulsed fashion [21]. The Orbitrap consists of an axial central electrode and a coaxial outer electrode. Tangentially injected ion packages from the C-cell start coherent rotation around the spindle-shaped, central electrode and harmonic oscillation along it. The frequency ω of these axial oscillations only depends on the m/z and the constant field curvature k :

$$\omega = \sqrt{\frac{k}{m/z}} \quad (B)$$

Frequencies and therefore mass-to-charge ratios of ions are obtained via fast Fourier transform (FFT), resulting in a full scan mass spectrum.

Tandem MS (MS/MS) enables the additional fragmentation of compounds. The detection of both precursor ion and fragmentation pattern increases identification confidence enormously. Filtering certain masses in the quadrupole is based on applying specific electromagnetic fields that allow only one m/z to pass. These ions are collected in the C-trap and transferred into the multipole collision cell. Higher-energy C-trap dissociation (HCD) is a collision-induced dissociation (CID) technique, where the fragmentation takes place outside the actual ion trap. Fragmentation is based on applying an electrical potential to accelerate and increase the kinetic energy of the selected ions. When they collide with neutral nitrogen molecules, the kinetic energy is partially converted into internal energy, leading to the breakage of covalent bonds and the generation of smaller fragments [22]. These fragments are then transferred back to the C-trap and injected into the Orbitrap.

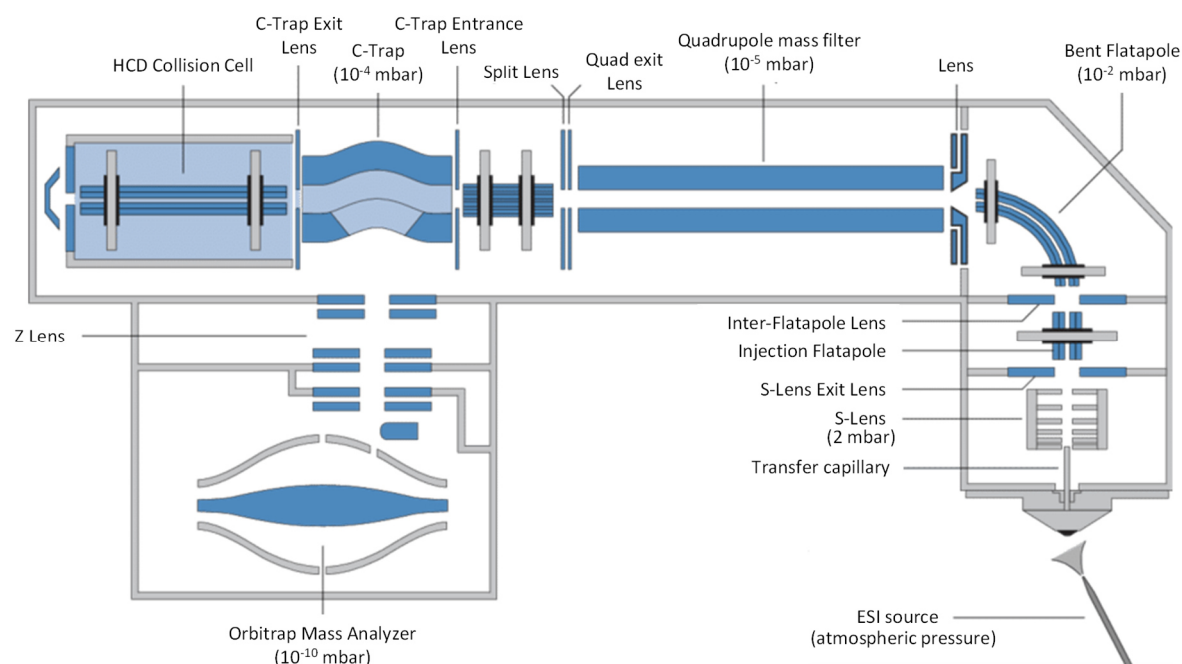


Figure 1.3 Schematic illustration of the Q Exactive mass spectrometer. Modified after Michalski et al., 2011 [19]. Proportions not to scale.

Multiple ways of data acquisition are possible. The fundamental experiment is a full scan, where all ions within a specific scan range are detected without fragmentation. To obtain fragmentation spectra, MS/MS (MS^2) experiments can be performed. Parallel Reaction Monitoring (PRM) is the method of choice for a preselected set of compounds. Here, a list of masses is prepared beforehand. As soon as these masses are present, an MS^2 scan is acquired. Another option is All Ion Fragmentation (AIF), where all detected masses get fragmented simultaneously. However, advanced data processing is required to interpret these MS^2 spectra. For untargeted analysis, a data-dependent approach offers great opportunities. Based on a full scan only the most abundant masses are fragmented to reduce redundant data.

1.2.3 Liquid chromatography

In so-called “shotgun” or direct infusion approaches, the biological sample or extract is directly injected into the mass spectrometer. Despite the need for ultra high-resolution, the setup is less elaborate and saves time. However, coupling liquid chromatography (LC) to MS has certain advantages [9]. First, the fractionation of the sample before entering the ESI source reduces ion suppression caused by matrix components and salts. Second, selectivity is increased because the separation of compounds via LC is based on their chemical properties. Consequently, the retention

time can be used as an orthogonal factor for compound identification, especially for isomers and isobars.

In general, the retention of compounds depends on the affinity towards the stationary and the mobile phase. The most frequently used techniques are reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) [9]. Hydrophobic and hydrophilic/electrostatic interactions are the predominant retention mechanisms in RP-LC and HILIC, respectively [23]. Depending on the focus of the analysis and the analytes' chemical properties, the chromatographic method has to be adjusted. For lipidomics, RP-LC achieves good separation of lipids due to their hydrophobic nature and the interaction with non-polar stationary phases, like C18 or C30 columns [4, 24]. Mechanisms in HILIC are more complex and not yet fully understood. It is suggested that hydrophilic portioning, hydrogen bonding and electrostatic interactions are the underlying principles [23]. Even though HILIC is less robust than RP, it is a popular option for metabolomics as it enables the retention of small hydrophilic and ionic metabolites [25, 26].

1.2.4 Compound identification

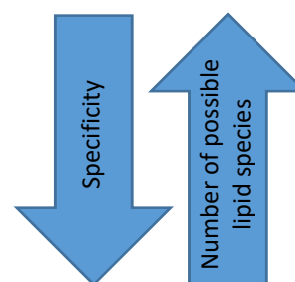
As mentioned above, untargeted approaches aim to detect as many compounds as possible. Consequently, reliable identification of lipids and metabolites is crucial. In LC-MS/MS, the assignment of peaks is usually based on the exact mass (m/z) and isotopic pattern, the fragmentation as well as the retention time. Via the exact mass, the molecular composition can be predicted. For structural information, fragmentation spectra and retention time are essential.

The identification of lipid classes is primarily based on characteristic fragmentation patterns. Mass and intensity of fragments reflect the class-specific functional group. The retention time is a complementary source of information, as more polar lipid classes elute earlier from RP columns. When it comes to the acyl chain length, the mass and the correlation of chain length and saturation with the retention time support an unambiguous peak assignment. Due to the structural diversity of the lipidome, it is necessary to define the level of identification depth one can achieve with the applied method (**Table 1.1**). The higher the structural specificity, the lower the number of possible compounds behind a mass [27, 28]. However, detailed structural elucidation like composition and position of acyl chains, location of double-bonds and cis/trans isomers can require highly sophisticated techniques.

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Table 1.1 Lipid nomenclature and structure specificity. Phosphatidylcholine (PC) lipid identification is shown as an example.

Nomenclature	Level of structural identification
PC (36:1)	Lipid class and bond type Sum composition
PC (18:1_18:0)	Composition of acyl chains
PC (18:0/18:1)	Position of acyl chains
PC (18:0/18:1[9])	Double-bond position
PC (18:0/18:1[9Z])	Stereochemistry



Sum composition nomenclature (e.g. PC (36:1)) is often used, as already the definition of acyl chain composition requires, that specific fragments are sufficiently abundant. Due to the concentration range of lipid species, it is not always possible to unambiguously identify the structural details.

For metabolomics, it is equally important to achieve consistent communication regarding metabolite identification confidence [7, 29]. Tentative structure identification on the m/z (MS^1) level should be supported by a putative identification on the MS^2 level. In contrast to lipidomics, where specific fragmentation patterns represent certain compound classes, metabolite fragmentation can be hard to predict. Significant progress has been achieved for computational methods like *in silico* fragmentation and machine learning [30]. Nevertheless, the comparison with experimentally generated fragmentation data is still the standard procedure. Several online databases are available, such as METLIN [31], HMDB and mzCloud (www.mzcloud.org). To ensure comparability, consistent mass accuracy, resolution, collision energy (CE) and fragmentation are needed [32-34]. As open-access libraries include spectra generated with different instrument types (e.g. time of flight (TOF), Orbitrap, Triple Quadrupole) or settings, the transfer to other equipment bears uncertainty [35, 36]. For ideal matching, the MS^2 spectra of reference material and samples should be generated under identical experimental conditions. Therefore, an in-house library is of great value for high-confidence metabolite identification. Information about the LC behavior of compounds can confirm compound assignments and is essential for method development. Varying chromatographic conditions affect metabolites differently, depending on their chemical structure. An LC library documenting the retention and peak quality of compounds under different conditions supports peak identification and helps assess the coverage bias in metabolomics method development.

1.3 Ischemic stroke

Stroke is one of the leading causes of mortality and adult disability worldwide [37]. It is classically defined as a sudden neurological deficit induced by a vascular cause [38]. Focal ischemia, caused by a disrupted blood flow to the affected brain area, is the common denominator of all frequent forms of stroke. Ischemic infarction represents about 85 % of all documented incidents, whereas hemorrhagic stroke, including intracerebral and subarachnoid hemorrhage, accounts for the minority of cases [39]. Known risk factors for ischemic stroke are age and sex, with an increased prevalence of stroke in advanced age and men. Modifiable risk factors are, among others, hypertension, smoking and diabetes mellitus (DM). Age and comorbidities like DM, high blood pressure and body mass index (BMI) have also been associated with increased morbidity, mortality and worse long-term stroke outcome [40]. The strongest predictor for the recovery of patients is the initial stroke severity [41, 42]. It is typically assessed by the National Institutes of Health Stroke Scale (NIHSS) at admission to the hospital. A neurological examination evaluates, among others, motor or sensory impairments, speech and cognition. The scale spans from 0 to 42 and can be categorized into five stages (no symptoms to severe stroke) [43]. To evaluate the long-term outcome of patients, the degree of disability is usually reported as modified Rankin scale, e.g. 90 days after ischemic stroke (mRS90) [44-46]. This scale includes seven levels (0 – 6), from no symptoms (0) or no significant disability (1) to slight disability (2), moderate (3) or moderately severe disability (4) to severe disability (5) and death (6). The rating is based on a patient questionnaire.

Depending on the cause of injury, ischemic stroke can be classified as cardioembolic, atherosclerotic, microvascular or cryptogenic [47]. Whereas the core of the ischemia is severely deprived of oxygen and glucose, perfusion increases gradually in the surrounding tissue [48]. This area, dynamic in size and distribution, is called penumbra. It is considered the primary target for treatment options, as the tissue is probably salvageable, depending on the restoration of complete perfusion and other pathophysiological factors. Despite extensive research, the only approved pharmacological treatment for ischemic stroke is thrombolysis. However, due to the small therapeutic window (3 - 4.5 hours) and several contraindications, only a minority of stroke patients is eligible for the administration of tissue-type plasminogen activator (tPA), a thrombolytic drug [49, 50]. Additional thrombectomy has been shown to prolong the time for tPA administration up to 24 hours [51, 52]. Restored perfusion resulted in a smaller infarct size, which correlated with a better neurological outcome.

1.3.1 The ischemic cascade

The biochemical reactions initiated by the obstructed blood vessel in the brain and the resulting ischemia can be described as a cascade of events (**Figure 1.4**) [53, 54]. The lack of oxygen and glucose in the ischemic tissue disrupts mitochondrial oxidative phosphorylation and rapidly depletes adenosine triphosphate (ATP). The decrease in cellular energy leads to a malfunction of ion pumps and consequently to depolarization of cells due to a high influx of sodium (Na^+) and calcium ions (Ca^{2+}). Depolarization is accompanied by an intracellular increase of water, causing a cytotoxic edema. Depolarization can also spread into the penumbra, reducing the chance of tissue recovery. Additionally, increasing intracellular Ca^{2+} concentrations trigger glutamate release, the main contributor to ischemia-induced excitotoxicity. An excessive release of the excitatory neurotransmitter and the subsequent activation of glutamate receptors aggravate Ca^{2+} influx in cells and mitochondria, potentiating the detrimental cascade of events. Damaged mitochondria produce increased levels of reactive oxygen species (ROS), like peroxides and oxygen radicals. Moreover, calcium-dependent activation of nitric oxide synthases (NOS) increases nitric oxide (NO) generation from the amino acid arginine. Oxidative stress and the formation of reactive nitrogen species (RNS) cause lipid peroxidation, protein oxidation and nitration, and DNA damage. Furthermore, Ca^{2+} overload activates other degradative enzymes, including proteases and phospholipases. Phospholipids are the main constituents of cell membranes. Excessive cleavage leads to the generation of free fatty acids (FAs) and disruption of cell membranes. Polyunsaturated fatty acids (PUFAs), like arachidonic acid, are further metabolized to eicosanoids, important inflammatory mediators. Finally, all these processes result in cell death in the ischemic core, marking an irreversible step in the ischemic cascade.

Under normal conditions, the neuronal microenvironment is protected and sustained by the blood-brain barrier (BBB) [55]. Tight junction proteins between adjacent brain endothelial cells, like occludin and claudins, form the initial barrier, restricting permeability and cellular transport. Endothelial cells, astrocytes, microglia, pericytes and neurons build the neurovascular unit (NVU) that regulates and maintains highly selective barrier functions as well as cerebral blood flow. During ischemia, dying cells secrete inflammatory mediators, including cytokines, free radicals and prostaglandins, stimulating pro-inflammatory signaling in neighboring astrocytes and microglia [56, 57]. In addition, immune cells like neutrophils, monocytes and lymphocytes from the systemic circulation are allured to the injury site [58]. These neuroinflammatory effects, oxidative stress, and the activation of proteases finally result in a BBB breakdown. Increased permeability promotes brain edema and potentiates neuroinflammation and immune cell infiltration [59].

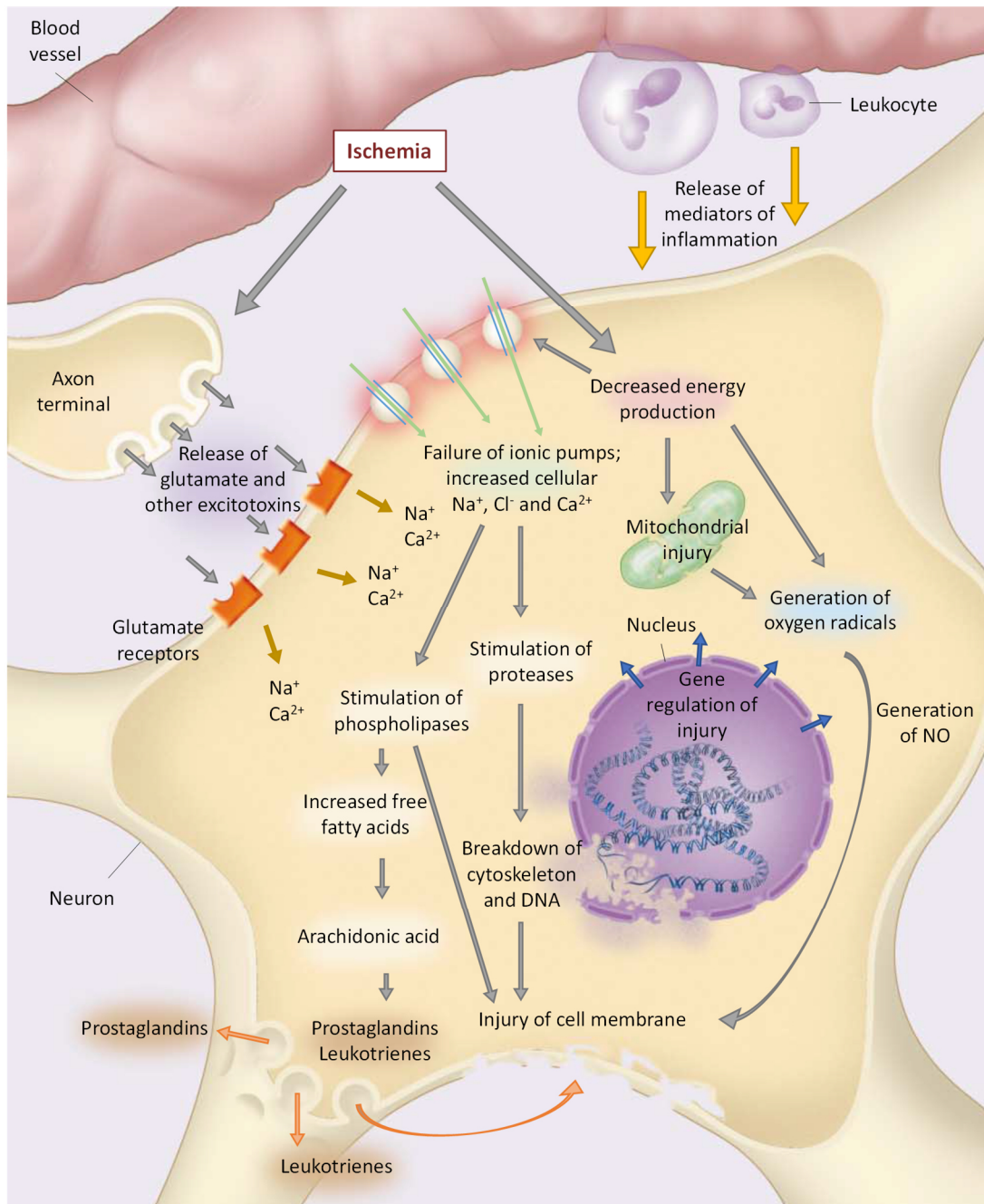


Figure 1.4 The ischemic cascade triggered in the brain by acute cerebral ischemia. Modified after Brott and Bogousslavsky, 2000 [53]. NO = nitric oxide.

Neuroprotective strategies, antagonizing oxidative stress and inflammation, have been investigated extensively. To explore molecular mechanisms and treatment options of ischemic stroke *in vivo*, middle cerebral artery occlusion (MCAO) in mice or rats is most utilized. Focal cerebral ischemia is induced, modelling either transient or permanent ischemia [60, 61]. Whereas transient models include effects of reperfusion, permanent occlusion focuses more on the ischemia itself.

1.3.2 Oxidative stress

Oxidative stress is defined as an imbalance of production and scavenging of ROS. It is considered one of the main contributors to ischemic and reperfusion injury, caused by an excessive mitochondrial, as well as xanthine oxidase- and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated, generation of ROS [62]. Scavenging is executed by the superoxide dismutase (SOD), an enzyme metabolizing superoxide anions to oxygen and hydrogen peroxide (H_2O_2), and glutathione peroxidase (GPx). Via the oxidation of the antioxidant glutathione, H_2O_2 or other peroxides are detoxified. The inhibition of NADPH oxidase has been reported as neuroprotective in an MCAO mouse model [63]. In mice deficient in SOD, exacerbated cerebral infarctions have been observed [64, 65]. In contrast, overexpression of SOD has been shown to be beneficial after focal cerebral ischemia [64, 66, 67]. Accordingly, GPx deficiency or overexpression resulted in detrimental or favourable outcomes [68, 69].

Superoxide anions can react with NO to generate highly reactive peroxynitrite. NO is an essential signaling molecule involved in numerous biological processes. However, in ischemia, Ca^{2+} -dependent activation of constitutive, endothelial or neuronal, isoforms (cNOS) and cytokine-mediated activation of inducible NOS (iNOS) lead to an excessive metabolism of arginine to citrulline and NO [70]. Mice deficient in iNOS or neuronal NOS (nNOS) showed a smaller infarct size and less neurological impairment [71, 72]. Interestingly, lack or less activity of endothelial NOS (eNOS) increased brain injury [73, 74]. In humans, NO metabolite concentrations in cerebrospinal fluid (CSF) were higher in ischemic stroke patients and were associated with larger infarcts and early neurological deterioration [75]. However, NO donors or highly selective NOS inhibitors have been considered for stroke treatment [76].

The impact of oxidative stress is reflected by increased oxidative stress markers in peripheral blood of ischemic stroke patients, correlating with stroke severity and infarct growth [77, 78]. Plasma lipid peroxides and the activity of antioxidant enzymes like SOD or GPx in erythrocytes were elevated in patients 24 hours after stroke onset [79]. Low plasma antioxidant activity has been associated with bigger infarct size and neurological impairment [80], and lower glutathione plasma levels were found in stroke patients compared to control subjects [81]. Intravenous injection of glutathione 10 min after MCAO in rats reduced infarct volume and attenuated ROS production as well as cell death [82]. However, the translation of antioxidant treatment from animal models to patients has failed so far [83].

1.3.3 Systemic inflammation

Concomitant with cerebral focal ischemia, acute systemic inflammation is induced [59]. Specific inflammatory gene expression patterns have been detected in the blood of ischemic stroke patients [84]. A correlation of the cytokine interleukin-6 (IL-6) and other peripheral inflammatory markers with infarct size, stroke severity and long-term outcome has been shown [85, 86]. However, findings in human cohorts are inconsistent, probably depending on the timing of blood sampling or stroke etiology [87]. In a rodent model, MCAO induced the expression of cytokines and other markers of inflammation in splenocytes, lymph node cells and blood cells after 6 and 22 hours [88]. Plasma levels of interferon gamma (IFN- γ), IL-6, and monocyte chemoattractant protein-1 (MCP-1) were elevated as well. Focal brain ischemia also affects peripheral organs, like the liver. It has been shown that activation of the sympathetic nervous system influences the function of hepatic natural killer T (NKT) cells, contributing to immunosuppression and poststroke infections [89]. Additionally, cerebral ischemia in rats induced the expression of tumor necrosis factor alpha (TNF- α) and activation of apoptotic pathways in the liver, potentially mediated by hepatic adrenergic signaling [90]. Administration of an unselective β -adrenergic receptor blocker mitigated the effects. Furthermore, an inflammatory response in the liver has been demonstrated in rats 10 and 20 hours after ischemia that could be attenuated when activating a vagus nerve-mediated, efferent cholinergic pathway [91].

1.3.4 Metabolomics and lipidomics in ischemic stroke

Although there has been great progress in the past, a deeper understanding of underlying molecular mechanisms and the search for biomarkers of ischemic stroke is essential for developing new treatment options. Recent studies performing metabolomics and lipidomics analysis provide large amounts of data, focusing not only on the brain but mainly on accessible biofluids like plasma and serum. Metabolite levels in the blood can be directly associated with changes in the brain, although the complexity of metabolite passage through the BBB has to be considered [92]. Additionally, BBB breakdown and described effects of ischemic stroke on the periphery make plasma and serum an interesting research target. Results from metabolomics and lipidomics studies in ischemic stroke patients are, to some extent, consistent. Differences can be caused by the study design, different time points of sample collection or other clinical parameters concerning included patients. Less variation is expected for animals. However, the applied model, time frame, species or genotype influence the observed metabolic alterations. As discussed above, analytical parameters determine the coverage of the metabolome and lipidome.

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Therefore, different publications reporting about different subsets of compounds make a comparison difficult.

Several studies have focused on the role of amino acids in ischemic stroke. Decreased levels of amino acids have been reported in plasma or serum of patients [93-96]. However, there have been findings reporting elevated levels of certain amino acids [96, 97]. In contrast, levels in the brain of MCAO animals have been reported to be increased [98, 99]. Another compound class that has been investigated are glycerophospholipids. However, contradictory results have been reported for phosphatidylcholines (PCs), phosphatidylethanolamines (PEs) and others [100]. PCs and PEs are key components of the phospholipid bilayer of all cell membranes. Degradation can therefore lead to impaired membrane integrity and cell function. Phospholipids also build the outer layer of lipoproteins, transporting hydrophobic lipids like cholesterol or triglycerides (TGs) in the blood. Biosynthetic pathways of PC and PE are demonstrated in **Figure 1.5**.

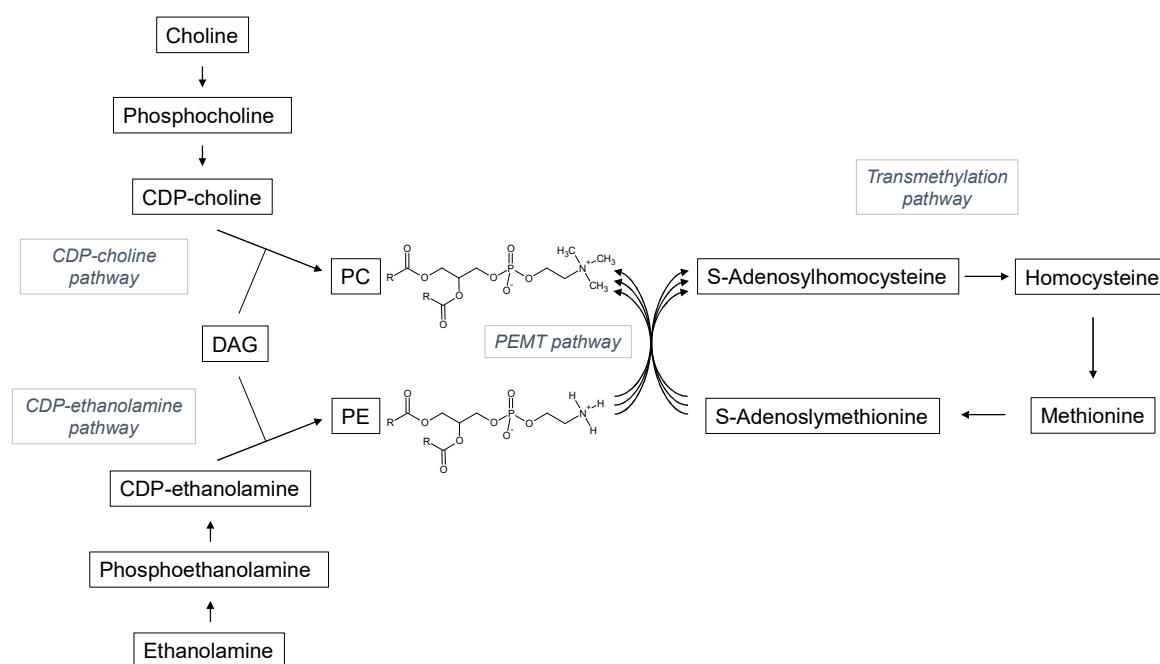


Figure 1.5 Schematic description of biosynthetic pathways of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). PEs can be generated via the CDP-ethanolamine pathway, PCs via the CDP-choline pathway or the PEMT pathway. The methyl donor S-adenosylmethionine is remethylated via the transmethylation pathway. CDP = cytidine diphosphate, DAG = diacylglycerol, PEMT = phosphatidylethanolamine N-methyltransferase.

Via the phosphatidylethanolamine N-methyltransferase (PEMT), PEs are directly metabolized to PCs [101]. The pathway is mainly active in the liver and responsible for converting long-chain polyunsaturated PEs to PCs. It includes a three-time methylation of the ethanolamine moiety of

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PE, with S-adenosylmethionine as methyl donor, resulting in PC and S-adenosylhomocysteine. In contrast, short- or medium-chain PCs are primarily generated by the cytidine diphosphate choline (CDP-choline) pathway, which is active in all cell types [102, 103]. PEMT-derived PCs account for about 30 % of total liver PC synthesis and they are essential for very low density lipoprotein (VLDL) excretion from the liver. However, according to the Human Protein Atlas, expression of PEMT has also been reported in the brain or immune cells like monocytes [104, 105].

Based on promising effects in animal studies, CDP-choline, generically named citicoline, has been proposed as a treatment for ischemic stroke patients [106-108]. It is the essential intermediate for PC synthesis via the CDP-choline pathway. The suggested neuroprotective mechanisms involve not only membrane stabilization by restoring PC brain levels, but also attenuation of glutamate excitotoxicity, oxidative stress, apoptosis and endothelial barrier dysfunction. However, clinical trials have failed to show a positive effect on mortality and recovery of ischemic stroke patients [109].

1.4 Aim of the study

Ischemic stroke and its effects on the brain and periphery are diverse and not yet fully understood. Especially the interaction of the affected brain with other organs, like the liver, has only been investigated in a limited number of studies. Metabolomics and lipidomics can provide new information about changed metabolite levels and potentially affected pathways. However, every study can only cover a subset of compounds. Therefore, our project focused on the development of an untargeted metabolomics screen, using the in-house MS² and LC libraries. Metabolomics and lipidomics profiling of ischemic stroke in human serum was performed to identify compounds and pathways of interest. Our data can provide insights into acute effects following ischemic stroke and into early prediction of post-stroke impairment. The implementation of an MCAO mouse model allowed for the analysis of plasma, brain and liver tissue and excluded confounding clinical parameters. Generating new hypotheses, e.g. in relation to the glycerophospholipid metabolism, could result in a better understanding of ischemic stroke and potentially in new treatment options.

2 Materials

2.1 Chemicals

Chemical

2,2,2-Tribromethanol (avertin)

2-Hydroxyethyl cellulose

2-Methyl-2-butanol

Acetic acid (LC-MS grade)

Acetone

Acetone (HPLC grade)

Acetonitrile (LC-MS grade)

Albumin bovine fraction V (BSA)

Ammonium acetate (LC-MS grade)

Ammonium formate ($\geq 99.995\%$)

Ammonium hydroxide (28 - 30 %)

Ammonium hydroxide $\geq 25\%$ (LC-MS grade)

Bepanthen eye and nose ointment

Bradford reagent

Chloroform (HPLC grade)

Formaldehyde

Formic acid 99 % (LC-MS grade)

Heparin

Hydroquinone

Isopropanol (LC-MS grade)

Lithium carbonate

Manufacturer

Sigma-Aldrich, St. Louis (USA)

Sigma-Aldrich, St. Louis (USA)

Sigma-Aldrich, St. Louis (USA)

Honeywell Speciality Chemicals GmbH (Fluka), Seelze (Germany)

Carl Roth GmbH & Co KG, Karlsruhe (Germany)

Honeywell Speciality Chemicals GmbH (Riedel-de Haën), Seelze (Germany)

Carl Roth GmbH & Co KG, Karlsruhe (Germany)

Merck KGaA, Darmstadt (Germany)

SERVA Electrophoresis GmbH, Heidelberg (Germany)

Sigma-Aldrich, St. Louis (USA)

Sigma-Aldrich, St. Louis (USA)

Thermo Fisher Scientific, Waltham (USA)

Honeywell Speciality Chemicals GmbH (Fluka), Seelze (Germany)

Bayer, Leverkusen (D)

Sigma-Aldrich, St. Louis (USA)

Honeywell Speciality Chemicals GmbH (Riedel-de Haën), Seelze (Germany)

Carl Roth GmbH & Co KG, Karlsruhe (Germany)

Biosolve B.V., Valkenswaard (Netherlands)

Ratiopharm, Ulm (D)

Honeywell Speciality Chemicals GmbH (Fluka), Seelze (Germany)

Honeywell Speciality Chemicals GmbH (Riedel-de Haën), Seelze (Germany)

Honeywell Speciality Chemicals GmbH (Riedel-de Haën), Seelze (Germany)

Materials

Mass Spectrometry Metabolite Library of Standards (MSMLS, Lot# 150-57)	Sigma-Aldrich, St. Louis (USA)
Metabolomics Amino Acid Mix Standard	Cambridge Isotope Laboratories, Andover, MA (USA)
Methanol (LC-MS grade)	Th. Geyer GmbH & Co KG, Renningen (Germany) Merck KGaA, Darmstadt (Germany)
Methyl tert-butyl ether (HPLC grade)	Honeywell Speciality Chemicals GmbH (Riedel-de Haën), Seelze (Germany)
Pierce™ LTQ ESI Negative Ion Calibration Solution	Thermo Fisher Scientific, Waltham (USA)
Pierce™ LTQ Velos ESI Positive Ion Calibration Solution	Thermo Fisher Scientific, Waltham (USA)
Ringer solution	Berlin-Chemie AG, Berlin (D)
Saline (sodium chloride 0.9%)	Berlin-Chemie AG, Berlin (D)
Silver nitrate	Carl Roth GmbH & Co KG, Karlsruhe (Germany)
Sodium citrate	J.T. Baker, Center Valley (USA)
SPLASH® LIPIDOMIX® Mass Spec Standard	Avanti Polar Lipids, Birmingham (USA)
Water (LC-MS grade)	Th. Geyer GmbH & Co KG, Renningen (Germany) Merck KGaA, Darmstadt (Germany)

2.2 Instruments

Instrument	Specification	Manufacturer
Centrifuges	5430R	Eppendorf AG, Hamburg (Germany)
	5810R	Eppendorf AG, Hamburg (Germany)
	Mikro 200R	Andreas Hettich, Tuttlingen (Germany)
Coagulator	ICC 50	Erbe Elektromedizin GmbH, Tübingen (Germany)
Cryotome	CM 3050	Leica Microsystems GmbH, Wetzlar (Germany)
Driller	Micromot 50/EF	Proxxon GmbH, Föhren (Germany)
Electric coagulation forceps	20195-013	Erbe Elektromedizin GmbH, Tübingen (Germany)
Freezers	MediLinie	Liebherr, Biberach an der Riß (Germany)
	HERAfreeze™ HFU T Series	Thermo Fisher Scientific, Waltham (USA)
	KM-DU73Y1 G5	Panasonic Corp., Osaka (Japan)

Materials

High performance liquid chromatography	Dionex Ultimate 3000 HPLC System	Thermo Fisher Scientific, Waltham (USA)
Homogenizer	Bullet Blender STORM BBY 24M	Next Advance, New York (USA)
Infrared lamp	230 V, 100 W, 1500 K Red	Paulmann Licht GmbH, Springe-Völksen (Germany)
Magnetic stirrer	Combimag MR Hei-Tec	IKA®-Werke, Staufen (Germany) Heidolph Instruments GmbH & Co. KG, Schwabach (Germany)
Mass spectrometer	Q-Exactive™ Hybrid Quadrupole-Orbitrap™	Thermo Fisher Scientific, Waltham (USA)
Ion source	Heated-electrospray ionization (HESI-II) probe	Thermo Fisher Scientific, Waltham (USA)
Multipette®	E3X	Eppendorf AG, Hamburg (Germany)
Pipettes	Research/Research plus (10 µl, 100 µl, 200 µl, 1000 µl)	Eppendorf AG, Hamburg (Germany)
Plate reader	CLARIOstar	BMG LABTECH GmbH, Ortenberg (Germany)
Sample concentrator	Savant SPD 111V (SpeedVac concentrator)	Thermo Fisher Scientific, Waltham (USA)
	Savant RVT 5105 (Refrigerated Vapor Trap)	Thermo Fisher Scientific, Waltham (USA)
	DVG50-UNV (Pressure sensor)	Thermo Fisher Scientific, Waltham (USA)
	VLP 120 (Vacuum Pump)	Thermo Fisher Scientific, Waltham (USA)
Scales	ENTRIS® Precision Balance (323I-1S)	Sartorius AG, Göttingen (Germany)
	ENTRIS® Analytical Balance (124I-1S)	Sartorius AG, Göttingen (Germany)
	MC1 LC 220S	Sartorius AG, Göttingen (Germany)
Scanner	Canscan 9000F	Canon, Tokio (Japan)
Stereomicroscope	SM33	Helmit Hund GmbH, Wetzlar (Germany)
Surgery tools		Fine Science Tools, Foster City (USA)

Materials

Temperature control module (with heating plate and rectal probe)	TKM-0904 EHE-3503-Cu-SWCR	FMI Föhr Medical Instruments GmbH (Germany)
Thermoblock	SmartBlock™ 1.5 ml/2.0 ml	Eppendorf AG, Hamburg (Germany)
Thermomixer	ThermoMixer® C	Eppendorf AG, Hamburg (Germany)
Ultrasonic bath	Sonorex Digiplus DL 514 BH	Bandelin electronic, Berlin (Germany)
Vortex	Vortex Genie 2	Scientific Industries, New York (USA)
Water purification system	Milli-Q Reference	Merck KGaA, Darmstadt (Germany)

2.3 Consumables

Consumable	Specification	Manufacturer
96-Well plates	Microplate 96/U Costar Assay Plate, flat bottom	Eppendorf AG, Hamburg (Germany) Corning Inc., Corning (USA)
96-Well plate lids	Microplate Sealing Films & Foils, self-adhesive	Eppendorf AG, Hamburg (Germany)
Blades	Leica 818 High profile Microtome Blades	Leica Microsystems GmbH, Wetzlar (Germany)
Butterfly cannula	Safety-Multifly 21 G	Sarstedt, Nümbrecht (Germany)
Filtering paper	Whatman	GE Healthcare Life Sciences, Little Chalfont (GB)
Glas vials	QsertVial™ 0.3 ml MRQ30™ 1.2 ml	Sigma-Aldrich, St. Louis (USA) Sigma-Aldrich, St. Louis (USA)
Liquid chromatography columns	Accucore C18 (2.6 µm, 100 x 2.1 mm) Accucore C30 (2.6 µm, 150 x 2.1 mm) Accucore HILIC (2.6 µm, 100 x 2.1 mm) Acquity UPLC BEH C18 (1.7 µm, 100 x 2.1 mm) Ascentis Express C18 (5 µm, 100 x 2.1 mm)	Thermo Fisher Scientific, Waltham (USA) Thermo Fisher Scientific, Waltham (USA) Thermo Fisher Scientific, Waltham (USA) Waters Corporation, Milford (USA) Supelco Inc., Bellefonte (USA)

Materials

	Ascentis Express ES-CN (2.7 µm, 100 x 2.1 mm)	Supelco Inc., Bellefonte (USA)
	Ascentis Express F5 (2.7 µm, 100 x 2.1 mm)	Supelco Inc., Bellefonte (USA)
	Ascentis Express Phenyl-Hexyl (2.7 µm, 100 x 2.1 mm)	Supelco Inc., Bellefonte (USA)
	Cortecs T3 (2.7 µm, 100 x 2.1 mm)	Waters Corporation, Milford (USA)
	Hypercarb (5 µm 150 x 2.1 mm)	Thermo Fisher Scientific, Waltham (USA)
	SeQuant ZIC-HILIC (5 µm, 150 x 2.1 mm)	Merck KGaA, Darmstadt (Germany)
	X-Bridge BEH Amide (2.5 µm, 100 x 2.1 mm)	Waters Corporation, Milford (USA)
Liquid chromatography guard columns	Accucore C30 Guard (2.6 µm, 10 x 2.1 mm) with universal uniguard holder for 2.1/3.0 mm ID	Thermo Fisher Scientific, Waltham (USA)
	SeQuant ZIC-HILIC Guard Kit (20 x 2.1 mm)	Merck KGaA, Darmstadt (Germany)
Microvette®	CB300 EDTA	Sarstedt, Nümbrecht (Germany)
Multipipette tips	Combitips advanced (0.5 ml/1 ml/ 5 ml / 10 ml)	Eppendorf AG, Hamburg (Germany)
Nitrile gloves	Peha-soft nitrile FI-NO Nitril Next Gen	Hartmann, Heidenheim (Germany) Meditrade GmbH, Kiefersgelden (Germany)
Object slides	Superfrost Plus	Thermo Fisher Scientific, Waltham (USA)
Pipette tips	10 µl/ 200 µl/ 1000 µl epT.I.P.S.® Standard, 2 - 200 µl/50 - 1000 µl	Sarstedt, Nümbrecht (Germany) Eppendorf AG, Hamburg (Germany)
Polypropylene tubes	CELLSTAR® 15 ml	Greiner Bio-One International GmbH, Kremsmünster (Austria)
Reaction tubes	1.5 ml/2 ml	Sarstedt, Nümbrecht (Germany)

Materials

	Safe-Lock Tubes 1.5 ml/2 ml	Eppendorf AG, Hamburg (Germany)
Scalpels	Typ 10/11/22	Feather, Osaka (Japan)
S-Monovette®	K3 EDTA, 2.7 ml	Sarstedt, Nümbrecht (Germany)
Suture material	H4F Seide, USP-3-0	RESORBA, Nürnberg (Germany)
Syringes	1 ml/50 ml	BD, Franklin Lakes (USA)
Vinyl gloves	Vinyl 2000 PF	Meditrade GmbH, Kiefersgelden (Germany)
Zirconium oxide beads	ZrOB05 (0.5 mm)	Next Advance, New York (USA)

2.4 Software

Software	Version	Manufacturer
Compound Discoverer	2.1/3.1	Thermo Fisher Scientific, Waltham (USA)
FreeStyle	1.1/1.6	Thermo Fisher Scientific, Waltham (USA)
Image J	1.48v	<i>Open Source Project</i>
Library manager	2.0	Thermo Fisher Scientific, Waltham (USA)
mzVault	2.3	Thermo Fisher Scientific, Waltham (USA)
Office365		Microsoft, Redmond (USA)
Prism	5.0/8.0	GraphPad, La Jolla (USA)
SPSS Statistics	25	IBM, Armonk (USA)
Thermo Xcalibur™	4.2/4.3	Thermo Fisher Scientific, Waltham (USA)
TraceFinder	3.2/4.0/5.0	Thermo Fisher Scientific, Waltham (USA)

3 Methods

3.1 MS² library generation

3.1.1 Standard compounds

For the MS² library generation, synthetic standard compounds from the Mass Spectrometry Metabolite Library of Standards (MSMLS, Sigma Aldrich) were used. The MSMLS contains 608 small molecule metabolites, provided in seven 96-deep well plates with 5 µg per well. Methanol (15 µl) and water (285 µl) were added to every well, lipid-like compounds (Plate 7) were dissolved in 300 µl chloroform:methanol:water (1:1:0.3, v/v/v). A final concentration of 16.7 µg/ml per metabolite stock solution was reached. Stock solutions were diluted 1:10 in 96-well plates with the corresponding solvent mixture (methanol:water (5:95, v/v) or chloroform:methanol:water (1:1:0.3, v/v/v)) before injection. Higher concentrated solutions for repeated injection were diluted 1:2 or 1:1 in glass vials.

3.1.2 Instrument method and software

Flow injection of 25 µl stock solution was performed in positive and negative ionization mode for 8 min using 0.02 ml/min of water:methanol (50:50, v/v) with 0.1 % formic acid. Targeted selected ion monitoring followed by data-dependent MS² (t-SIM/dd-MS², Top 10) was performed for normalized collision energies (NCEs) of 10, 30, 50, 75, 100 % and stepped NCE (10/50/100 %). Inclusion lists contained 10 masses per instrument method. Instrument settings are shown in **Table 3.1**. Raw data was processed using FreeStyle 1.1 and manually curated by choosing high-quality MS² spectra. The MS² library was administrated using Library Manager 2.0 and mzVault 2.3.

Table 3.1 Instrument settings for MS² library generation. Settings for electrospray ionization (ESI) source, targeted selected ion monitoring (t-SIM) and data-dependent MS² (dd-MS²). Sugar like compounds were fragmented with different ionization settings. RF level = radio frequency level, AGC = automatic gain control, IT = injection time, NCE = normalized collision energy.

ESI source	General settings	Sugar like compounds
Sheat gas flow rate	20	20
Auxiliary gas flow rate	5	5
Spray voltage [kV]	4.5 (+ve mode) 3.5 (-ve mode)	4 (+ve mode)
Capillary temp. [°C]	300	320
S-lens RF level	75	30
Auxiliary gas heater temp. [°C]	-	200

Methods

t-SIM	
Resolution	70,000
AGC target	1.00E+05
Maximum IT [ms]	200
Loop count	10
Isolation window	1.0 m/z
dd-MS²	
Resolution	17,500
AGC target	1.00E+05
Maximum IT [ms]	200
Loop count	10 (= Top10)
Isolation window	0.4 m/z
NCE [%]	10 30 50 75 100 10/50/100
dd-settings	
Minimum AGC target	1.00E+03
Exclude isotopes	on

3.2 LC library generation and method development

A metabolite standard mix (MSM), with all MSMLS stock solutions added in equal volumes, was used for analysis. The MSM was dried under vacuum and reconstituted in either methanol, methanol:water (1:1, v/v) or methanol:acetonitrile (1:1, v/v), resulting in 167 ng/ml of every standard. Separation of compounds was performed under different LC conditions. Details are listed in **Table S6.1**. Full scan followed by data-dependent MS² scans (Full MS/dd-MS², Top 10) for positive and negative ionization mode was applied for detection. Instrument settings are shown in **Table S6.2**. TraceFinder 3.2 was used for data processing. The compound database for peak detection included all masses from the MS² library (**Table S6.3**). Isomeric compounds and duplicates were excluded, resulting in 300 compounds tested for the LC library. Exact mass ($\Delta\text{ppm} < 5 \text{ ppm}$) and isotopic pattern for identification and matching fragmentation with the MS² library for confirmation of metabolites was adduced. Chromatographic peak quality was defined by individual scores (sensitivity, peak width, peak asymmetry, retention) and a total score (mean of individual scores), calculated for each confirmed compound (**Table 3.2**).

Methods

Table 3.2 Calculation and score system for the LC library. *S/N* = signal-to-noise ratio, *RT* = retention time, *t₀* = *RT* of non-retained compound. The total score for a compound equals the mean of all individual scores.

Score	Sensitivity	Peak width	Peak asymmetry (fronting/tailing)	Retention (retention factor <i>k</i>)
	<i>S/N ratio</i>	ΔRT	$A_s = \frac{right\ RT - RT}{RT - left\ RT}$	$k = \frac{t_R - t_0}{t_0}$
1	≥ 50000	≤ 0.5	0.85 - 1.2	2 - 5
2	5000 - 49999	0.51 - 1	0.7 - 0.84 1.21 - 1.5	1 - 1.99 5.01 - 10
3	500 - 4999	1.1 - 1.5	0.5 - 0.69 1.51 - 2.0	0.5 - 0.99 10.01 - 15
4	50 - 499	1.51 - 2	0.25 - 0.49 2.1 - 4.0	0.2 - 0.49 15.01 - 20
5	< 50	> 2	< 0.25 > 4	< 0.2 > 20
∅ = total score				

The mean total score for certain LC conditions represents the average score from all confirmed compounds. When combining data from positive and negative ionization mode, the lower score was selected for duplicate compounds. Chemical properties of metabolites (octanol/water coefficient (log *P*), physiological charge, *pK_a*, all calculated by ChemAxon (www.chemaxon.com)) were obtained from HMDB. Venn diagrams were created using BioVenn [110]. The chromatographic separation of 83 isomeric compounds was tested for five different LC methods (RP 4, 5, 6, 19; HILIC 19; **Table S6.1**). Likely separable isomers were selected based on acquired chromatograms of the MSM. Stock solutions from the MSMLS were diluted 1:10 with methanol:water (1:1, v/v) and methanol:acetonitrile (1:1, v/v) for RP and HILIC, respectively. We analyzed the individual isomers to confirm chromatographic separation and determine the retention factor (**Table S6.4**). If successful, identified isomers in the MSM were included in the LC library for the tested methods. MSM spiked human plasma samples for method development were extracted as described in chapter 3.3.1.

3.3 Metabolomics analysis

3.3.1 Metabolite extraction protocol

Metabolite extraction was performed by adding 400 µl acetone:acetonitrile:methanol (1:1:1, v/v/v) containing a mixture of internal standards (**Table 3.3**) to 100 µl plasma, serum or tissue homogenate [25, 111]. Samples were vortexed, mixed (15 min/4 °C) and incubated (2 h/-20 °C) to

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allow protein precipitation. Supernatant was collected after centrifugation (14,000 rpm/10 min/4 °C) and evaporated under vacuum. Dried extracts were reconstituted in 50 µl of methanol:acetonitrile (1:1, v/v). Samples were centrifuged again (14,000 rpm/10 min/4 °C) and supernatants were transferred to glass vials. Instead, MSM spiked plasma samples for method development were reconstituted in MSM, either dissolved in methanol:acetonitrile (1:1, v/v) for HILIC or methanol:water (1:1, v/v) for RP-LC.

Table 3.3 ¹³C- and ¹⁵N-labeled internal standards for metabolomics screen.

Metabolomics Amino Acid Mix (Cambridge Isotope Laboratories)	Exact mass	m/z	Adduct	Concentration per sample [µM]
Glycine (¹³ C ₂ ¹⁵ N)	78.03582	79.0431	[M+H]	2.5
L-Alanine (¹³ C ₃ ¹⁵ N)	93.05482	94.0621	[M+H]	2.5
L-Serine (¹³ C ₃ ¹⁵ N)	109.04968	108.0424	[M-H]	2.5
L-Aspartate (¹³ C ₄ ¹⁵ N)	138.04798	137.0407	[M-H]	2.5
L-Threonine (¹³ C ₄ ¹⁵ N)	124.06868	123.0614	[M-H]	2.5
L-Valine (¹³ C ₅ ¹⁵ N)	123.09282	124.1001	[M+H]	2.5
L-Methionine (¹³ C ₅ ¹⁵ N)	155.06482	156.0721	[M+H]	2.5
L-Proline (¹³ C ₅ ¹⁵ N)	121.07712	122.0844	[M+H]	2.5
L-Glutamic acid (¹³ C ₅ ¹⁵ N)	153.06692	154.0742	[M+H]	2.5
L-Cystine (¹³ C ₆ ¹⁵ N ₂)	248.03808	247.0308	[M-H]	1.25
Leucine (¹³ C ₆ ¹⁵ N)	138.11182	139.1191	[M+H]	2.5
L-Isoleucine (¹³ C ₆ ¹⁵ N)	138.11182	139.1191	[M+H]	2.5
L-Lysine (¹³ C ₆ ¹⁵ N ₂)	154.11972	155.127	[M+H]	2.5
L-Arginine (¹³ C ₆ ¹⁵ N ₄)	184.11992	185.1272	[M+H]	2.5
L-Histidine (¹³ C ₆ ¹⁵ N ₃)	164.08068	163.0734	[M-H]	2.5
L-Phenylalanine (¹³ C ₉ ¹⁵ N)	175.10622	176.1135	[M+H]	2.5
L-Tyrosine (¹³ C ₉ ¹⁵ N)	191.10112	192.1084	[M+H]	2.5

3.3.2 Metabolomics screen

An extraction blank (100 µl LC-MS water) and quality controls (QCs) were included in every analysis. For that purpose, all samples of an experiment were pooled in equal volumes and aliquots were used for every batch. The QC was diluted before and after the extraction with water or methanol:acetonitrile (1:1, v/v), respectively (**Table 3.4**). The extraction protocol was performed for extraction blanks, QCs and samples as described in 3.3.1.

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Table 3.4 Dilution of quality controls (QCs) for metabolomics screen. Dilution of QC before extraction and after extraction.

Step	QC [μ l]	LC-MS water [μ l]	Relative concentration
1	10	90	0.1
2	20	80	0.2
3	50	50	0.5
4	80	20	0.8
5	100	-	1

Step	QC extracted [μ l]	Methanol/acetonitrile (1:1)	Relative concentration
1	5	45	0.1
2	10	40	0.2
3	25	25	0.5
4	40	10	0.8
5	50	-	1

Extracted compounds were separated on a SeQuant ZIC-HILIC column (5 μ m, 200 Å, 150 x 2.1 mm) equipped with a corresponding Guard Kit (20 x 2.1 mm). We used water with 5 mM ammonium acetate (= eluent A) and acetonitrile:eluent A (95:5, v/v) (= eluent B) as mobile phases or the same eluents with 0.1 % formic acid. The gradient elution was set as follows: isocratic step of 100 % B for 3 min, 100 % B to 60 % B in 15 min, held for 5 min, returned to initial conditions in 5 min and held for 5 min. Flow rate was 0.5 ml/min, injection volume was 5 μ l, column temperature was maintained at 35 °C. Data was acquired using Full MS/dd-MS² (Top 10) in positive and negative ionization mode via polarity switching. Instrument settings are listed in **Table 3.5**.

Table 3.5 Instrument settings for metabolomics screen. Settings for electrospray ionization (ESI) source, Full MS and data-dependent MS² (dd-MS²). RF level = radio frequency level, AGC = automatic gain control, IT = injection time, NCE = normalized collision energy.

ESI source	General settings
Sheat gas flow rate	30
Auxiliary gas flow rate	10
Spray voltage [kV]	3.5 (+ve mode) 3.0 (-ve mode)
Capillary temp. [°C]	320
S-lens RF level	75
Auxiliary gas heater temp. [°C]	50
Full MS	
Mass range	60 – 900 m/z
Resolution	70,000

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AGC target	3.00E+06
Maximum IT [ms]	200
dd-MS²	
Resolution	17,500
AGC target	5.00E+05
Maximum IT [ms]	50
Loop count	10 (= Top 10)
Isolation window	1.0 m/z
Stepped NCE [%]	10/50/100
dd-settings	
Minimum AGC target	2.50E+02
Dynamic exclusion [s]	20
Exclude isotopes	on

3.3.3 Data processing

Compound Discoverer 2.1 was used for data processing in an untargeted workflow (**Figure S6.1**). Identification was based on exact mass ($\Delta\text{ppm} < 5$), retention time and matching the fragmentation with the MS² library and mzCloud. Raw areas under the curve were normalized to the internal standards (**Table 3.3**). Additionally, we performed a QC based normalization for signal drift correction, as reported before [112]. The non-diluted QC sample was measured at least every 10th sample and at the beginning and end of a batch. Linear regression was used to calculate a correction factor F . For a compound c each sample had a different correction factor F_c according to its run order R as defined by equation (C):

$$F_c = \beta R + \alpha \quad (C)$$

where β and α are the slope and the intercept of the regression line, respectively. For inter-batch comparability, corrected areas were divided by the mean of corrected areas of QCs per batch, if necessary. Additionally, QCs were used to assure linearity and stability of the signal for each confirmed compound.

3.4 Lipidomics analysis

3.4.1 Lipid extraction protocol

Lipid extraction was performed by adding 1000 μl methanol/methyl tert-butyl ether/chloroform (1.33:1:1, v/v/v) containing butylated hydroxytoluene (100 mg/l) and SPLASH® LIPIDOMIX® Mass Spec Standard (2.5 ml/l, **Table 3.6**), to 50 μl of plasma, serum or tissue homogenate.

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Table 3.6 Deuterium-labeled internal standards for lipidomics screen. CE = cholesterylester, DAG = diacylglyceride, LPC = lysophosphatidylcholine, LPE = lysophosphatidylethanolamine, MAG = monoacylglyceride, PA = phosphatidic acid, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PI = phosphatidylinositol, PS = phosphatidylserine, SM = sphingomyelin, TG = triacylglyceride.

SPLASH Lipidomix (Avanti)	Exact mass	m/z	Adduct	Concentration per sample [ng]
CE 18:1(d7)	657.6436	675.6779	[M+NH ₄]	875
Cholesterol (d7)	393.3988	394.4061	[M+H]	250
DAG 15:0-18:1(d7)	587.5501	605.5844	[M+NH ₄]	25
LPC 18:1(d7)	528.3921	529.3994	[M+H]	62.5
LPE 18:1(d7)	486.3451	487.3524	[M+H]	12.5
MAG 18:1(d7)	363.3366	364.3439	[M+H]	5
PA 15:0-18:1(d7)	667.5164	685.5508	[M+NH ₄]	17.5
PC 15:0-18:1(d7)	752.6061	753.6134	[M+H]	400
PE 15:0-18:1(d7)	710.5591	711.5664	[M+H]	12.5
PG 15:0-18:1(d7)	741.5537	740.5465	[M-H]	75
PI 15:0-18:1(d7)	829.5698	828.5625	[M-H]	25
PS 15:0-18:1(d7)	754.5490	755.5563	[M+H]	12.5
SM 18:1(d9)	737.6397	738.6470	[M+H]	75
TG 15:0-18:1(d7)-15:0	811.7641	829.7985	[M+NH ₄]	137.5

Samples were vortexed and mixed for 30 min at 25 °C. After centrifugation (2,000 g/10 min/20 °C) supernatant was collected and evaporated under vacuum. Dried extracts were reconstituted in 50 µl methanol:isopropanol (1:1, v/v), centrifuged (14,000 rpm/10 min/20 °C) and the supernatant was transferred into a glass vial.

3.4.2 Lipidomics screen

An extraction blank (50 µl LC-MS water) and QCs were extracted with every batch as mentioned in chapter 3.3.2. Dilution patterns are specified in **Table 3.7**. The extraction protocol was performed for extraction blanks, QCs and samples as described in chapter 3.4.1.

Table 3.7 Dilution of quality controls (QCs) for lipidomics screen. Dilution of QC before extraction and after extraction.

Step	QC [µl]	LC-MS water [µl]	Relative concentration
1	5	45	0.1
2	10	40	0.2
3	25	25	0.5
4	40	10	0.8
5	50	-	1

Methods

Step	QC extracted [μ l]	Methanol/isopropanol (1:1)	Relative concentration
1	5	45	0.1
2	10	40	0.2
3	25	25	0.5
4	40	10	0.8
5	50	-	1

Extracted compounds were separated on an Accucore C30 column (2.6 μ m, 150 Å, 150 mm x 2.1 mm) equipped with a corresponding guard column (2.6 μ m, 10 mm x 2.1 mm). Acetonitrile:water (6:4, v/v) (= eluent A) and isopropanol:acetonitrile (9:1, v/v) (= eluent B), both with 10 mM ammonium acetate and 0.1 % formic acid, were used. The following gradient was applied: from 30 – 43 % B in 2.0 min, 43 – 55 % B in 0.1 min, to 65 % B in 9.9 min, to 85 % B in 6 min and to 100 % B in 2 min. An isocratic step of 100 % B was held for 15 min and returned to initial conditions in 0.1 min and held for 2.9 min. Flow rate was 0.26 ml/min, injection volume was 5 μ l, column temperature was maintained at 45 °C. Data was acquired using a Full MS/dd-MS² (Top 15) experiment in positive and negative ionization mode via polarity switching. Instrument settings are listed in **Table 3.8**.

Table 3.8 Instrument settings for lipidomics screen. Settings for electrospray ionization (ESI) source, Full MS and data-dependent MS² (dd-MS²). RF level = radio frequency level, AGC = automatic gain control, IT = injection time, NCE = normalized collision energy.

ESI source	General settings
Sheat gas flow rate	40
Auxiliary gas flow rate	10
Spray voltage [kV]	3.5 (+ve mode) 3.2 (-ve mode)
Capillary temp. [°C]	320
S-lens RF level	100
Auxiliary gas heater temp. [°C]	320
Full MS	
Mass range	250 – 3000 m/z
Resolution	70,000
AGC target	3.00E+06
Maximum IT [ms]	200
dd-MS ²	
Resolution	17,500
AGC target	1.00E+05
Maximum IT [ms]	50
Loop count	15 (= Top 15)
Isolation window	1.0 m/z
Stepped NCE [%]	10/30/50

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dd-settings	
Minimum AGC target	5.00E+03
Dynamic exclusion [s]	20
Exclude isotopes	on

3.4.3 Data processing

Data were processed by using TraceFinder 3.2, 4.0 and 5.0. Lipids were identified by exact mass ($\Delta\text{ppm} < 5$), the isotopic pattern, retention time and by matching of specific MS^2 fragments to an in-house database. The area under the peak was normalized to the corresponding internal standard (**Table 3.6**). The QCs were used to assure linearity and stability of the signal of each confirmed compound.

3.5 Human samples

3.5.1 Human plasma for method development

After collection in EDTA containing tubes, whole blood was centrifuged (2,500 g/10 min) at room temperature within 30 min. The plasma of all volunteers was pooled, aliquoted and stored at -80°C .

3.5.2 Human serum cohort (Heidelberg)

After approval of the study by the ethical committee of the medical faculty of the University of Heidelberg, the collection of clinical data and blood took place upon informed consent by the patient or legal guardian following the Declaration of Helsinki. Under the direction of Dr. Oliver Herrmann (Department of Neurology, University of Heidelberg), patients with an acute ischemic stroke were included in the study. Patients without evidence for stroke from an ophthalmological ward in the same hospital were included as a control group. Blood samples from all patients were collected on admission to the hospital and from a subset of stroke patients also on two consecutive days. The ethical committee of the University of Lübeck approved the investigation of serum samples. Control and stroke patients were matched for age, gender, and diabetic status before the analysis.

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3.5.3 Human serum cohort (Lübeck)

After approval of the study by the ethical committee of the University of Lübeck, the collection of clinical data and blood took place upon informed consent by the patient or legal guardian following the Declaration of Helsinki. Under the direction of Prof. Georg Roysl (Department of Neurology, University of Lübeck), patients with an acute ischemic stroke were included in the study. Blood samples were collected on admission to the hospital.

3.6 Animal experiments

All following experiments were conducted by Zaib A. Shaheryar and Ines Stölting, both members of the Institute for Experimental and Clinical Pharmacology and Toxicology at the University of Lübeck.

3.6.1 Mouse line and animal housing

All animal experiments were performed according to the guidelines of the *Ministerium für Energiewende, Landwirtschaft, Umwelt und ländliche Räume des Bundeslandes Schleswig-Holstein*. Mice with a genetic C57BL/6N background were obtained from JANVIER LABS (France). If not mentioned otherwise, animals were housed in groups of three to five. They had free access to water and food (#1314, Altromin Spezialfutter GmbH & Co. KG) and were held under a 12 hour circadian rhythm. At the time of the experiment, animals were approximately 8 weeks old. Both males and females were included.

3.6.2 Permanent distal middle cerebral artery occlusion

A permanent distal middle cerebral artery (arteria cerebri media) occlusion (MCAO) was performed on mice to induce cerebral ischemia. Animals were anaesthetized with an intraperitoneal (i.p.) injection of 15 - 20 µl/g bodyweight of 1.5 % avertin (dissolved in 2-methyl-2-butanol). Under full anaesthesia, animals were fixed under a stereomicroscope and the eyes were protected with an ointment against dehydration. To avoid changes in body temperature during the operation, animals were put on a heating plate (37 °C) and temperature was controlled with a rectal probe. For the MCAO, an incision of about 2 cm was made between left eye and ear. The underlying tissue was treated with saline to keep it moist and cool. The musculus temporalis was removed using electric coagulation forceps. The exposed cranial bone above the arteria cerebri media was thinned out by careful trepanation until only a thin lamella was left. The lamella

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was taken out cautiously to avoid bleeding. After removing the meninges, the arteria cerebri media was occluded at three positions using the electric coagulation forceps at the lowest voltage level. The blocked blood flow was visually controlled and the lesion was closed with a skin suture. During the anaesthetic recovery, animals were protected from hypothermia by an infrared lamp. Blood collection and transcardial perfusion were performed after 2 hours or 24 hours. In case of death during the experiment, animals were excluded from all analyses.

3.6.3 Plasma collection, transcardial perfusion and organ harvesting

At 2 hours or 24 hours after MCAO, mice were anaesthetized with 1.5 % avertin as described above and the thoracic cavity was opened. Whole blood was collected by cardiac puncture from the right ventricle and transferred to an EDTA containing tube. After centrifugation (14,000 rpm/5 min/4 °C), the supernatant (plasma) was collected, snap-frozen in liquid nitrogen and stored at -80 °C. A butterfly cannula was inserted into the left ventricle and the right atrium was opened with a small incision. The animal was slowly perfused with 20 ml of Ringer solution containing 10 U/ml heparin. Liver tissue was collected in a reaction tube and snap-frozen in liquid nitrogen. The brain was harvested and frozen on dry ice to avoid deformation. All organs were stored at -80 °C.

3.6.4 Brain sectioning

Brain sectioning was performed using a cryotome at -20 °C. The brain was fixed with the cerebellum at the cutting block and covered with 1.5 % 2-hydroxyethyl cellulose. Coronal sections (20 µm) were obtained in triplicates from the olfactory bulb to the cerebellum with a distance of 400 µm. After transfer to object slides, dried sections were stored at -20 °C for histological analyses. The intermediate tissue slices were separated into left and right hemisphere and collected in separate reaction tubes for homogenization and MS analysis.

3.6.5 Infarct size measurement

The ischemic tissue was determined by a high-contrast silver infarct staining [113]. The impregnation solution was prepared by mixing aqueous solutions of lithium carbonate and silver nitrate. Ammonium hydroxide was added until the emerging precipitant was redissolved. Finally, distilled water (aqua dest.) was added. The solution was filtered and protected from light. The developing solution was prepared by mixing filtered formaldehyde and a sodium citrate solution.

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Hydroquinone and acetone were added and the solution was stirred at room temperature for 30 – 60 min.

Table 3.9 Composition of impregnation solution for silver staining. Volume for approximately one cohort of MCAO brains.

<i>Impregnation solution</i>		
Chemical	Concentration	Volume
Silver nitrate	0.1 g/ml	33.75 ml
Lithium carbonate	0.012 g/ml	67.5 ml
Ammonium hydroxide	25 %	Approx. 3.6 ml
Aqua dest.		506.25 ml

Table 3.10 Composition of developing solution for silver staining. Volume for approximately one cohort of MCAO brains.

<i>Developing solution</i>		
Chemical	Concentration	Volume
Sodium citrate	0.016 g/ml	420 ml
Formaldehyde	37 %	120 ml
Hydroquinone		1.8 g
Acetone		90 ml

Brain sections were incubated in the impregnation solution for 2 min. After washing (3 x 1 min) with deionized water, they were incubated in the developer solution for 1 – 3 min. After washing (3 x 1 min) with deionized water, sections dried overnight at room temperature. Stained brain sections were scanned and areas of right hemisphere (contralateral), left hemisphere (ipsilateral) and silver deficit (infarct area) were measured. To consider the edema, the Swanson corrected infarct area per brain slice (Swanson area) and Swanson corrected infarct size (Swanson infarct) per animal were calculated as follows [114]:

$$\text{Swanson area [mm}^2\text{]} = \text{right hemisphere} + \text{silver deficit} - \text{left hemisphere} \quad (D)$$

$$\text{Swanson infarct [mm}^2\text{]} = \sum \text{Swanson area} \times 0.4 \text{ mm} \quad (E)$$

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3.6.6 Latency to move test

A latency to move test was performed before and 24 hours after the MCAO. One day before the experiment, mice were single-housed. On the day of the test, the mouse was placed in the middle of a circular plate and time was recorded until it moved out of a defined area (diameter = 14 cm, measured distance = 7 cm, which is equivalent to an average body length). The test was repeated three times. If 30 seconds were exceeded, the recording was stopped. The ratio of the time taken after MCAO (average of three recordings) and the time taken before MCAO (average of three recordings) was used for analysis.

3.7 Sample preparation and protein determination

Frozen liver tissue (50 – 150 mg) was collected in a reaction tube containing a similar amount of zirconium oxide beads and 500 µl LC-MS grade water. Liver tissue was homogenized for 3 min at speed level 8 (out of 12) with an homogenizer and centrifuged (2,000 g/10 min/4 °C). The supernatant (400 µl) was transferred into a new reaction tube. Homogenates were diluted accordingly to reach a protein concentration between 0.4 mg/ml and 1.25 mg/ml. Zirconium oxide beads and 500 µl of LC-MS grade water were added to the collected brain slices (chapter 3.6.4). Homogenization at speed level 8 for 3 min was followed by centrifugation (2,000 g/10 min/4 °C). The supernatant (400 µl) was transferred into a new reaction tube and diluted 1:10. Protein content of diluted homogenates was determined using the Bradford reagent following a 96-well plate assay protocol. Protein standards ranging from 0.4 – 1.25 mg/ml were prepared from a bovine serum albumin (BSA) standard solution (10 mg BSA/ml, **Table 3.11**).

Table 3.11 Standard solutions for protein determination.

Protein content [mg/ml]	1.25	1
10 mg/ml BSA [µl]	50	50
H ₂ O [µl]	350	450

Protein content [mg/ml]	0.8	0.6	0.4
1 mg/ml BSA [µl]	80	60	40
H ₂ O [µl]	20	40	60

Protein standards or samples (5 µl) were added to separate wells in a 96-well plate (flat bottom). The Bradford Reagent (250 µl) was added and the absorbance at 595 nm was measured after 10 min. Absorbance (blank corrected) of protein standards was plotted against the protein

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content to determine the protein content of samples. Relative peak areas obtained from the metabolomics and lipidomics analysis of tissue samples were normalized to the absorbance.

3.8 Statistics

Sample size (n) and the applied statistical test is stated under the respective graph. Significance was assumed when the p-value was less than 0.05, if not stated otherwise.

Pathway analyses were performed with MetaboAnalyst 4.0 [115, 116], referring to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway library for homo sapiens [5]. Pathway over representation analysis (ORA) was performed based on the Small Molecule Pathway Database (SMPDB) for normal human metabolic pathways [117]. ORA was implemented using the hypergeometric test to evaluate whether a particular metabolite set is represented more than expected by chance within the given compound list.

For human serum samples, technical outliers were determined based on the Z-score, with a data point x , the mean level of a compound in all samples $\bar{x}$ and the corresponding standard deviation σ (equation F).

$$Z = \frac{x - \bar{x}}{\sigma} \quad (F)$$

In case of $Z \geq 4.5$ or $Z \leq -4.5$, the data point was excluded. Statistical analyses were performed using MetaboAnalyst 4.0 and 5.0 and GraphPad Prism 5.0 and 8.0. Missing values were replaced with the median of all samples for the implementation of MetaboAnalyst. Features with over 20 % missing values were excluded. To correct for multiple testing, the p-value was adjusted for the false discovery rate (FDR) by the Benjamin-Hochberg procedure [118]. For the comparison of two groups, non-parametric testing (Wilcoxon Mann Whitney test) was applied as data were often not normally distributed. To compare three groups, a Kruskal-Wallis test with a Dunn's post hoc test was performed. Data from consecutive days were analyzed with one way repeated-measures analysis of variance (ANOVA). Cluster analysis was based on Pearson distance measure and Ward clustering algorithm. A Friedman test and Dunn's post hoc test were performed for a detailed analysis of paired samples. Two groups at different time points were analyzed with a two-way repeated measures ANOVA, using the Geisser-Greenhouse correction to adjust for lack of sphericity, and the Sidak's post test.

Sparse partial least squares regression discriminant analysis (sPLS-DA) was performed considering five components and 10 variables per component. Receiver operating characteristics (ROC-)

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models were computed with a linear support vector machines (SVM) algorithm and 100 cross validations were performed for a smooth curve.

All mouse data (chapter 4.3) were analyzed without an outlier test, if not stated otherwise. The comparison of two groups was performed with a paired or unpaired Student's t-test. FDR adjustment was conducted when appropriate. For comparing more than two groups, a two-way repeated measures ANOVA with Sidak's post test was used. Correlation analysis was based on a Pearson correlation.

4 Results

Parts of the following results have been published before in the article *“MS² and LC libraries for untargeted metabolomics: Enhancing method development and identification confidence”* by Folberth et al. in 2020 [119].

4.1 Metabolomics screen

LC-MS/MS is the method of choice for untargeted metabolomics. However, metabolite identification remains one of the main challenges. Development and implementation of a fast workflow for experimental in-house MS² library generation improved identification confidence. Additionally, we generated an LC library to develop a metabolomics screening method with maximum metabolome coverage.

4.1.1 Generation of an in-house MS² library

We developed a method to facilitate the generation of experimental in-house MS² libraries from pure synthetic standards (**Figure 4.1 A**). We used a flow injection workflow to circumvent manual injection and a targeted selected ion monitoring followed by data-dependent fragmentation (t-SIM/dd-MS²). Acquired spectra were manually curated and selected for the MS² library.

The used Mass Spectrometry Metabolite Library of Standards (MSMLS) provides a broad assortment of small, primary metabolites in a convenient 96-well plate format. According to HMDB, almost 90 % of the MSMLS metabolites have been quantified, detected or are expected in biological matrices, 83 % of them in blood, confirming the biological relevance of the library (**Figure 4.1 B, C**).

Results

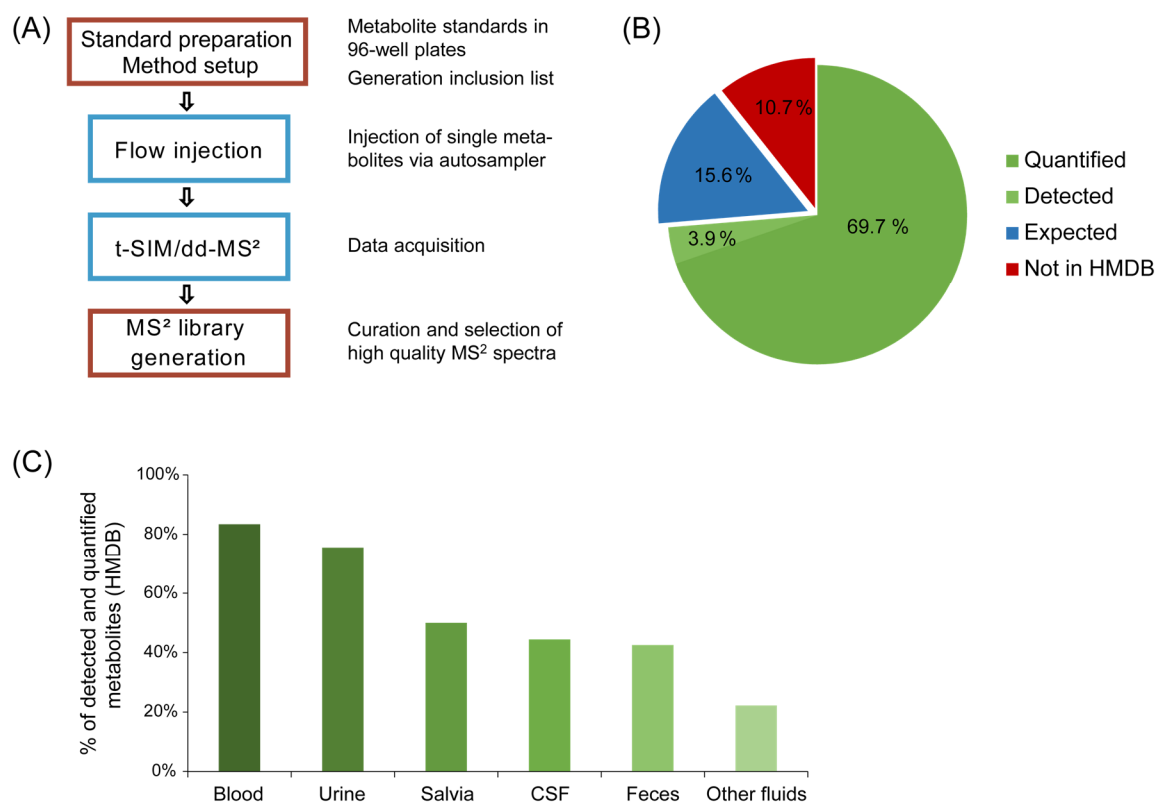


Figure 4.1 MS² library generation from synthetic standards. (A) Flow injection/targeted SIM/data-dependent MS² (t-SIM/dd-MS²) workflow. Manually conducted steps are displayed with a red frame, automated steps with a blue frame. (B) Biological relevance of the Mass Spectrometry Metabolite Library of Standards (MSMLS), data acquired from the Human Metabolome Database (HMDB). Almost 90 % of the included metabolites have been quantified, detected or are expected in biological matrices. (C) Occurrence of detected and quantified metabolites in different biological matrices. Data obtained from HMDB. CSF = cerebrospinal fluid. Figures adopted and modified from Folberth et al., 2020 [119].

The automatic acquisition of MS² spectra at multiple normalized collision energies (NCEs) is an advanced strategy to build a comprehensive library. However, combining positive and negative mode in one run resulted in a long cycle time and fewer NCEs (**Figure 4.2 A**). Separate instrument methods for each ionization mode require two runs per metabolite, but cycle time could be reduced to 5 s with the benefit of six different NCEs. The t-SIM/dd-MS² experiment offers high-resolution MS¹ spectra and distinct MS² spectra, but an inclusion list of masses for t-SIM scans is mandatory. By adjusting the optimized MS method to 10 masses in the inclusion list (Top 10), method setup got less time-consuming, but cycle time increased to 20 s. However, the 0.02 ml/min flow injection of a single standard offered MS¹ and MS² spectra in the required quality and quantity for manual selection of high-quality spectra (**Figure 4.2 B – D**). The curation process was based on reproducibility and coherence of spectra (**Figure 4.2 D**). MS² spectra (e.g. for L-aspartate [M-H]⁻, NCE = 10 % (spectrum A) were included in the library when the fragmentation was reproducible, based on replicate spectra within the same run (NCE = 10 % (spectra B and C). Additionally, the fragmentation at consecutive NCEs had to be coherent.

Results

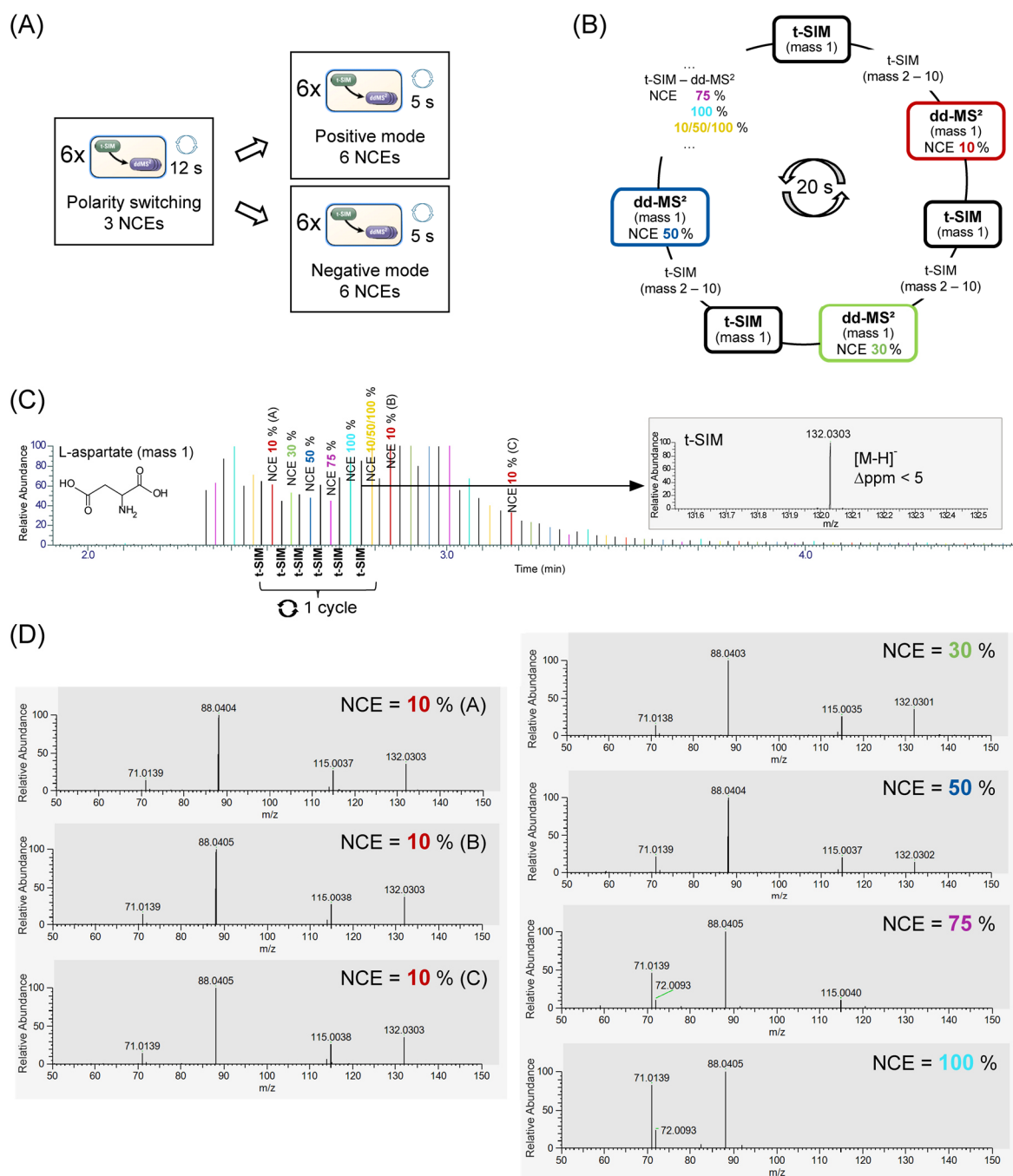


Figure 4.2 Method development and MS² library generation. (A) Development of the experimental design of the t-SIM/dd-MS² instrument method. Cycle time was reduced from 12 s to 5 s for one compound in the inclusion list (Top 1) when having separate methods for positive and negative ionization mode. (B) Concept of t-SIM/dd-MS² (Top 10) data acquisition. One cycle comprises t-SIM scans for all masses of the inclusion list (mass 1 – 10) followed by optimally one dd-MS² scan for the injected mass 1 at NCE = 10 %, followed by the same procedure for NCE = 30, 50, 75, 100 and 10/50/100 %. Cycle time is 20 s. (C) Example for data acquisition, L-aspartate (-ve mode) related scans: black = t-SIM, colored = dd-MS² for NCEs = 10, 30, 50, 75, 100, 10/50/100 %. Inset shows a t-SIM spectrum of L-aspartate. (D) Example for curation process. A spectrum for L-aspartate (NCE = 10 % (A)) was included in the library after comparison to replicate spectra (NCE = 10 % (B) and (C)) and spectra of consecutive NCEs (30, 50, 75, 100 %; 10/50/100 % not shown here). Figures adopted and modified from Folberth et al., 2020 [119].

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External databases like mzCloud, METLIN or HMDB were used for comparison of fragments in case of inconsistency. An automatic threshold was applied for every selected spectrum to remove noise before it was included in the library.

The developed flow injection/t-SIM/dd-MS² method offers a simple, reproducible and effective way to determine the fragmentation of numerous standard compounds for different NCEs. Over 4,000 MS² spectra for 506 metabolites at up to 6 NCEs are included in the MS² library. Different metabolic pathways are represented, for example purine and pyrimidine metabolism, amino acid metabolism, carbohydrate-related pathways or the citric acid cycle (**Figure 4.3 A**). Compared to the online mass spectral database mzCloud, our in-house library provides new information for over 40 % of the 506 metabolites (**Figure 4.3 B**, **Table S6.3**).

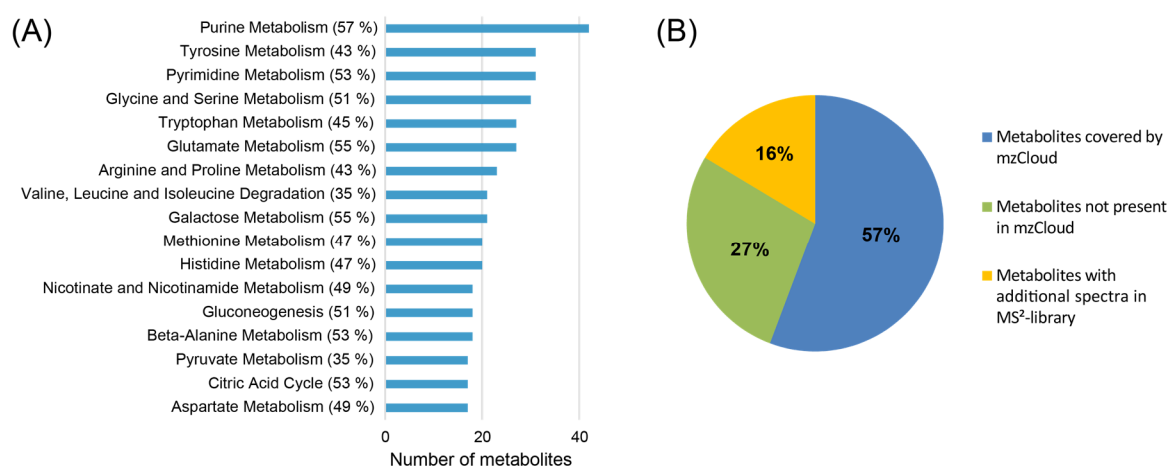


Figure 4.3 MS² library coverage. (A) Metabolic pathways represented by the MS² library (top 17, MetaboAnalyst). Coverage of pathways (metabolites in MS² library/total number of metabolites in pathway) varies between 35 % and 57 %. (B) Comparison of MS² library with mzCloud. Over 40 % of metabolites are not present in mzCloud or the MS² library includes spectra for an additional ionization mode. Figures adopted and modified from Folberth et al., 2020 [119].

4.1.2 LC library

The analytical parameters strongly influence the chromatographic behavior of individual metabolites [7]. Column chemistry and eluent additives, which determine the pH value and buffer system, are the primary factors. In addition, flow rate and injection volume affect coverage, peak quality and retention of compounds. To investigate the influence of these parameters, 10 stationary phases, 3 pH-ranges, 4 injection volumes, 7 flow rates as well as 5 eluent additives and buffer components in different concentrations were tested, resulting in 57 LC-MS conditions (**Table 4.1**, **Table 4.2**, **Table S6.1**, **Table S6.2**). A Full scan/dd-MS² (Top 10) approach with a stepped NCE (10/50/100 %) was used.

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Table 4.1 Main parameters tested for LC library generation (reversed-phase conditions). A = acidic, n = neutral, b = basic pH of eluents, Inj V = injection volume, MeOH = methanol.

Columns	pH values	Flow rate [ml/min]	Inj V [μ l]	Sample solvent
BEH C18	a, n, b	0.1, 0.2	5, 10, 15, 20	MeOH, MeOH/H ₂ O (1:1)
C18	a, n, b	0.1, 0.2	5	MeOH, MeOH/H ₂ O (1:1)
Cortecs T3	a, n, b	0.1, 0.15, 0.2, 0.25, 0.3	5, 10, 15, 20	MeOH, MeOH/H ₂ O (1:1)
ES-CN	a	0.1	5	MeOH/H ₂ O (1:1)
F5	a	0.1	5	MeOH/H ₂ O (1:1)
Phenyl-Hexyl	a	0.1	5	MeOH/H ₂ O (1:1)
Hypercarb	a	0.1	5	MeOH/H ₂ O (1:1)

Table 4.2 Main parameters tested for LC library generation (HILIC conditions). A = acidic, n = neutral, b = basic pH of eluents, HILIC = hydrophilic interaction liquid chromatography.

Columns	pH values	Buffer components	Buffer concentration [mM]
HILIC	a, n	Ammonium acetate Ammonium formate Acetic acid Formic acid	5, 10, 15
BEH Amide	a, n, b	Ammonium acetate Ammonium formate Acetic acid Formic acid Ammonium hydroxide	5, 10, 15
ZIC HILIC	a, n	Ammonium acetate Ammonium formate Acetic acid Formic acid	5, 10, 15

We worked with a metabolite standard mix (MSM) that included all 506 metabolites from the MS² library (**Table S6.3**). Identification of compounds was based on the exact mass (Δ ppm < 5 ppm), isotopic pattern and matching fragmentation (stepped NCE 10/50/100 %) with the MS² library (**Figure 4.4 A**). A score threshold of 30 % was applied for the library search and each match was confirmed manually. A scoring system was introduced to assess the chromatographic peak quality considering sensitivity (signal to noise (S/N) ratio), peak width (Δ RT), peak asymmetry (A_s) and retention (retention factor k) (chapter 3.2, **Table 3.2**). A low score would represent a sharp and symmetric peak with a high S/N ratio and a good - but not too strong - retention, whereas a high score reflects a not entirely satisfactory peak (**Figure 4.4 B, C**). The retention factor k considers the solvent volume preceding the actual sample (dead volume) of a method and is therefore more suitable to compare different LC methods than the retention time.

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A certain retention on the column is important, however, if the retention is too strong, the peak shape can suffer or a compound is not eluted at all.

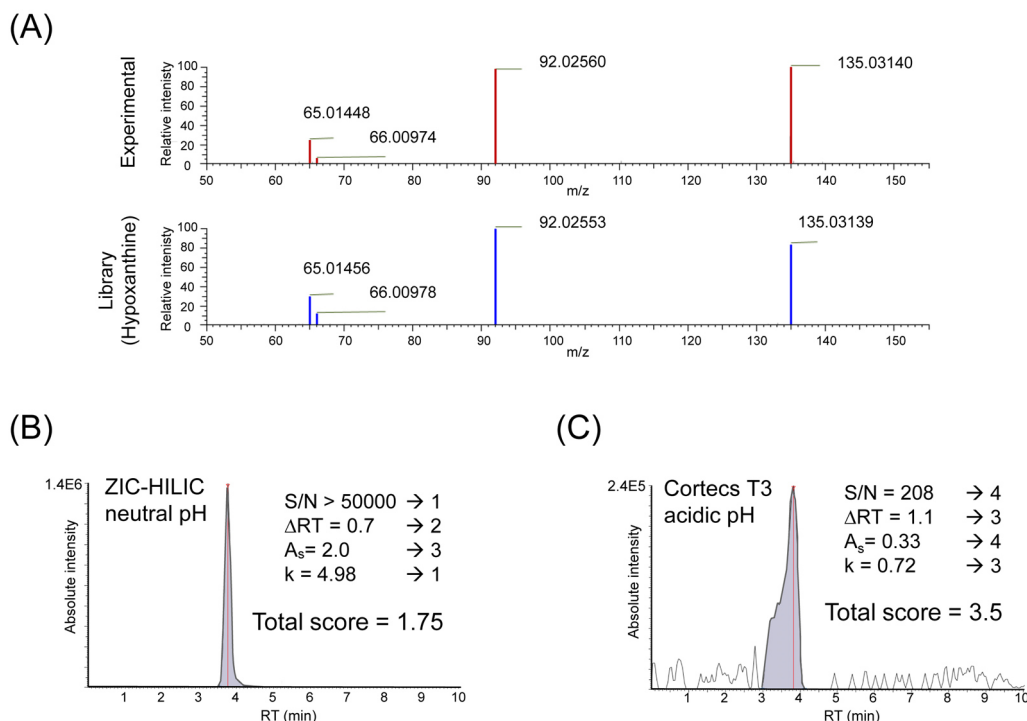


Figure 4.4 Peak identification and assessment of peak quality. (A) The experimental fragmentation spectrum from the metabolite standard mix (MSM) matches the MS² library spectrum for hypoxanthine ([M-H]⁻). (B) Chromatographic peak for hypoxanthine analyzed with a HILIC method and (C) with a RP method. Calculated scores reflect peak qualities. S/N = signal-to-noise ratio; ΔRT = peak width; A_s = peak asymmetry; k = retention factor. Retention times were similar here, but k value varied due to different dead volumes of the methods. Figures adopted and modified from Folberth et al., 2020 [119].

By testing 57 LC-MS conditions in positive and negative ionization mode, we built a comprehensive database for LC behavior of 294 metabolites, including 46 separable isomers tested for 5 different conditions (**Table S6.3**, **Table S6.4**). The LC library includes scores to characterize the chromatographic behavior of each confirmed compound as well as retention times and k values as a resource for elution order.

Due to the different retention mechanisms and the chemical diversity of the metabolome, the choice of chromatographic conditions for LC-MS-based untargeted metabolomics comes with an inevitable coverage bias [9-11]. To illustrate this bias, data from the LC library was plotted based on the partition coefficient (log P value, **Figure 4.5**). Poorly retained compounds ($k < 1$) were excluded, data from positive and negative mode was combined. As expected, the coverage of metabolites was dependent on the log P value, for both HILIC and RP mode. Nonpolar compounds

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(log $P > 0$) were detected preferably with RP, whereas HILIC is more suitable for polar compounds (log $P < 0$).

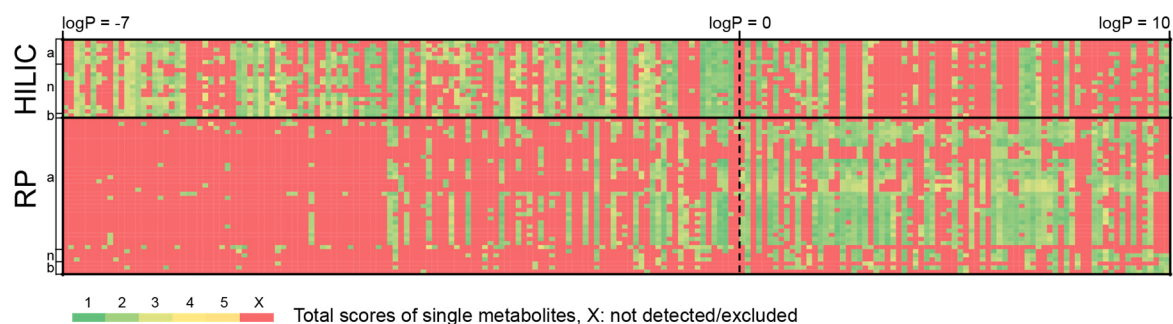


Figure 4.5 Polarity dependent coverage of metabolites by different LC methods. Heatmap shows the total scores of single metabolites analyzed by different LC methods. Metabolites were sorted by their log P value according to HMDB. Isomers, compounds with retention factor $k < 1$ or missing information about chemical properties (HMDB) were excluded (see Table S6.3). A = acidic, n = neutral, b = basic pH of eluents. Figure adopted from Folberth et al., 2020 [119].

Next to the polarity of compounds, charged functional groups and other chemical properties determine chromatographic behavior and ionization [120]. Therefore, the same data was plotted according to physiological charge and acid-base characteristics (**Figure 4.6**).

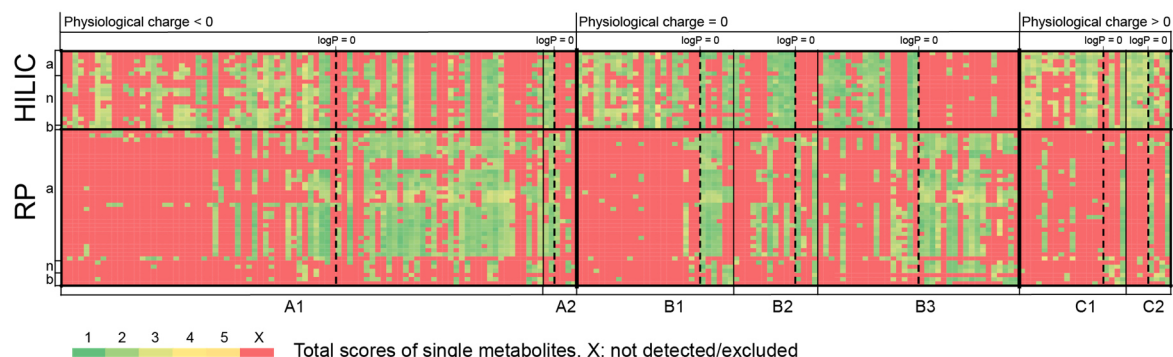


Figure 4.6 Coverage of metabolites by different LC methods based on additional chemical properties. Heatmap shows the total scores of single metabolites analyzed by different LC methods. Metabolites were sorted by physiological charge, acid-base characteristics and log P value, all according to HMDB. Isomers, compounds with retention factor $k < 1$ or missing information about chemical properties (HMDB) were excluded (see Table S6.3). A = acidic, n = neutral, b = basic pH of eluents. A1 = strong acids, A2 = medium-strong acids, B1 = zwitterions, B2 = medium-strong acids/bases, not dissociated, B3 = no acidic/basic groups, C1 = strong bases, C2 = no acidic/basic groups. Figure adopted from Folberth et al., 2020 [119].

Strong acids (A1) and zwitterions (B1) were detectable using HILIC rather independent of the log P effect that was, however, dominant in RP mode, suggesting that the primary interactions of the HILIC stationary phase with these metabolites are electrostatic ones. Both compound groups are probably charged or have at least one charged functional group, independent of the applied pH

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value. Uncharged metabolites of groups B2, B3 as well as A2 showed a strong dependence on the log P value in HILIC, indicating mainly hydrophilic interactions. Group A2 metabolites are negatively charged under physiological conditions but probably not dissociated under experimental conditions; neither are group B2 metabolites. Cationic metabolites (C1, C2) were better detected using HILIC, likely due to electrostatic interactions. In this study, the pH value used for HILIC only showed an effect for a subgroup of metabolites in C1. They represent secondary and tertiary amines that were better detectable under acidic conditions, probably because of strong interactions with the stationary phase under non-acidic conditions and consequently a lack of elution. For RP, acidic conditions were preferable, because of improved ionization in the ESI source of the MS, especially in the positive mode [11]. In general, positively and negatively charged compounds were better detected in the positive or negative ionization mode, respectively.

The representation of different metabolic pathways can also reflect the coverage bias. For example, 18 % of the histidine metabolism was covered using a HILIC-based method (HILIC 19, **Table S6.1**), but only 5 % with RP (**Figure 4.7 A**). Instead, tryptophan-related metabolites were covered up to 14 % by RP methods (RP 5, 6 and 19, **Table S6.1**) compared to 8 % for HILIC. Applying a RP method under acidic conditions in the positive ionization mode resulted in the best coverage for that pathway, as highlighted in **Figure 4.7 B**.

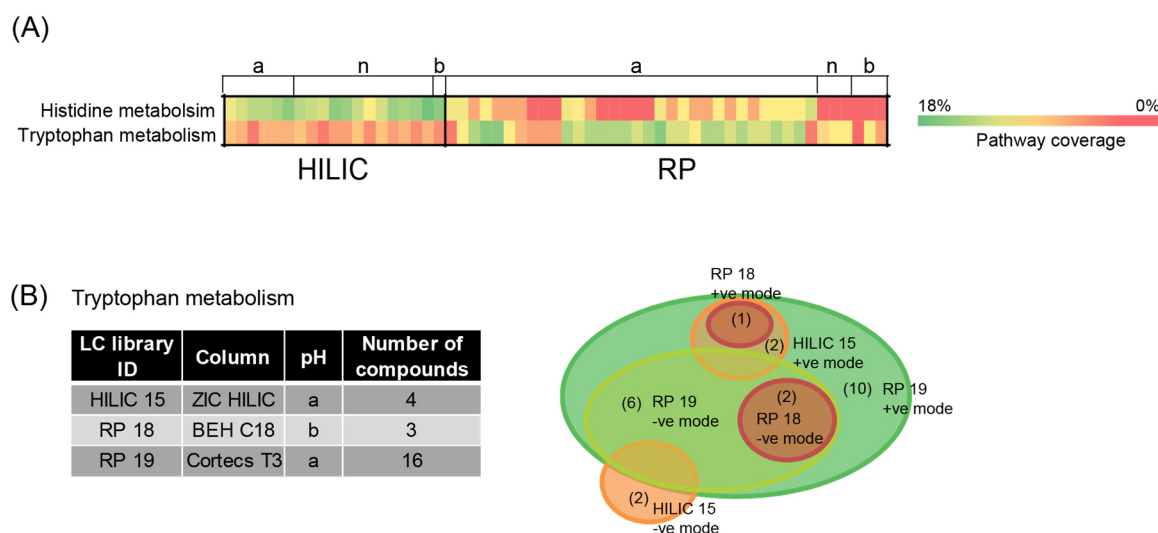
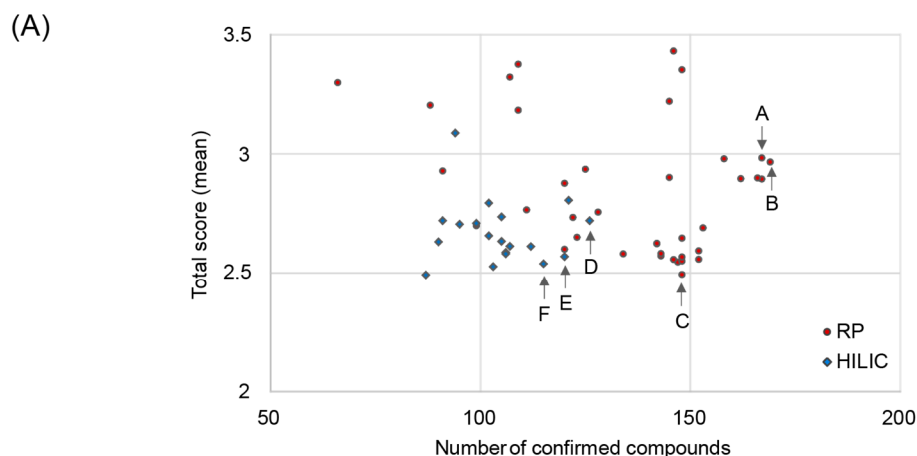


Figure 4.7 Pathway coverage of different liquid chromatography conditions. (A) Coverage of histidine and tryptophan metabolism for different LC methods. a = acidic, n = neutral, b = basic pH of eluents. (B) Overlapping of confirmed metabolites related to the tryptophan metabolism, analyzed by different LC-MS methods. Number of confirmed compounds in parenthesis. Details about LC-MS methods are shown in the enclosed table and Table S6.1. Figures adopted from Folberth et al., 2020 [119].

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4.1.3 Method development

The LC library was further implemented to choose a method for maximum coverage of the metabolome. Crucial selection criteria were the number of confirmed compounds and the chromatographic peak quality, represented by the calculated scores. Three conditions with high numbers and/or low scores were chosen from both RP (conditions A, B, C) and HILIC (conditions D, E, F) methods (**Figure 4.8, Table S6.1**).



(B)

Condition	Mode	Column	pH	Additive	Flow rate [ml/min]	Number of confirmed compounds	Total score	
A	RP	F5	a	0.1 % FA	0.1	A 167	3.0	A
B	RP	Cortecs T3	a	0.1 % FA	0.1	B 169	3.0	B
C	RP	Cortecs T3	a	0.1 % FA	0.3	C 148	2.5	C
D	HILIC	ZIC HILIC	n	5 mM Ammonium acetate	0.75	D 126	2.7	D
E	HILIC	ZIC HILIC	n	10 mM Ammonium acetate	0.75	E 120	2.6	E
F	HILIC	ZIC HILIC	a	10 mM Ammonium acetate, 0.1 % AA	0.75	F 115	2.5	F

Figure 4.8 Selection of promising LC conditions for metabolomics screen. (A) Number of confirmed compounds plotted against mean total scores of all confirmed compounds for 57 tested LC methods. Arrows point to LC conditions (A – F) that were selected based on a high number of confirmed compounds and/or a low score. (B) Overview of selected conditions A – F with corresponding number of confirmed compounds in the metabolite standard mix (MSM) and mean total score for confirmed compounds (positive and negative ionization mode combined); a = acidic, n = neutral, b = basic pH of eluents, FA = formic acid, AA = acetic acid. Figure adopted from Folberth et al., 2020 [119].

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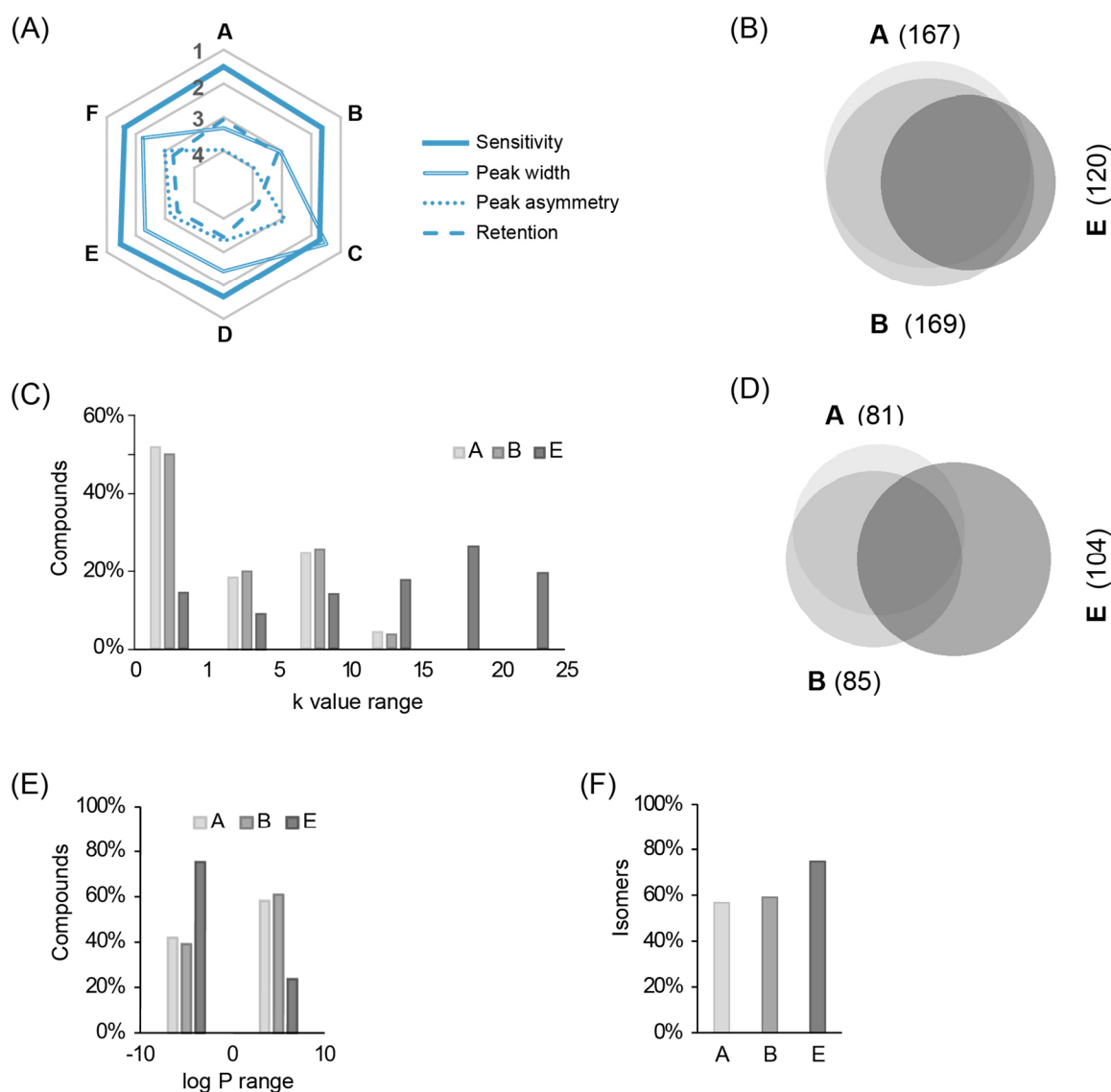


Figure 4.9 Detailed consideration of possible LC conditions for metabolomics screen. (A) Radar chart with individual scores for confirmed compounds in the MSM of conditions A – F. (B) Venn Diagram of confirmed compounds in the MSM for conditions A, B and E. (C) k value range of confirmed compounds in the MSM for conditions A, B and E. (D) Venn Diagram of confirmed compounds in the MSM for conditions A, B and E, compounds with $k < 1$ were excluded. (E) Log P value range of confirmed compounds in the MSM for conditions A, B and E. (F) Percentage of separated isomers per condition. Figure adopted from Folberth et al., 2020 [119].

Regarding the individual scores, sensitivity was similar for all conditions, with the best score for HILIC condition E (**Figure 4.9 A**). Peak width was narrower in HILIC conditions D – F, except for RP condition C. In contrast to conditions A and B, the flow rate in C was increased, leading to a decreased peak width and a better peak symmetry. However, the score for retention was compromised in condition C. Conditions A, B and E were selected for a more detailed comparison, which revealed that 90 % of confirmed compounds in E were overlapping with A and B. (**Figure 4.9 B**). It has to be considered, though, that about 50 % of the confirmed compounds were not or poorly retained (retention factor $k < 1$) under RP conditions A and B (**Figure 4.9 C**). The detection

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of these metabolites is likely to be compromised in biological samples due to ion suppression effects caused by unretained matrix components. Excluding all compounds with $k < 1$ resulted in a superior number for HILIC condition E with an overlap of only 50 % with conditions A and B, while they still overlapped by 80 % (**Figure 4.9 D**). As already demonstrated in chapter 4.1.2, the log P values of confirmed compounds ($k > 1$) using HILIC (condition E) were mainly in the negative log P range. In contrast, the distribution was shifted towards the positive log P range for both RP conditions A and B (**Figure 4.9 E**). The separation of isomeric compounds was best under condition E (**Figure 4.9 F**, **Table S6.4**). From 83 separable isomers, 75 % were baseline-separated using condition E compared to 56 % and 58 % under conditions A and B, respectively. As there were no critical differences between A and B, condition A was excluded.

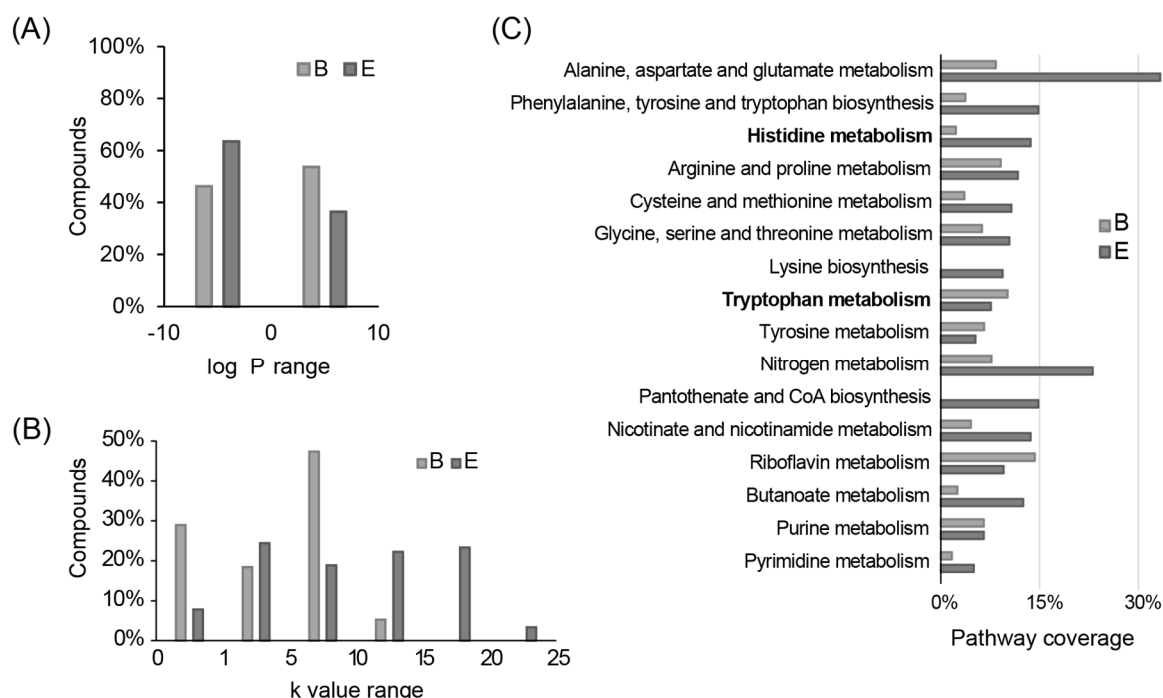


Figure 4.10 Metabolomics of MSM spiked plasma under two different LC conditions. (A) Log P value range of confirmed compounds found in metabolite standard mix (MSM) spiked plasma for conditions B and E. (B) k value range of confirmed compounds found in MSM spiked plasma for conditions B and E. (C) Pathway analysis (MetaboAnalyst) of confirmed compounds found in MSM spiked plasma for conditions B and E. Figure adopted from Folberth et al., 2020 [119].

Using the data from the LC library limited the variety of conditions to one RP- and one HILIC-based method, which were tested with human plasma. Samples were extracted with a protein precipitation protocol and spiked with the MSM after the extraction. Via fast polarity switching, spectra in both positive and negative ionization mode were acquired in one run. Applying LC conditions B and E resulted in 76 and 90 confirmed compounds, respectively, with only 14 % overlap. As before, the ZIC HILIC method (E) covered more hydrophilic compounds (**Figure 4.10 A**).

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The retention of compounds was better distributed on the HILIC column (E) than on the RP column (B) and less than 10 % of confirmed compounds had a $k < 1$ (**Figure 4.10 B**). As demonstrated in chapter 4.1.2, high coverage of the histidine and other amino acid pathways could be achieved by applying condition E (HILIC), whereas condition B (RP) was superior for the tryptophan metabolism (**Figure 4.10 C**). Based on the more comprehensive coverage of hydrophilic metabolites and better distribution of compound elution, condition E was chosen for metabolomics analysis.

4.1.4 Method adjustment and validation

An unexpected spray current pattern led to minor changes in the method (**Figure 4.11**). Even if only solvent blanks were measured (methanol:acetonitrile, 1:1, v/v), the current between ESI needle and source inlet was increased at the beginning of the gradient compared to identical conditions (95 % acetonitrile) at the end (curve A). After 12 min, it reached a peak of almost 50 μA that would not fit the gradient. Lowering the buffer concentration from 10 mM ammonium acetate to 5 mM (curve B) decreased the current. However, the inconsistent pattern remained the same. Shortening the gradient (curve C) and lower spray voltage settings (curve D) did not show the intended effect either. Limiting buffer presence to the water phase (eluent A) normalized the spray current pattern eventually (curve E). The method's general characteristics, described in chapter 4.1.3, are not expected to be affected by the adaptations.

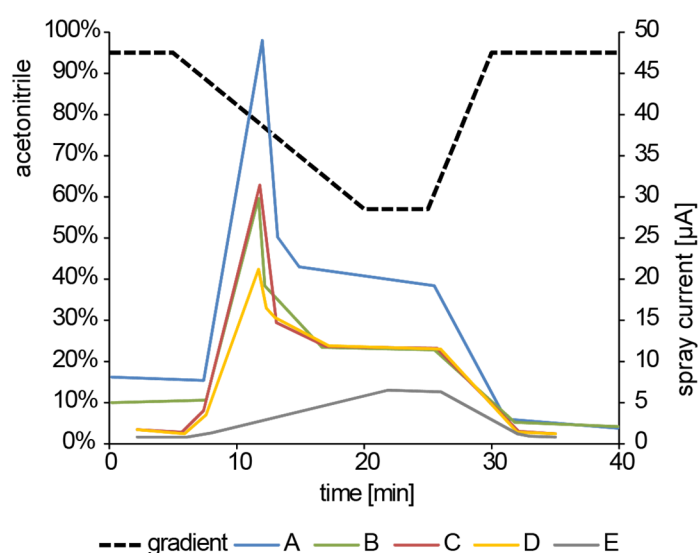


Figure 4.11 Spray current pattern and method adjustment. Spray current pattern from the measurement of solvent blanks (methanol:acetonitrile 1:1, v/v) and adjustment of the method. A: 10 mM ammonium acetate in eluent A and B, spray voltage +ve mode: 4.5 kV, -ve mode: 3.5 kV; B: 5 mM ammonium acetate in eluent A and B; C: changed gradient, D: spray voltage +ve mode: 3.5 kV, -ve mode: 3 kV; E: 5 mM ammonium acetate in eluent A only. The black dotted line represents the gradient. Figure adopted from Folberth et al., 2020 [119].

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Further measurements revealed a signal drift over time (**Figure 4.12 A**). When analyzing extracted plasma samples (9 biological replicates, 7 technical replicates), a decrease in signal intensity could be observed. A linear relation between signal and time was assumed to correct the changes in intensity for each analyte individually. The deviation of a signal to the mean of all signals is used to calculate a correction factor based on a linear regression [112]. The signal drift correction counteracts the decrease in signal intensity over time (**Figure 4.12 B**).

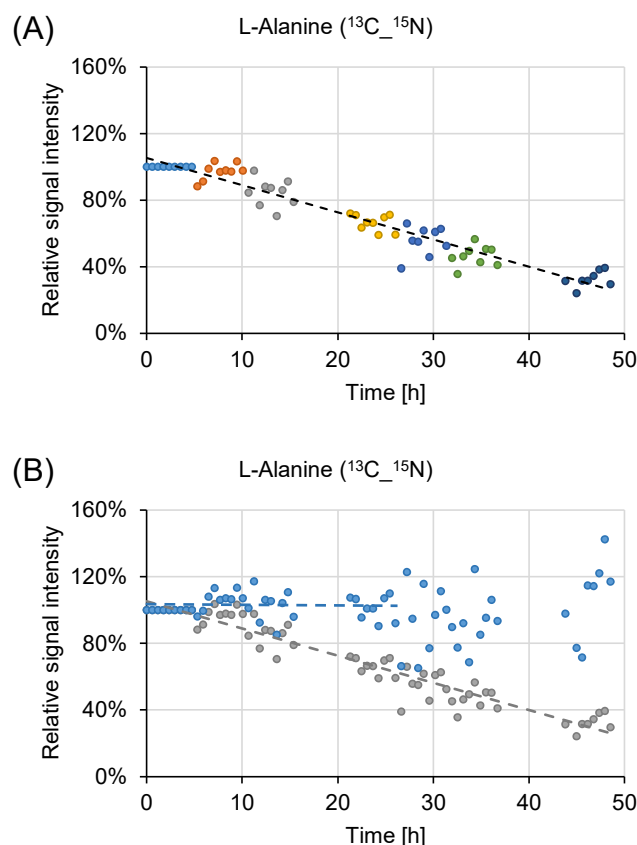


Figure 4.12 Signal development over time for L-alanine ($^{13}\text{C}_3\text{-}^{15}\text{N}$). (A) Nine extracted plasma samples were measured seven times over 48 h. The signal intensity relative to the first acquisition decreased by about 70 % for the internal standard L-alanine ($^{13}\text{C}_3\text{-}^{15}\text{N}$). Technical replicates presented in different colors. (B) Signal over time with and without signal drift correction. The correction counteracts the signal drift and leads to stable values.

To avoid increased signal variability after a certain number of runs, the run time was limited to approximately 24 – 30 h per batch. Signal drift correction was based on technical replicates of the same quality control (QC) sample (see chapter 3.3.3). Cleaning the instrumentation after every batch led to a recovery of the signal intensity.

Five hundred and forty serum samples were measured in 30 batches. Retention times were stable with $\Delta\text{RT} < 9\%$ for internal standards. Metabolites were identified based on exact mass, expected retention time and matching fragmentation with the MS^2 library and mzCloud. Every match was

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checked manually, resulting in 179 confirmed metabolites. After normalization, data quality was assessed (**Figure 4.13 A**, **Table S6.5**). Overall and intra-batch variability of QCs was required to be < 40 %, overall variability of diluted QCs (0.8:1, v/v; repeatability A and B) was required to be < 30 %. Additionally, the signal had to be linear ($R^2 > 0.9$) over all 30 batches. Within these quality criteria, 94 confirmed metabolites remained. Overall and intra-batch variability were < 20 % in QC samples for 72 % and 82 % of the 94 metabolites, respectively (**Figure 4.13 B**). When QCs were diluted before the extraction (repeatability A), variability was higher compared to QCs diluted after the extraction (repeatability B).

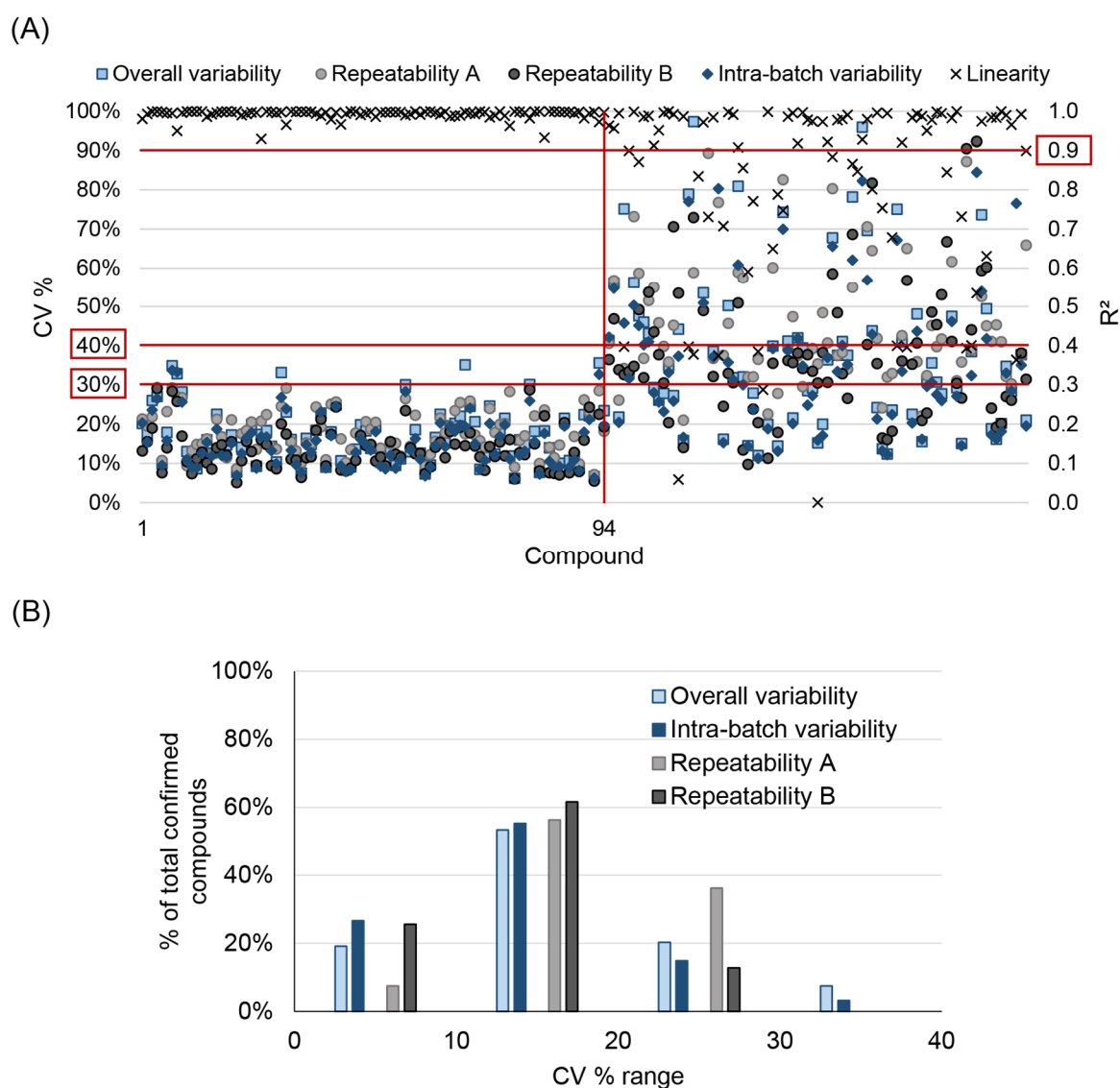


Figure 4.13 Metabolomics of human serum samples. (A) Statistical quality criteria applied for the 179 confirmed compounds. Linearity was required ($R^2 > 0.9$). Overall and intra-batch variability as well as repeatability were required to be < 40 % and < 30 %, respectively. 94 compounds remained. CV: coefficient of variation; R^2 : coefficient of determination; repeatability A: QC diluted (0.8:1 (v/v)) before extraction; repeatability B: QC diluted (0.8:1 (v/v)) after extraction. (B) Distribution of the coefficient of variation (CV %) for 94 confirmed compounds (Overall and intra-batch variability, repeatability A/B).

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As suggested by our MSM-based results in chapter 4.1.3, over representation analysis confirmed that mainly amino acid-related pathways, but also other metabolites related to carnitine or glutathione, are covered by the metabolomics screen (**Figure 4.14**).

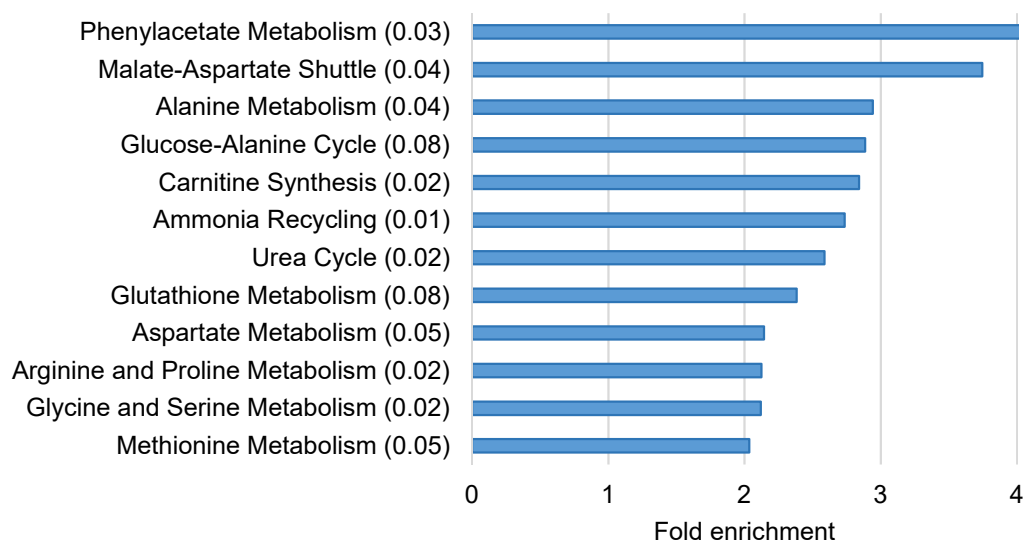


Figure 4.14 Pathway over representation analysis of 94 confirmed metabolites in human serum samples. P values (one-tailed) in parenthesis. Analysis was implemented using the hypergeometric test to evaluate whether a particular metabolite set is represented more than expected by chance within the given compound list.

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4.2 Metabolomics and lipidomics analysis of ischemic stroke patients

The blocked blood flow in the brain during an ischemic attack leads to oxygen and nutrient deprivation in the affected area. Complex signaling cascades are activated, affecting the brain and the periphery. To investigate metabolic alterations, serum samples of ischemic stroke patients and matched controls (**Table 4.3**) were analyzed with the described metabolomics method and a lipidomics screen.

Table 4.3 Human serum cohort. Ischemic stroke patients and matched control patients. Mean $\pm$ SD.

Clinical data	Ischemic stroke (n = 112)	Control (n = 140)
Age (years)	69.6 $\pm$ 12.3	69.1 $\pm$ 12.2
Sex (m/f)	83 (74 %)/29 (26 %)	108 (77 %)/32 (23 %)
Diabetes (m/f)	19/5 (21 %)	27/7 (24 %)
BMI (kg/m ²)	26.6 $\pm$ 4.1	26.5 $\pm$ 4.0
Blood sampling		
Day 1 (n)	112	140
Day 2 (n)	94	-
Day 3 (n)	68	-

We identified 188 lipid species out of 12 lipid classes (**Table 4.4**). Additionally, 76 metabolites, 10 PC/PE ratios and 7 sphingomyelin/ceramide (SM/Cer) ratios were analyzed. All compounds are listed in **Table S6.6**.

Table 4.4 Identified lipids in the human serum cohort. Lipid class, corresponding abbreviation and number of identified lipid species.

Lipid class	Abbreviation	Lipid species
Acylcarnitines	AcCa	16
Cholesterylester	CE	5
Ceramides	Cer	10
Cerebrosides	CerG	7
Lysophosphatidylcholines	LPC	20
Lysophosphatidylethanolamines	LPE	6
Phosphatidylcholines	PC	57
Phosphatidylethanolamines	PE	10
Phosphatidylinositols	PI	2
Sphingomyelins	SM	34
Triacylglycerides	TG	19
Ethanolamides	EA	2

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4.2.1 Changed metabolite levels in serum after ischemic stroke

On day 1 after ischemic stroke, the serum levels of 54 lipids and metabolites were significantly changed compared to the control group (**Figure 4.15**). In our study, day 1 was defined as the time between symptom onset and blood sampling being less than 30 hours.

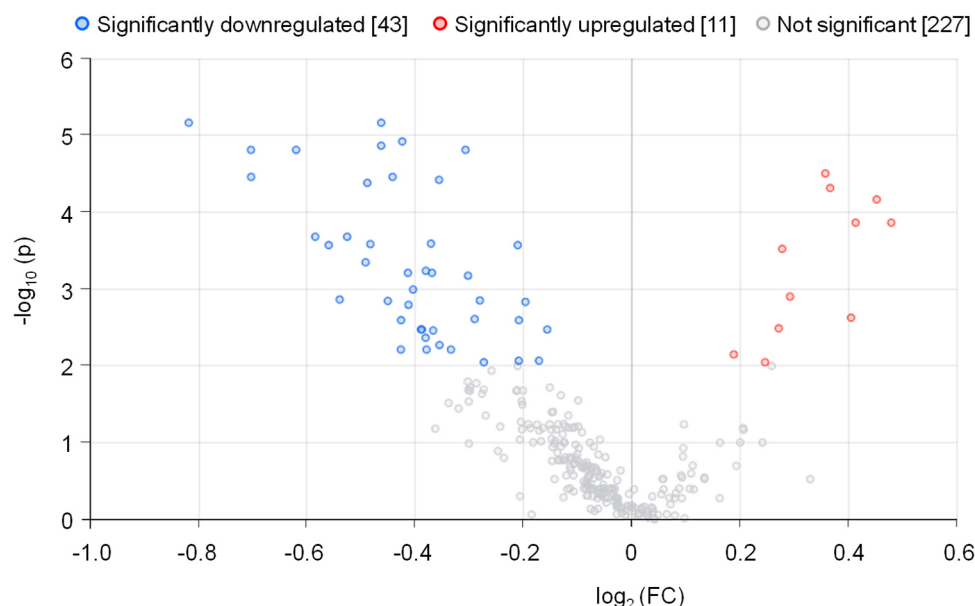


Figure 4.15 Significant changes in serum of ischemic stroke patients. Volcano plot shows increased and decreased levels of metabolites and lipids. P-value false discovery rate (FDR) adjusted. P-value threshold: 0.01, fold change threshold: 1.1. Wilcoxon Mann Whitney test, $n = 140/112$ (control/stroke). All 281 compounds with corresponding p-values and fold changes are listed in Table S6.6.

Decreased serum levels were observed for several odd-chain AcCas and glycerophospholipids (LPCs, LPEs, PCs and PEs) (**Table 4.5, Figure 4.16**). Residues of phospholipids were primarily medium-chain, saturated or monounsaturated FAs. Almost all measured TGs were downregulated, independent of the chain length. Several metabolites, like proteinogenic amino acids proline, threonine, tryptophan and tyrosine, were decreased as well. Other downregulated amino acids were α -aminoadipate, kynurenine and methionine sulfoxide. 4-Guanidinobutanoate and 5'-methylthioadenosine belong to amino acid-related pathways as well, trigonelline (N-methylnicotinate) to the nicotinate and nicotinamide metabolism.

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Table 4.5 Decreased lipids and metabolites in serum of ischemic stroke patients. Fold change (FC) and p-value (false discovery rate (FDR) adjusted) according to Figure 4.15. Wilcoxon Mann Whitney test, n = 140/112 (control/stroke).

Compound	Fold change (FC)	log ₂ (FC)	p-value	-log ₁₀ (p)
AcCa (3:0)	0.73	-0.46	6.81e-06	5.17
AcCa (11:1)	0.75	-0.42	1.19e-05	4.92
AcCa (13:0)	0.77	-0.38	6.19e-03	2.21
AcCa (13:1)	0.73	-0.46	1.35e-05	4.87
LPC (12:0)	0.61	-0.70	3.44e-05	4.46
LPC (14:0)	0.77	-0.37	6.07e-04	3.22
LPC (14:1)	0.65	-0.62	1.53e-05	4.81
LPC (15:1)	0.77	-0.38	5.72e-04	3.24
LPE (16:0)	0.89	-0.17	8.64e-03	2.06
LPE (18:1)	0.86	-0.21	8.65e-03	2.06
PC (28:0)	0.74	-0.43	6.19e-03	2.21
PC (28:1)	0.61	-0.70	1.53e-05	4.81
PC (30:0)	0.83	-0.27	9.05e-03	2.04
PC (30:1)	0.78	-0.37	3.50e-03	2.46
PC (31:2)	0.82	-0.29	2.49e-03	2.60
PE (34:2)	0.82	-0.28	1.39e-03	2.86
PE (36:1)	0.71	-0.49	4.12e-05	4.39
PE (36:2)	0.74	-0.44	3.44e-05	4.46
TG (48:2)	0.57	-0.82	6.81e-06	5.17
TG (50:1)	0.75	-0.41	1.58e-03	2.80
TG (50:2)	0.76	-0.40	1.00e-03	3.00
TG (50:3)	0.72	-0.48	2.57e-04	3.59
TG (52:2)	0.78	-0.36	5.41e-03	2.27
TG (52:3)	0.79	-0.33	6.19e-03	2.21
TG (52:4)	0.74	-0.43	2.57e-03	2.59
TG (52:5)	0.68	-0.56	2.65e-04	3.58
TG (53:2)	0.67	-0.59	2.06e-04	3.69
TG (53:3)	0.71	-0.49	4.43e-04	3.35
TG (53:4)	0.69	-0.53	2.06e-04	3.69
TG (54:4)	0.76	-0.39	3.40e-03	2.47
TG (54:5)	0.77	-0.38	4.35e-03	2.36
TG (54:6)	0.73	-0.45	1.41e-03	2.85
TG (58:6)	0.76	-0.39	3.40e-03	2.47
4-Guanidinobutanoate	0.77	-0.37	2.53e-04	3.60
5'-Methylthioadenosine	0.87	-0.20	1.45e-03	2.84
α-Aminoadipate	0.75	-0.41	6.07e-04	3.22
Kynurenine	0.81	-0.30	6.61e-04	3.18
Methionine sulfoxide	0.78	-0.36	3.76e-05	4.42
Proline	0.9	-0.16	3.40e-03	2.47
Threonine	0.87	-0.21	2.57e-03	2.59
Trigonelline	0.69	-0.54	1.35e-03	2.87
Tryptophan	0.81	-0.31	1.53e-05	4.81
Tyrosine	0.86	-0.21	2.65e-04	3.58

Results

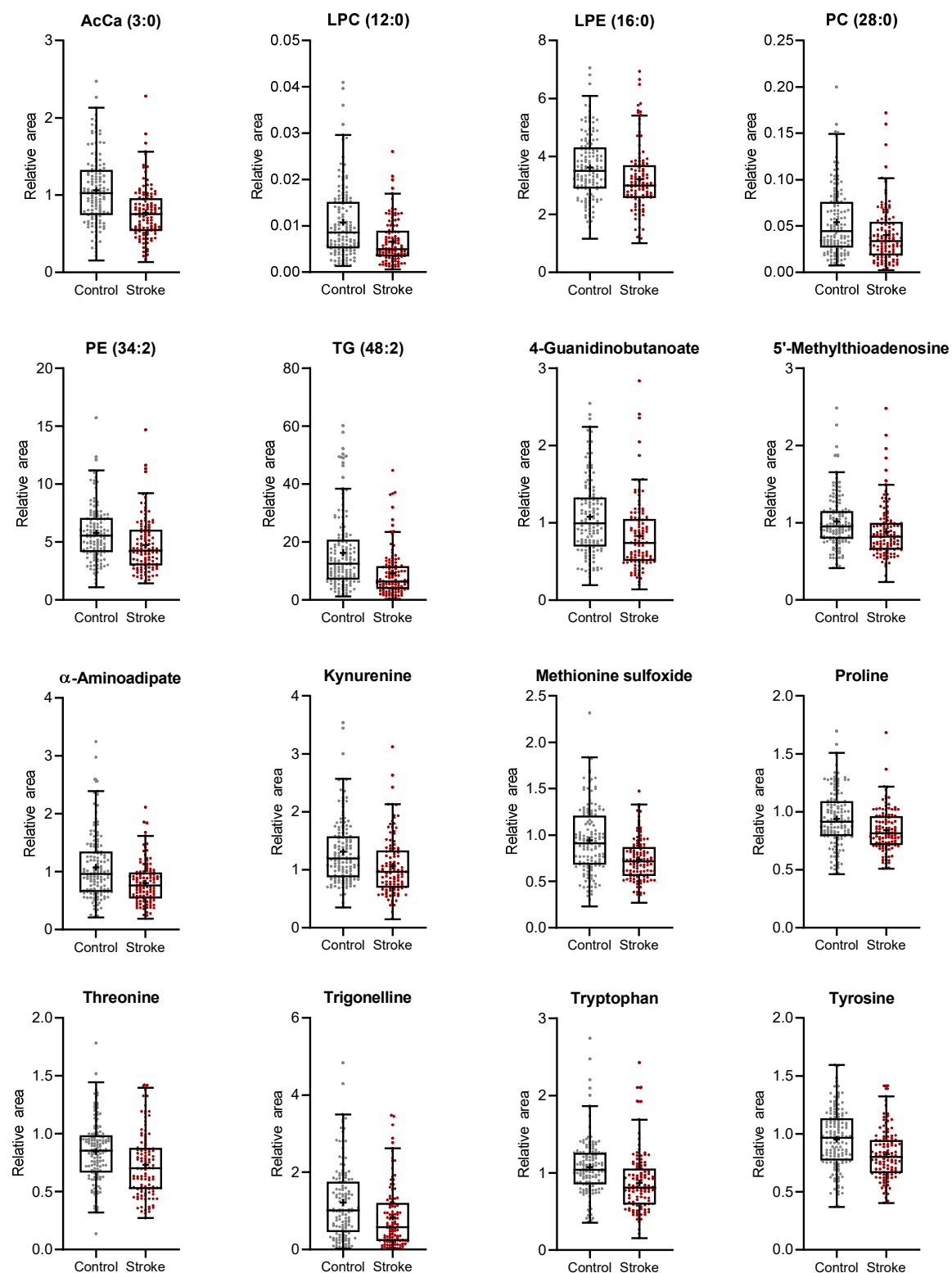


Figure 4.16 Decreased lipid classes (representative) and metabolites in serum of ischemic stroke patients. Box plots (Tukey) show the distribution of data points. Boxes extend from the 25th to 75th percentiles. The line represents the median; the plus-sign the mean value. Dots correspond to the relative area of individual samples. Extreme values are located above or below the whiskers. N = 140/112 (control/stroke), p-values and fold changes see Table 4.5.

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Table 4.6 Increased lipids and metabolites in serum of ischemic stroke patients. Fold change (FC) and p-value (false discovery rate (FDR) adjusted) according to Figure 4.15. Wilcoxon Mann Whitney test, $n = 140/112$ (control/stroke).

Compound	Fold change (FC)	$\log_2(\text{FC})$	p-value	$-\log_{10}(\text{p})$
AcCa (14:1)	1.39	0.48	1.35e-04	3.87
AcCa (14:2)	1.32	0.40	2.39e-03	2.62
AcCa (16:1)	1.33	0.41	1.35e-04	3.87
AcCa (18:1)	1.21	0.28	2.96e-04	3.53
Palmitoyl-EA	1.21	0.27	3.29e-03	2.48
Oleoyl-EA	1.19	0.25	9.05e-03	2.04
2-Hydroxybutyric acid	1.37	0.45	6.75e-05	4.17
PC (34:2)/PE (34:2)	1.22	0.29	1.24e-03	2.91
PC (36:1)/PE (36:1)	1.29	0.37	4.79e-05	4.32
PC (36:2)/PE (36:2)	1.28	0.36	3.10e-05	4.51
PC (38:4)/PE (38:4)	1.14	0.19	7.16e-03	2.15

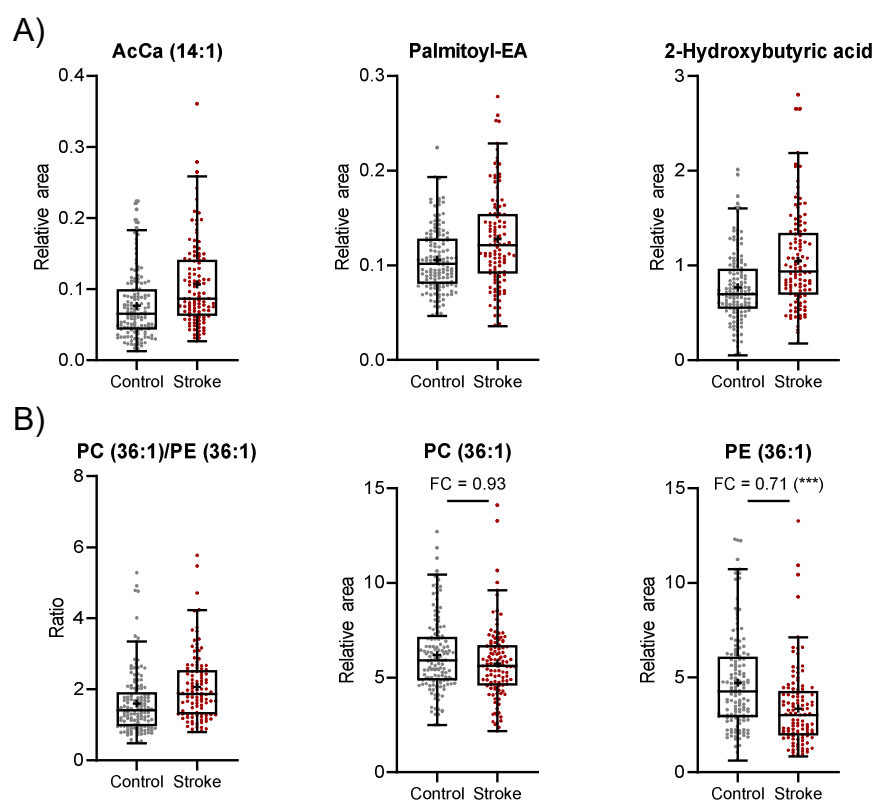


Figure 4.17 Increased compounds and ratios (representative) in serum of ischemic stroke patients. (A) Increased AcCa (14:1), palmitoyl-EA and 2-hydroxybutyric acid. (B) Increased PC/PE ratio with corresponding PC and PE. Box plots (Tukey) show the distribution of data points. Boxes extend from the 25th to 75th percentiles. The line represents the median; the plus-sign the mean value. Dots correspond to the relative area of individual samples. Extreme values are located above or below the whiskers. $N = 140/112$ (control/stroke), p-values and fold changes see Table 4.6, for PE (36:1) see Table 4.5.

Results

Some even-chain AcCas were upregulated in serum (**Table 4.6, Figure 4.17 A**). The chain length of the FAs bound to carnitine was higher for increased AcCas than decreased AcCas (**Table 4.5**). Palmitoyl-EA and oleoyl-EA were both elevated. 2-Hydroxybutyric acid was the only significantly increased metabolite and it belongs to the propanoate metabolism. Additionally, ratios of PC to PE with the same chain length were significantly changed between stroke patients and the control group (**Figure 4.17 B**). The increase originated from a more pronounced decrease of PEs than the corresponding PCs.

4.2.2 Time-dependent development of serum levels after ischemic stroke

Over three consecutive days, the development in lipid and metabolite serum levels was investigated. Significant changes occurred for 34 compounds. A cluster analysis revealed four main groups (**Figure 4.18, Figure 4.19**).

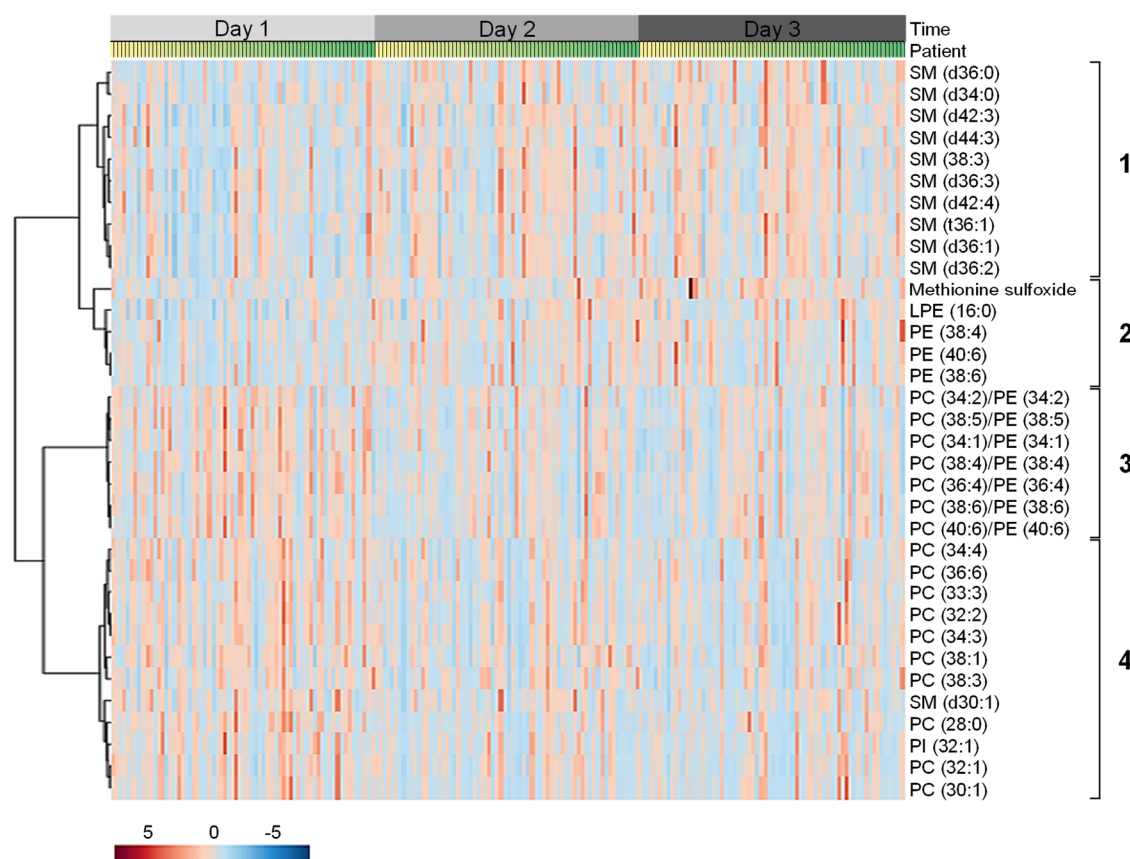


Figure 4.18 Time-dependent differences in serum after ischemic stroke. Heatmap and cluster analysis of significantly changed compounds. Four main clusters can be identified for 34 compounds. Serum concentrations of these compounds significantly changed over time as shown by one-way repeated measures ANOVA for days 1, 2 and 3, $n = 68$, p -value false discovery rate (FDR) adjusted, p -value threshold: 0.001. Colour code represents autoscaled, relative concentration of lipids and metabolites in each sample. Distance measure: Pearson, clustering algorithm: Ward.

Results

Cluster 1 represents SMs with different chain lengths. These lipids were not significantly changed between control and stroke group (chapter 4.2.1), but increased on day 2 and day 3 compared to day 1 (**Figure 4.19**). The same pattern was observed for three polyunsaturated, long-chain PEs in cluster 2. Compared to the control group, methionine sulfoxide (met-S-oxide) and LPE (16:0) were decreased on day 1 (**Table 4.5**) but increased again on day 2 and day 3. Cluster 3 comprises PC/PE ratios, which first increased after stroke (day 1, **Table 4.6**) and later decreased over time. Several PCs were already reduced on day 1 compared to the control group and they further decreased on day 2 and day 3 (cluster 4). Interestingly, PC (28:0), PC (32:1) and PC (30:1) are forming a sub-cluster, which indicates some dissociation from the longer, unsaturated PCs in cluster 4.

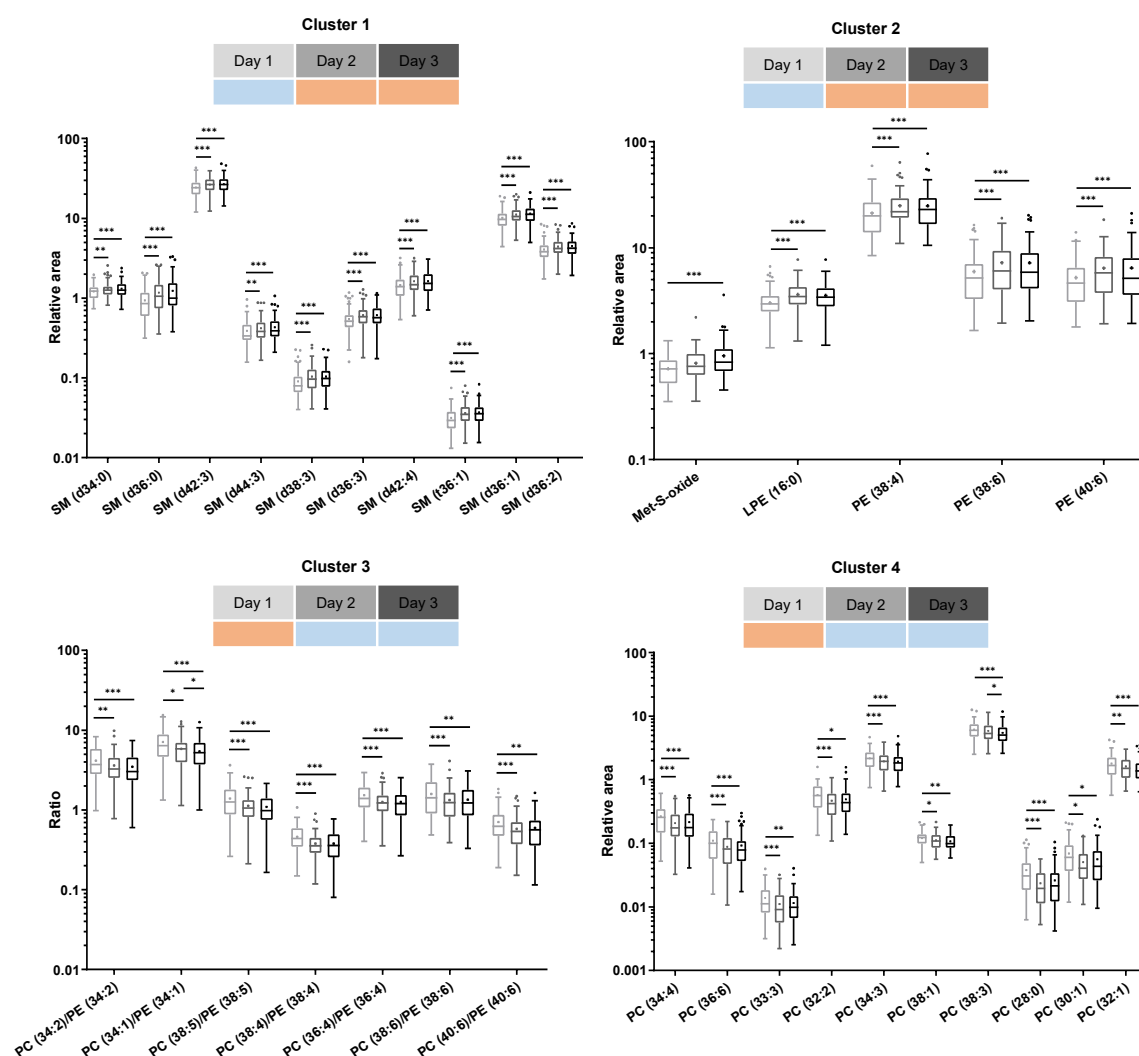


Figure 4.19 Detailed analysis of time-dependent changes in serum after ischemic stroke. Clusters 1 to 4 according to Figure 4.18, colored bars represent a relative increase (light red) or decrease (light blue). Box plots (Tukey) show the distribution of data points. Boxes extend from the 25th to 75th percentiles. The line represents the median; the plus-sign the mean value. Extreme values are located above or below the whiskers. $N = 68$, Friedman test and Dunn's post hoc test, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Met-S-oxide = methionine sulfoxide.

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4.2.3 Early prediction of post-stroke impairment

4.2.3.1 Serum lipid levels of patients with different outcome

To investigate the impact of certain lipid levels on the outcome of stroke patients, we included a second serum cohort, consisting of 51 ischemic stroke patients (25 male, 26 female) with an average age of 73. From the original cohort ($n = 112$), only patients with reported mRS90 score were included ($n = 107$). The patients were categorized based on their post-stroke impairment after 90 days. A good outcome was represented by an mRS90 ≤ 2 , a bad outcome by an mRS90 ≥ 3 (**Table 4.7**). Patients with a good outcome were, on average, younger and showed a lower NIHSS at admission to the hospital. The good outcome group contained fewer women and fewer diabetic patients (% of total patients).

Table 4.7 Patients with different outcomes included in the analysis. Groups based on the modified Rankin scale after 90 days (mRS90). Mean $\pm$ SD, NIHSS: National Institutes of Health Stroke Scale.

Clinical data	Good outcome ($n = 106$)	Bad outcome ($n = 52$)
	mRS90 ≤ 2	mRS90 ≥ 3
Age (years)	67.0 $\pm$ 12.1	77.0 $\pm$ 10.6
Sex (m/f)	79 (75 %)/27 (25 %)	29 (56 %)/23 (44 %)
Diabetes (% of total patients)	14 (13%)	13 (25%)
NIHSS (admission)	4.2 $\pm$ 4.1	9.0 $\pm$ 6.1

We checked for significant differences in serum levels between the cohorts to avoid batch or study effects, resulting in 101 analyzed lipids and 5 lipid ratios (**Table S6.7**). Fifteen lipids were significantly changed between patients with a good and patients with a bad outcome (**Figure 4.20**). Additionally, these lipids were significantly correlated to the actual mRS90 value, ranging from 0 to 6 (**Table 4.8**).

Results

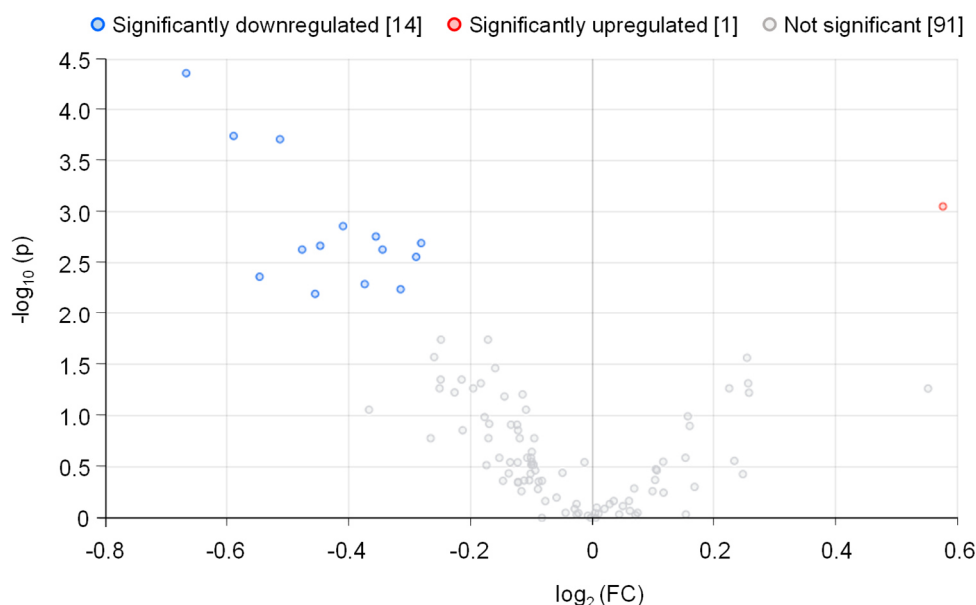


Figure 4.20 Significant changes in serum lipids regarding the outcome of ischemic stroke patients. Volcano plot shows increased and decreased lipid levels. P-value false discovery rate (FDR) adjusted. P-value threshold: 0.01, fold change threshold: 1.1. Wilcoxon Mann Whitney test, $n = 106/52$ (good outcome/bad outcome). All compounds with corresponding p-values and fold changes are listed in Table S6.7.

Five PC/PE ratios, polyunsaturated long-chain PCs, saturated LPCs and one SM were downregulated in patients with a bad outcome (**Table 4.8, Figure 4.21**). In comparison to the control group, SM (d40:1) and LPC levels were increased in patients with a bad outcome, but not significantly changed in patients with a good outcome (**Figure 4.21**). In contrast, long-chain PCs showed a significant downregulation in those patients, too. PE (38:6) was significantly upregulated in patients with a bad outcome compared to the control and the good outcome group. PC/PE ratios were increased in patients with a good outcome and decreased in patients with a bad outcome.

Table 4.8 Altered lipids and ratios in serum of ischemic stroke patients with a bad outcome. Fold change and p-value (false discovery rate (FDR) adjusted) according to Figure 4.20. Wilcoxon Mann Whitney test, $n = 106/52$ (good outcome/bad outcome). All correlations with the modified Rankin scale after 90 days (mRS90) were significant with $p \leq 0.001$ (Spearman rank correlation), r = correlation coefficient.

Compound	Fold change (FC)	$\log_2(\text{FC})$	p-value	$-\log_{10}(p)$	Correlation mRS90 (r)
LPC (18:0)	0.82	-0.28	2.00e-03	2.70	-0.36
LPC (19:0)	0.77	-0.38	4.47e-03	2.35	-0.39
LPC (22:0)	0.79	-0.34	2.33e-03	2.63	-0.35
LPC (24:0)	0.78	-0.36	1.74e-03	2.76	-0.36
PC (38:3)	0.8	-0.32	5.71E-03	2.24	-0.36
PC (40:8)	0.73	-0.45	2.14E-03	2.67	-0,37

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PC (42:9)	0.73	-0.46	6.34E-03	2.20	-0.33
PC (42:10)	0.68	-0.55	4.31E-03	2.37	-0.33
PE (38:6)	1.49	0.58	8.81e-04	3.05	0.33
SM (d40:1)	0.82	-0.29	2.75e-03	2.56	-0.33
PC (34:2)/PE (34:2)	0.70	-0.51	1.93e-04	3.71	-0.31
PC (36:4)/PE (36:4)	0.75	-0.41	1.37e-03	2.86	-0.31
PC (38:5)/PE (38:5)	0.72	-0.48	2.33e-03	2.63	-0.27
PC (38:6)/PE (38:6)	0.63	-0.67	4.35e-05	4.36	-0.39
PC (40:6)/PE (40:6)	0.66	-0.59	1.80e-04	3.75	-0.37

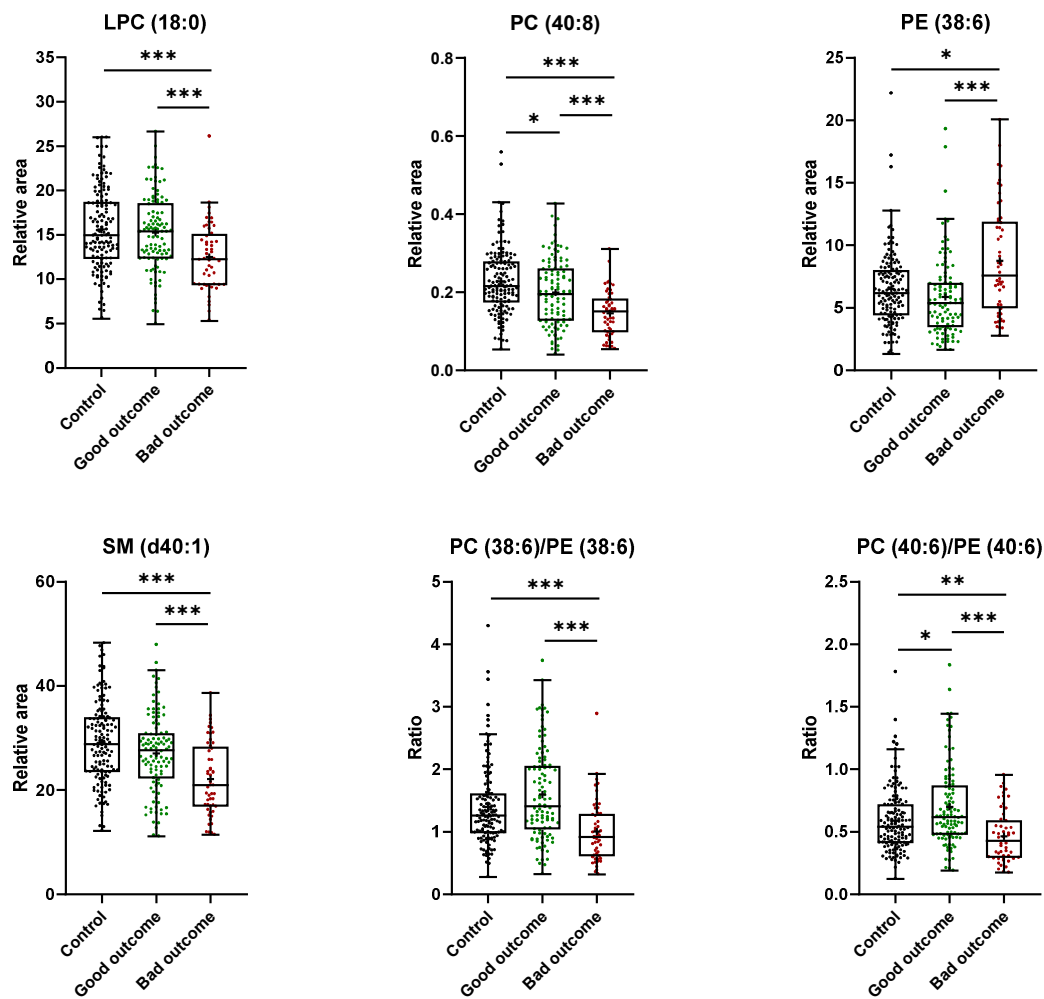


Figure 4.21 Altered lipids and ratios (representative) in serum of ischemic stroke patients with a bad outcome. Box plots (Tukey) show the distribution of data points. Boxes extend from the 25th to 75th percentiles. The line represents the median; the plus-sign the mean value. Dots correspond to the relative area of individual samples. Extreme values are located above or below the whiskers. N = 140/106/52 (control/good outcome/bad outcome), Kruskal-Wallis test with Dunn's post hoc test, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

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The differences in PC/PE ratios were consistent over three days (**Figure 4.22**). The increase on day 1 compared to the control group (**Figure 4.21**) was followed by a decrease on day 2 for patients with a good outcome. However, PC/PE ratios remained lower in patients with a bad outcome.

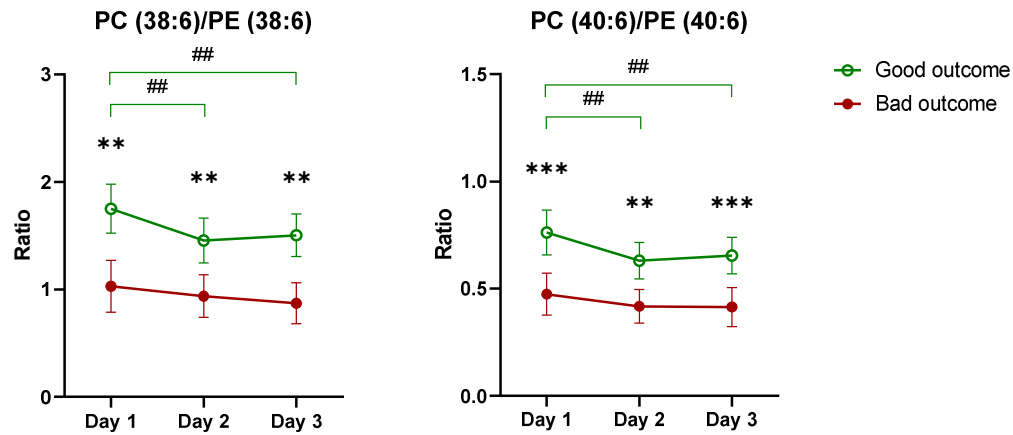


Figure 4.22 Time-dependent development of PC/PE ratios (representative) regarding the outcome of ischemic stroke patients. PC (38:6)/PE (38:6) and PC (40:6)/PE (40:6) on days 1, 2 and 3 after ischemic stroke, divided into patients with a good and patients with a bad outcome. Mean $\pm$ 95% confidence interval (CI), $n = 50/14$ (good outcome/bad outcome), two-way repeated measures ANOVA with Sidak's post test, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ## $p \leq 0.01$.

4.2.3.2 Biomarker-based model for early prediction of post-stroke impairment

An aim of the analysis was to identify a promising biomarker for the early prediction of post-stroke impairment. A sparse partial least squares regression discriminant analysis (sPLS-DA) was performed to determine variables discriminating between patients with a good and a bad outcome. Dimensionality reduction resulted in the organization of samples based on two components (**Figure 4.23 A**). Two clusters were formed, but no distinct separation of the two groups. Loading plots showed that the NIHSS at admission (stroke severity) and age, as well as PC (38:6)/PE (38:6), contributed most to component 1 (**Figure 4.23 B**). The association of stroke severity and age with the long-term outcome has been reported before [40, 41]. The PC (38:6)/PE (38:6) ratio already showed significant differences between patients with good and bad outcome in our study (chapter 4.2.3.1) and was therefore further investigated.

Results

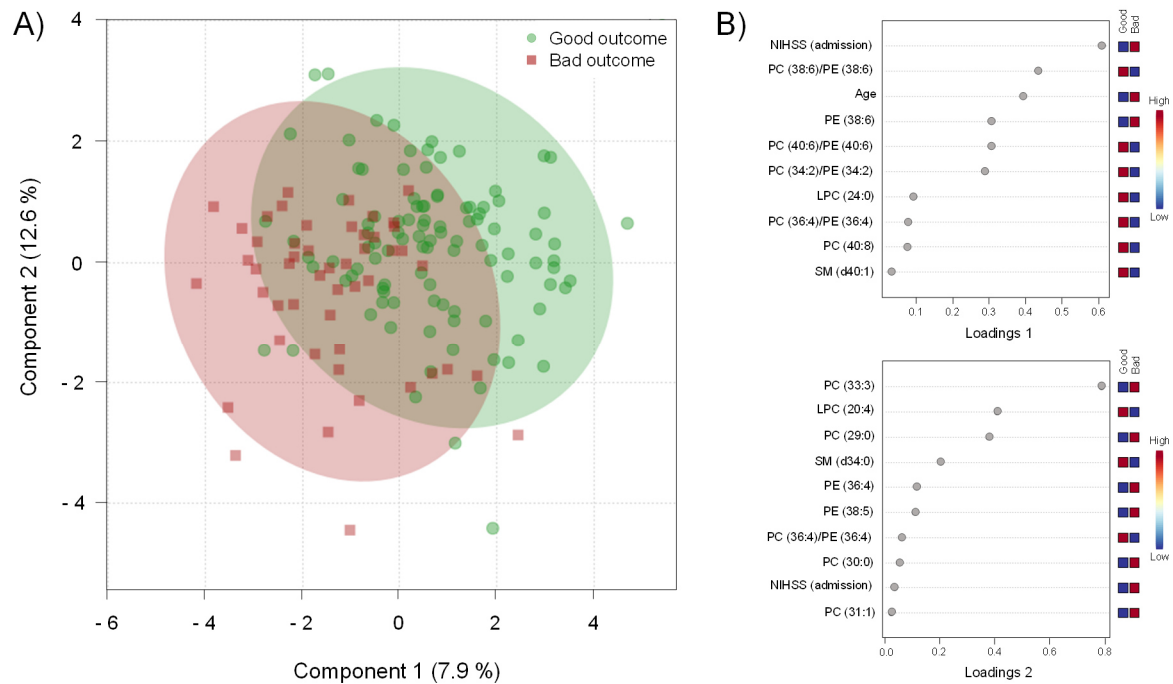


Figure 4.23 sPLS-DA for feature selection to predict the outcome of stroke patients. (A) 2D scores plot with 95 % confidence region. Explained variation for each component in parenthesis. Each symbol represents a patient. (B) Loading plots of components 1 and 2. Variables are ranked by the absolute values of their loadings. Blue and red squares indicate high or low serum levels.

Receiver operating characteristics (ROC-) analysis is helpful to visualize the diagnostic power of disease-related factors. Therefore, we performed a univariate ROC analysis for PC (38:6)/PE (38:6) (**Figure 4.24 A**). The ROC curve plots the true positive rate (sensitivity) against the false positive rate (specificity) of different decision thresholds for a binary output (good/bad outcome of patients). The box plot on the left illustrates each datapoint and the decision threshold (blue line), with correctly and falsely classified patients above and below the line, respectively. The area under the curve (AUC) was 0.75. An AUC of 0.5 would indicate a random assignment of samples; the ideal value would be 1. The combination of NIHSS and age in a ROC curve based model resulted in an AUC of 0.82 and a predictive accuracy of 76 % (**Figure 4.24 B**). Adding the PC (38:6)/PE (38:6) ratio to the model increased the AUC, but not the predictive power. Therefore, changes in the PC/PE ratio are likely to be directly related to stroke severity and/or age. Indeed, PC (38:6)/PE (38:6) was significantly correlated with NIHSS and age ($r = -0.24$ (NIHSS) and $r = -0.36$ (age), based on a Spearman rank correlation).

Results

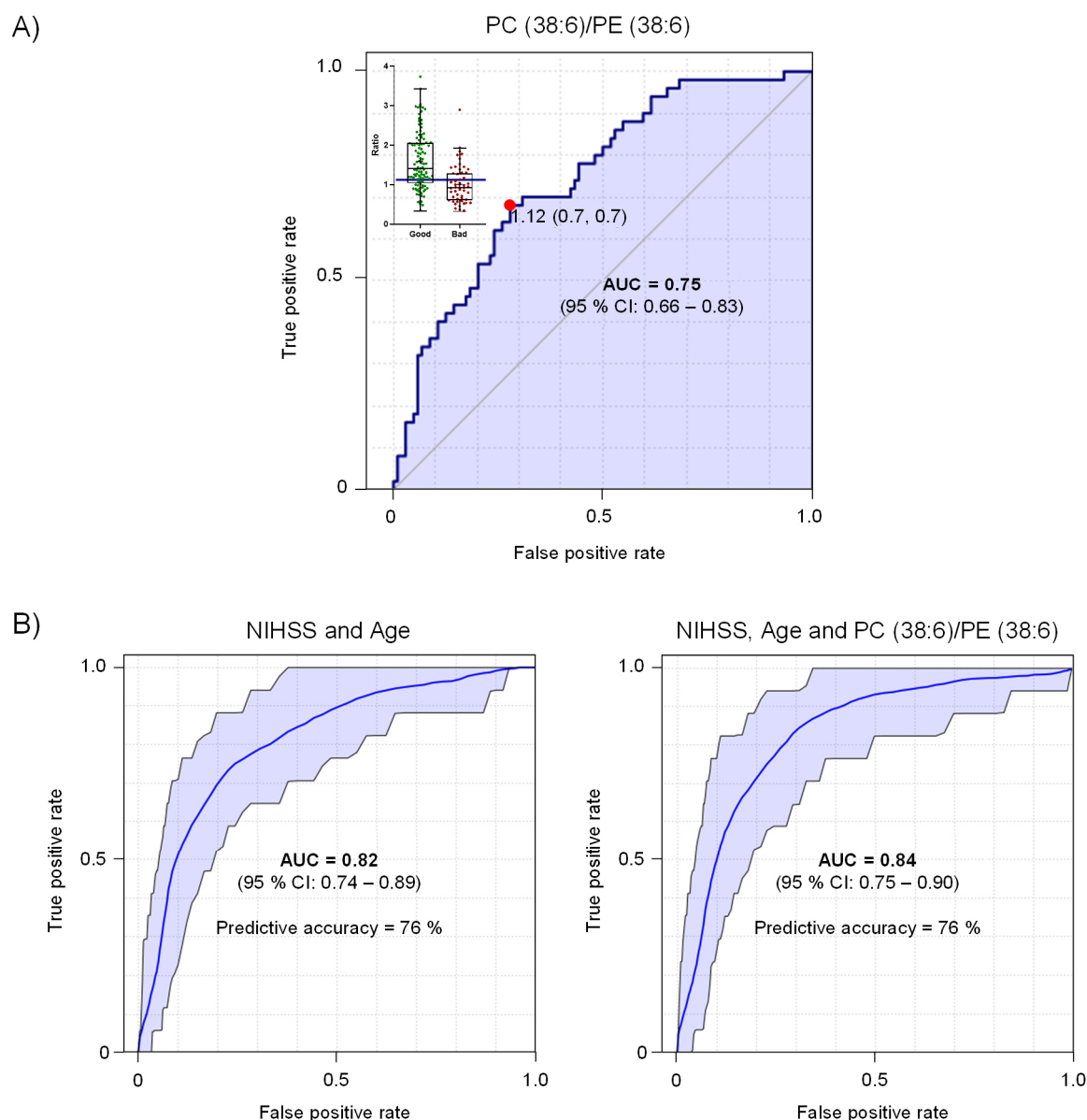


Figure 4.24 Predictive power of PC (38:6)/PE (38:6) to determine the outcome of patients. (A) Univariate ROC analysis of PC (38:6)/PE (38:6). Red mark represents the best cut-off/decision threshold, chosen based on closest to top left corner, sensitivity and specificity in parenthesis. Area under the curve (AUC) with 95% confidence interval (CI) in parenthesis. Box plot shows data distribution. Blue line marks the best decision threshold. (B) ROC curve based model for NIHSS and age, with and without inclusion of PC (38:6)/PE (38:6). ROC curve with 95 % CI. Predictive accuracy and ROC curve based on 100 cross validations.

4.3 Changed metabolite levels in a mouse model of ischemic stroke

To further investigate metabolic processes involved in stroke, we incorporated a stroke mouse model. The permanent distal middle cerebral artery occlusion (pMCAO) induces an ischemic injury

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and allows the analysis of metabolite and lipid levels under controlled conditions. We performed a lipidomics and metabolomics analysis of plasma, liver and brain tissue from MCAO mice. Two different time points were investigated (2 hours and 24 hours after MCAO).

4.3.1 Changed metabolite levels in the brain of MCAO animals

The brain is the primary organ affected by MCAO. As expected, numerous alterations in metabolite and lipid levels were observed on the affected, ipsilateral side compared to the contralateral side of the brain (**Figure 4.25**, **Figure 4.26**). Acute changes already occurred after 2 hours, although alterations were more pronounced after 24 hours. Significantly changed compounds relevant to our study are listed in **Table 4.9** and **Table 4.10**. A more comprehensive list can be found in the supplement (**Table S6.8**).

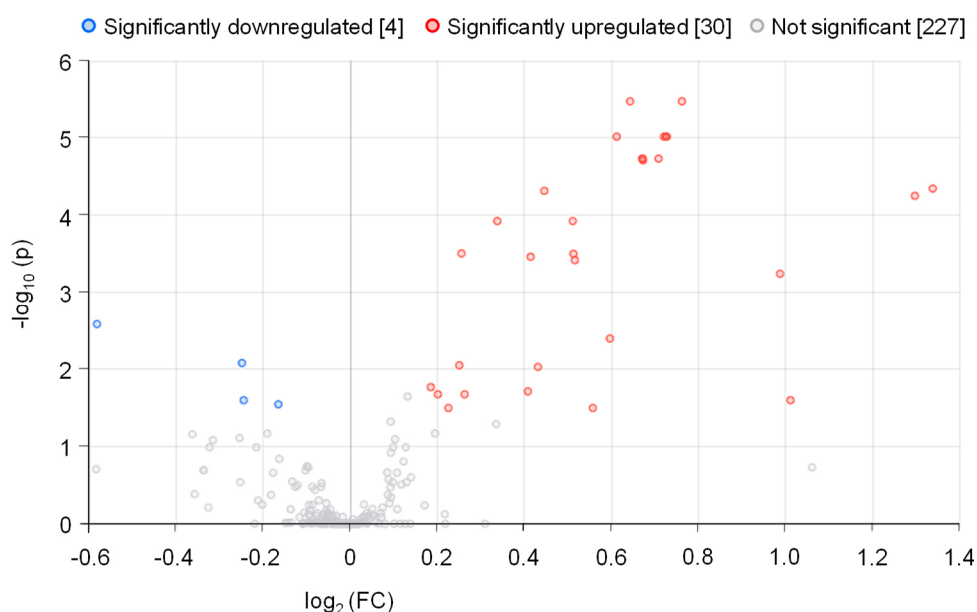


Figure 4.25 Significant changes in the brain of MCAO mice after 2 hours. Volcano plot shows increased and decreased levels of metabolites and lipids on the ipsilateral side of the brain compared to the contralateral side. P-value false discovery rate (FDR) adjusted. P-value threshold: 0.05, fold change threshold: 1.1. Paired Student's t-test, $n = 16$.

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Table 4.9 Selection of significantly changed compounds in the brain of MCAO mice after 2 hours. Fold change and *p*-value (false discovery rate (FDR) adjusted) according to Figure 4.25. Paired Student's *t*-test, *n* = 16.

Compound	Fold change (FC)	log ₂ (FC)	p value	-log ₁₀ (p)
α-Aminoadipate	1.59	0.67	1.84e-05	4.73
Argininosuccinic acid	1.43	0.51	3.15e-04	3.50
Asparagine	1.19	0.26	3.11e-04	3.51
Citrulline	2.46	1.30	5.58e-05	4.25
Cystathionine	1.36	0.45	4.82e-05	4.32
Dimethylarginine	1.35	0.43	9.39e-03	2.03
Glycine	1.19	0.25	8.94e-03	2.05
Histidine	1.7	0.76	3.34e-06	5.48
Leucine	1.65	0.72	9.58e-06	5.02
Methionine	1.66	0.73	9.58e-06	5.02
Phenylalanine	1.59	0.67	1.84e-05	4.73
Phosphoethanolamine	0.84	-0.24	2.52e-02	1.60
Proline	1.65	0.72	9.58e-06	5.02
S-Adenosylhomocysteine	1.26	0.34	1.19e-04	3.93
Serine	1.15	0.20	2.12e-02	1.67
Threonine	1.56	0.64	3.34e-06	5.48
Tryptophan	1.59	0.67	1.94e-05	4.71
Tyrosine	1.63	0.71	1.84e-05	4.73

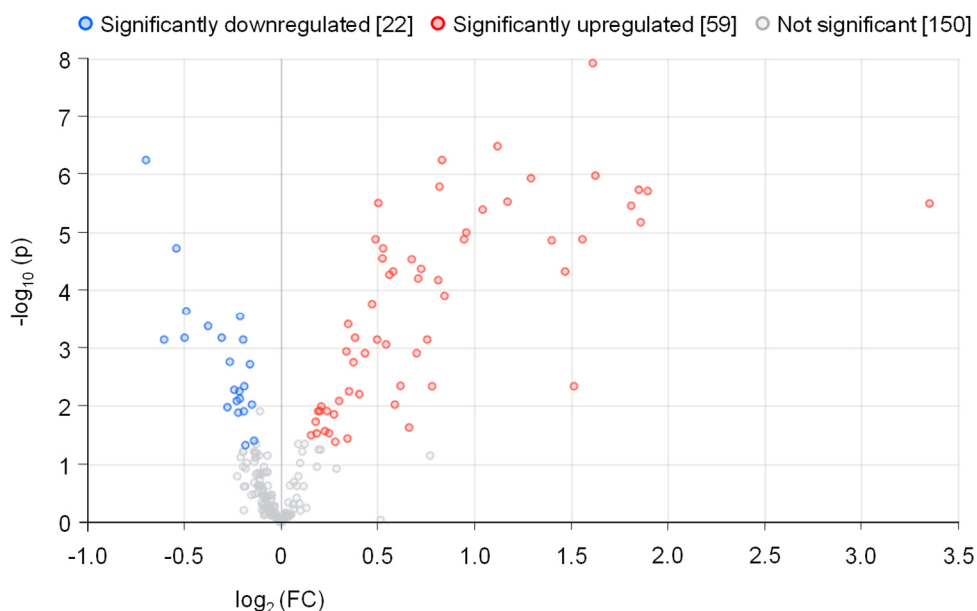


Figure 4.26 Significant changes in the brain of MCAO mice after 24 hours. Volcano plot shows increased and decreased levels of metabolites and lipids on the ipsilateral side of the brain compared to the contralateral side. *P*-value false discovery rate (FDR) adjusted. *P*-value threshold: 0.05, fold change threshold: 1.1. Paired Student's *t*-test, *n* = 15.

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Table 4.10 Selection of significantly changed compounds in the brain of MCAO mice after 24 hours Fold change and p-value (false discovery rate (FDR) adjusted) according to Figure 4.26. Paired Student's t-test, n = 15.

Compound	Fold change (FC)	log ₂ (FC)	p value	-log ₁₀ (p)
5'-Methylthioadenosine	0.82	-0.28	1.04e-02	1.98
α-Aminoadipate	2.76	1.47	4.66e-05	4.33
Arginine	1.42	0.50	3.07e-06	5.51
Betaine	3.05	1.61	1.18e-08	7.93
Choline	1.13	0.18	1.85e-02	1.73
Citrulline	3.62	1.86	6.59e-06	5.18
Cystathionine	2.17	1.12	3.21e-07	6.49
Glycine	1.3	0.37	1.77e-03	2.75
Histidine	1.62	0.70	1.24e-03	2.91
Leucine	1.58	0.66	2.33e-02	1.63
Methionine	1.47	0.56	5.31e-05	4.28
Phenylalanine	1.53	0.62	4.49e-03	2.35
Proline	1.35	0.43	1.24e-03	2.91
Tryptophan	1.69	0.75	7.20e-04	3.14
Tyrosine	1.41	0.49	7.20e-04	3.14
Valine	1.94	0.96	9.90e-06	5.00
Carnitine	1.44	0.52	2.78e-05	4.56
Acetylcarnitine	2.25	1.17	2.92e-06	5.53
AcCa (3:0)	3.08	1.62	1.03e-06	5.99
AcCa (14:0)	1.21	0.27	1.38e-02	1.86
AcCa (16:0)	1.6	0.67	2.87e-05	4.54
AcCa (18:0)	2.06	1.04	3.99e-06	5.40
LPC (16:0)	1.65	0.72	4.21e-05	4.38
LPC (17:0)	1.63	0.71	6.16e-05	4.21
LPC (18:0)	1.45	0.54	8.72e-04	3.06
LPC (20:0)	1.21	0.28	4.10e-02	1.39
LPC (20:4)	0.77	-0.38	4.21e-04	3.38
LPC (22:6)	0.71	-0.50	6.69e-04	3.17
PC (31:0)	0.86	-0.21	2.83e-04	3.55
PC (33:0)	0.9	-0.15	9.42e-03	2.03
PC (34:3)	1.15	0.21	1.01e-02	1.99
PC (37:4)	0.87	-0.20	7.20e-04	3.14
PC (39:6)	0.86	-0.22	5.57e-03	2.25
PC (40:8)	1.13	0.18	2.94e-02	1.53
Palmitoyl-EA	10.19	3.35	3.12e-06	5.51
Oleoyl-EA	2.94	1.56	1.30e-05	4.89

After 2 hours, numerous amino acids were upregulated on the ipsilateral side of the brain. Interestingly, argininosuccinic acid, dimethylarginine and citrulline were all elevated (**Figure 4.27**, **Figure 4.28**). Arginine, a metabolite of all three, could not be detected in this cohort. Furthermore, methionine, cystathionine and S-adenosylhomocysteine (SAH) were increased on the side of the ischemic injury. These metabolites are involved in the transmethylation and transsulfuration

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pathway. Phosphoethanolamine, which is part of the CDP-ethanolamine pathway that generates PEs, was downregulated.

The increase of most amino acids on the ipsilateral side was persistent 24 hours after MCAO (**Figure 4.28**). In this cohort, we could not detect argininosuccinic acid or dimethylarginine, but we observed an increase in arginine (**Figure 4.29**). Citrulline and cystathionine levels were even more strongly elevated on the ipsilateral side after 24 hours, compared to the 2 hours cohort. The increased betaine, a metabolite of the likewise elevated choline, is also part of the transmethylation pathway and acts as a methyl donor in the remethylation of homocysteine to methionine. 5'-Methylthioadenosine was decreased on the ipsilateral side.

Carnitine and all detected acylcarnitines were increased after 24 hours on the ipsilateral side (**Figure 4.29**). LPCs with saturated FA residues were upregulated. In contrast, LPCs with PUFAs were decreased. For PCs, changes were not consistent. Apparent changes could be observed for palmitoyl-EA and oleoyl-EA. Both ethanolamides were strongly upregulated after ischemia.

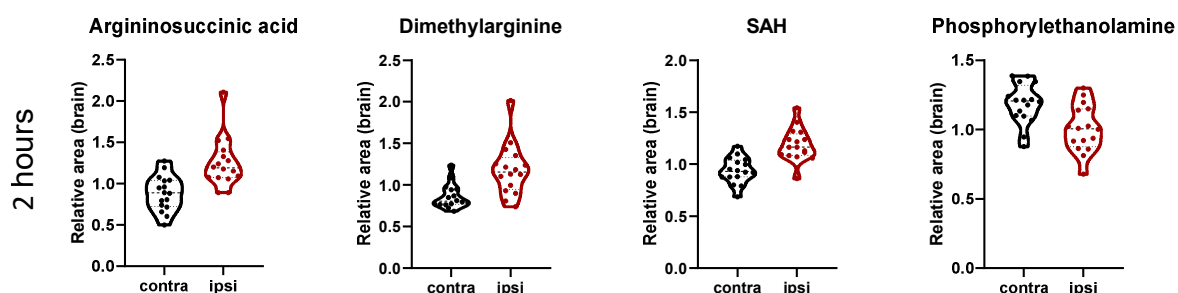


Figure 4.27 Metabolites (representative) changed in the brain 2 hours after MCAO. Fold changes and *p*-values see Table 4.9. Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. *N* = 16, SAH = S-adenosylhomocysteine.

Results

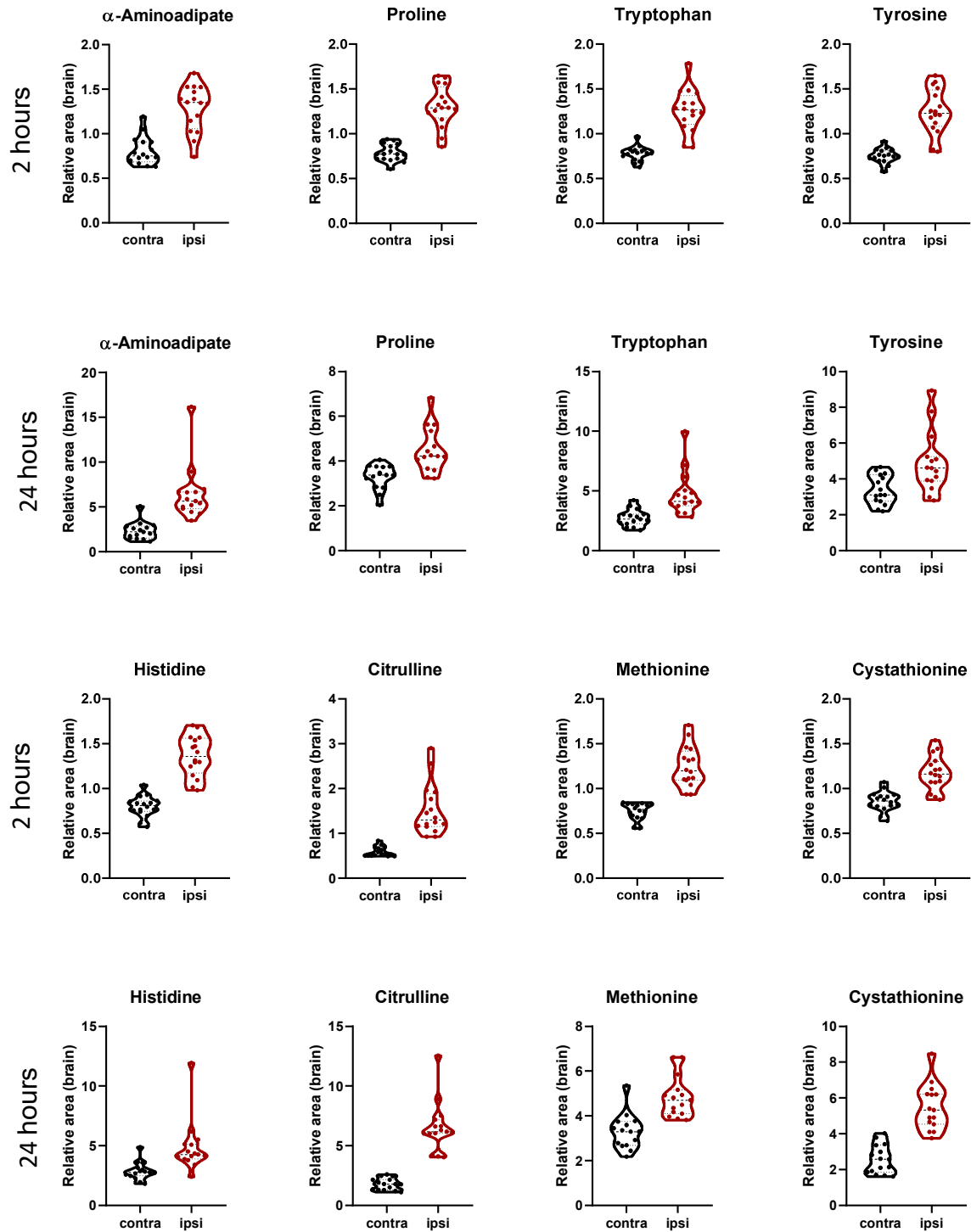


Figure 4.28 Metabolites (representative) changed in the brain both 2 hours and 24 hours after MCAO. Fold changes and p-values see Table 4.9 and Table 4.10. Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. N = 16 (2 hours), n = 15 (24 hours).

Results

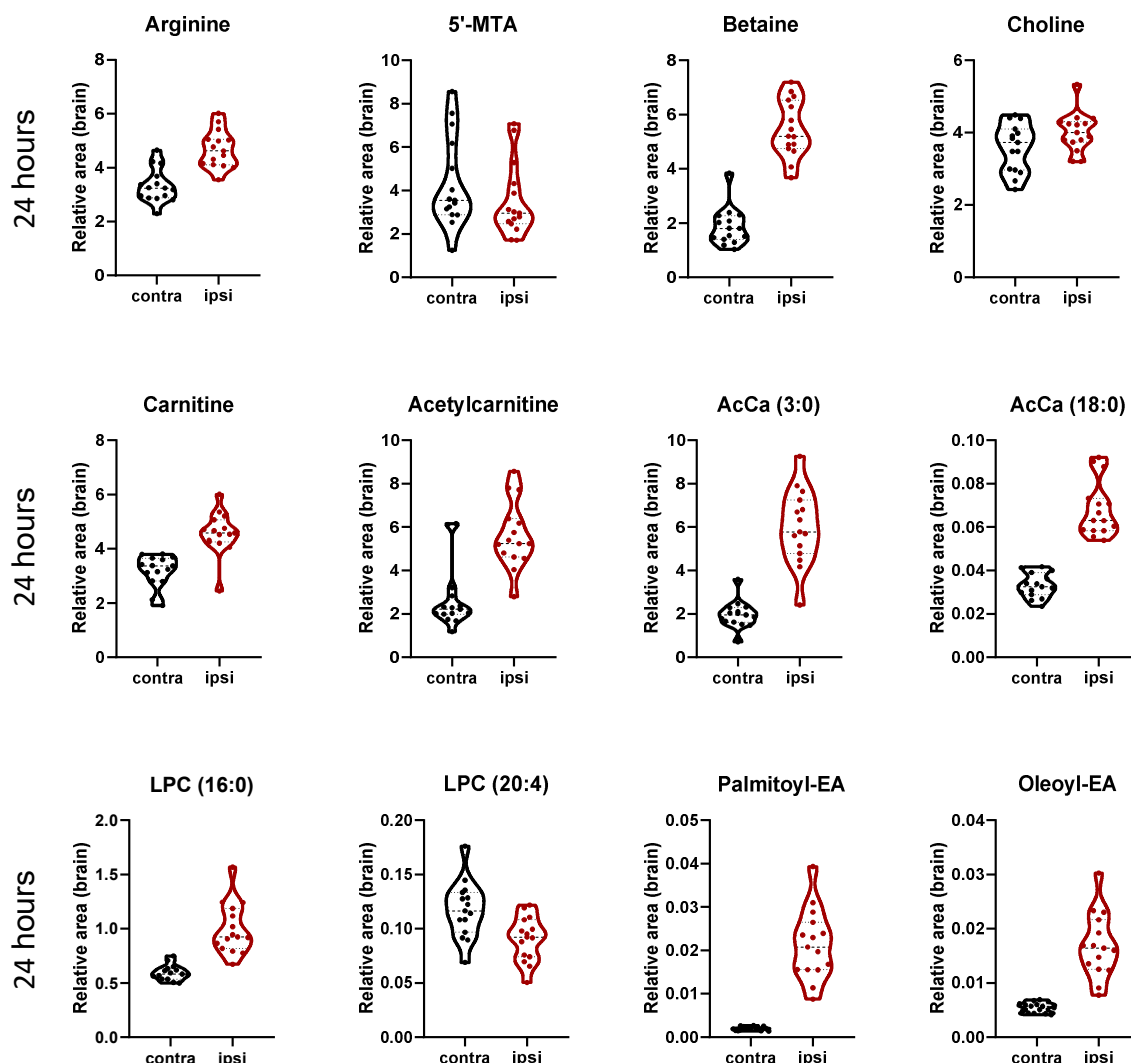


Figure 4.29 Metabolites (representative) changed in the brain 24 hours after MCAO. Fold changes and *p*-values see Table 4.10. Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. *N* = 15, 5'-MTA = 5'-methylthioadenosine.

4.3.2 Changed metabolite levels in relation to the infarct size

Corresponding to the outcome-related results from the human study (chapter 4.2.3.1), the changes of lipids and metabolites in relation to the measured infarct size (2 hours and 24 hours after MCAO) were analyzed. The Swanson corrected infarct volume was determined based on a silver staining. Additionally, the neurological deficit was tested with a latency to move test after 24 hours. The time mice needed to move one body length was measured before and after the MCAO, and the ratio (time taken after/time taken before MCAO) was used for analysis.

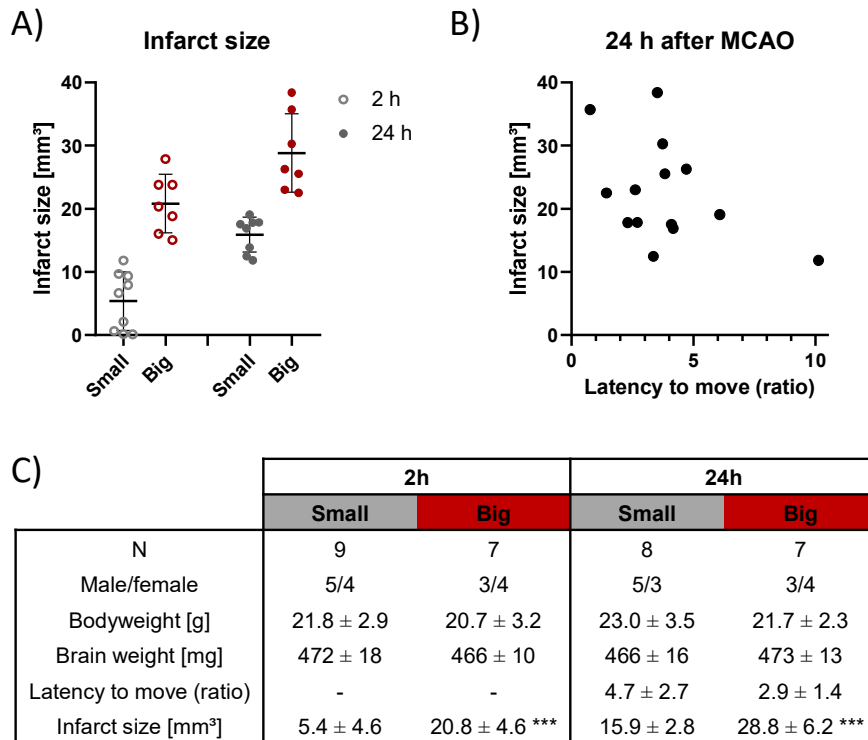


Figure 4.30 Infarct size and latency to move 2 hours and 24 hours after MCAO. (A) Classification of mice (small/big infarct) based on the infarct volume (Swanson corrected), $n = 16$ (2 hours), $n = 15$ (24 hours). Mean $\pm$ SD. (B) No correlation (Pearson) between infarct size and latency to move was observed 24 hours after MCAO. (C) Group allocation of mice according to (A). Significant changes are marked (unpaired Student' t -test, *** $p \leq 0.001$). One animal was excluded for the analysis of the latency to move, because it did not move during the test at baseline level. Data reported as mean $\pm$ SD.

As expected, the overall infarct volume after 24 hours was more pronounced than 2 hours after MCAO (**Figure 4.30 A**). Interestingly, there was no correlation between the infarct size and the latency to move 24 hours after stroke (**Figure 4.30 B**). Based on the median, mice were separated into animals with a small or a big infarct (**Figure 4.30 A, C**). No differences were observed regarding the defined groups for body and brain weight, and the sex distribution was similar. After 24 hours, there was no significant difference in the latency to move when comparing the small and big infarct group.

Already after 2 hours, various lipids and metabolites in the plasma and the brain showed a significant correlation with the infarct size, indicating an immediate effect of the MCAO not only on the affected tissue but also on the peripheral metabolism (**Table 4.11**). To corroborate these findings, absolute changes between small and big infarct animals were also analyzed.

Results

Table 4.11 Correlation and fold change analysis in relation to the infarct size after 2 hours. Metabolites and lipids in plasma and brain (ipsilateral side) correlated to the measured infarct size (Swanson corrected). Pearson correlation coefficient (*r*) and *p*-value (*p*), *n* = 16. Fold change analysis between mice with a big or small infarct. For classification see Figure 4.30 A. Student's *t*-test for plasma, two-way repeated measures ANOVA with Sidak's post test for the brain. *N* = 9/7 (small infarct/big infarct). **p* < 0.05, ***p* < 0.01, ****p* < 0.001. A list of all analyzed compounds can be found in Table S6.9.

		<i>r</i> (infarct size)	<i>p</i> (correlation)	FC (big/small)	<i>p</i> (big/small)
Plasma	LPC (22:0)	-0.52	*	0.91	
	LPC (26:1)	-0.55	*	0.92	
	PC (37:3)	-0.52	*	0.83	
	PC (38:4)	-0.56	*	0.90	
	PC (38:5)	-0.60	*	0.87	
	PC (39:5)	-0.57	*	0.81	
	PC (39:7)	-0.53	*	0.75	
	PC (40:7)	-0.60	*	0.85	
	PC (38:6)/PE (38:6)	-0.67	**	0.89	*
	PC (40:7)/PE (40:7)	-0.53	*	0.90	
Brain (ispi)	PE (36:4)	-0.58	*	0.92	
	PE (38:4)	-0.62	**	0.91	
	PE (38:6)	-0.63	**	0.89	*
	PE (40:4)	-0.73	**	0.89	*
	PE (40:6)	-0.73	**	0.89	*
	PE (40:7)	-0.62	**	0.91	
	α-Aminoadipate	0.53	*	1.25	**
	Glutathione	-0.60	*	0.81	
	Citrulline	0.64	**	1.77	***

Several LPC and PC levels in the plasma were negatively correlated with the infarct size. Nevertheless, no significance was reached when plasma levels were compared between animals with a small and animals with a big infarct (**Table 4.11**). Two PC/PE ratios decreased in plasma with increasing infarct size (**Figure 4.31 A, B**). The lower ratio in the big infarct group originated from slightly bigger changes in PC than PE levels (**Figure 4.31 C**). These results are similar to the observation in ischemic stroke patients, where PC/PE ratios were decreased in the serum of patients with a bad outcome and negatively correlated with the mRS90 score and stroke severity (**Figure 4.21, Table 4.8**).

Results

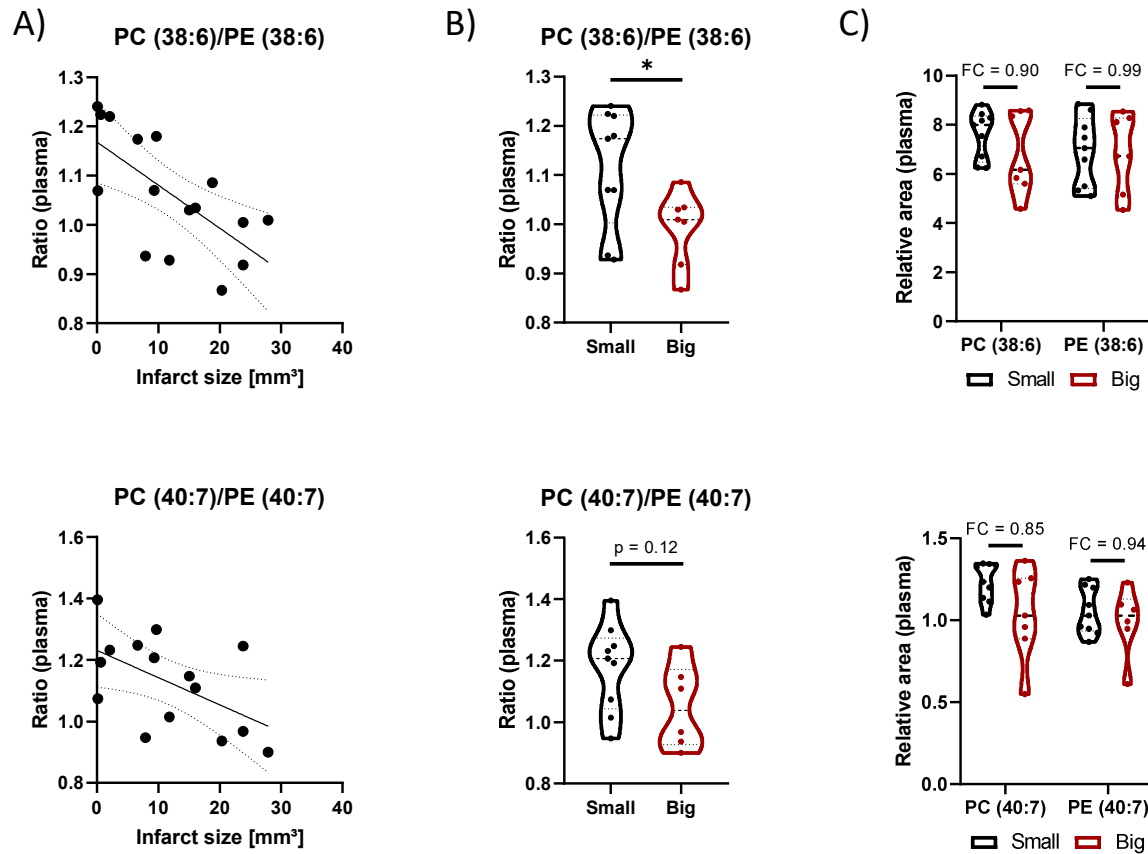


Figure 4.31 Changed PC/PE ratios in plasma in relation to the infarct size after 2 hours. (A) Correlation with the infarct size, correlation coefficient (r) and p -value according to Table 4.11. Linear regression lines and 95% confidence intervals (CI) are shown. (B) Comparison of animals with a small infarct and animals with a big infarct. Student's t -test, fold changes (FC) and p -values according to Table 4.11. (C) Changes of PCs and PEs contributing to the PC/PE ratios shown in (A) and (B). $N = 9/7$ (small infarct/big infarct). Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

PE levels on the ipsilateral brain side showed a strong negative correlation with the infarct size. Thus, decreased levels in animals with a big infarct were detected in comparison to mice with small infarcts (Table 4.11, Figure 4.32). The same pattern was observed for glutathione, an antioxidant, whereas α -amino adipate and citrulline were positively correlated, with a significant increase in the big infarct group. On the contralateral side of the brain, there was no significant difference in PE levels in comparison to the ipsilateral side or between the small and big infarct size groups. For glutathione, a downward trend on the ipsilateral compared to the contralateral side of animals with a big infarct was indicated. α -Amino adipate and citrulline were upregulated on the ipsilateral side in both groups. A difference on the contralateral side depending on the infarct size was only observed for α -amino adipate.

Results

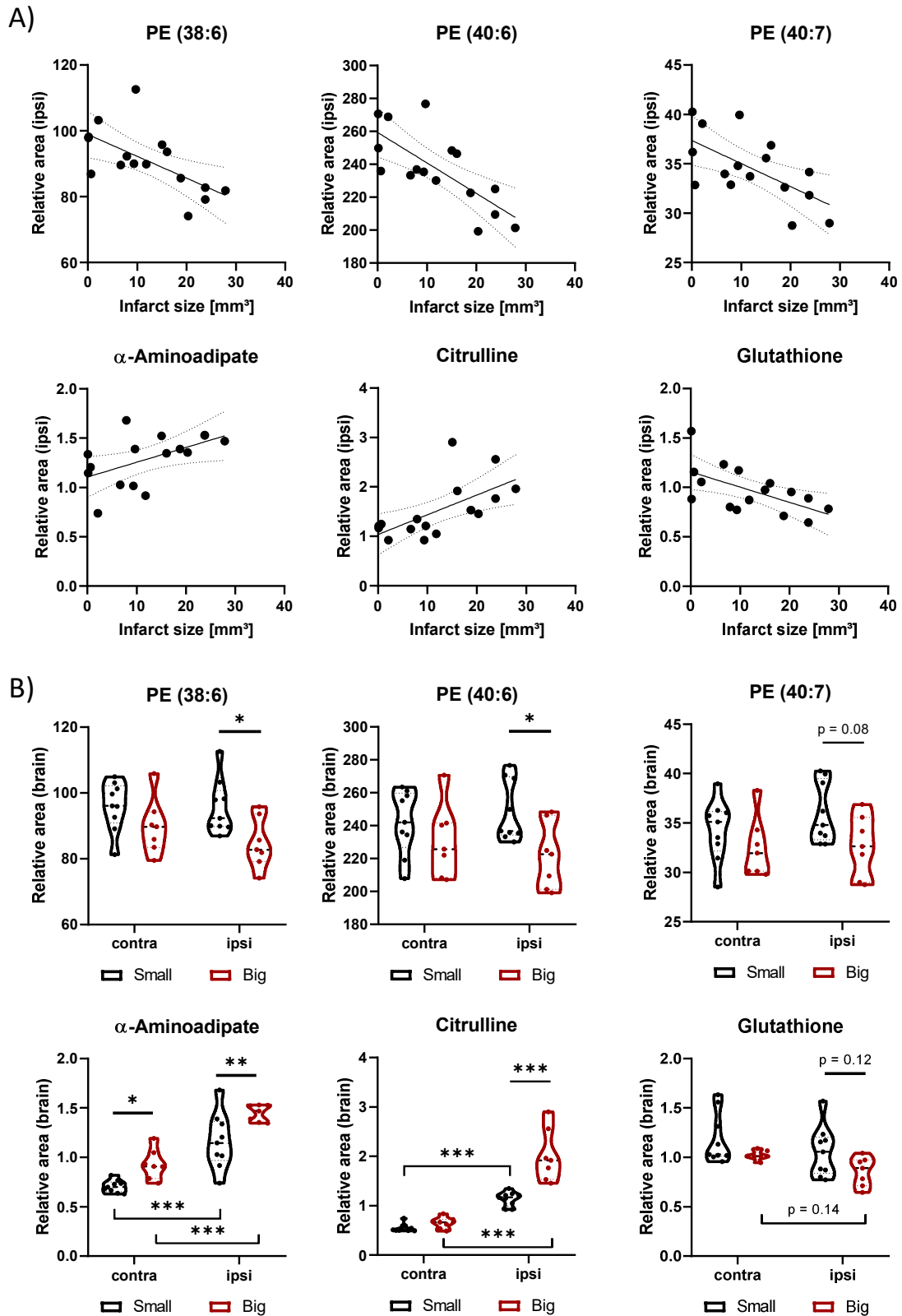


Figure 4.32 Changed PEs (representative) and metabolites in the brain in relation to the infarct size after 2 hours. (A) Correlation with infarct size, correlation coefficient (r) and p -value according to Table 4.11. Linear regression lines and 95% confidence intervals (CI) are shown. (B) Comparison of animals with a small and a big infarct for contra- and ipsilateral side of the brain. Fold changes (FC) and p -values for the ipsilateral side according to Table 4.11. Two-way repeated measures ANOVA with Sidak's post test. $N = 9/7$ (small infarct/big infarct). Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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Table 4.12 Correlation and fold change analysis in relation to the infarct size after 24 hours. Metabolites and lipids in plasma, brain (ipsilateral side) and liver correlated to the measured infarct size (Swanson corrected). Pearson correlation coefficient (*r*) and *p*-value (*p*), *n* = 15. Fold change analysis between mice with a big or small infarct. Student's *t*-test for plasma/liver, two-way repeated measures ANOVA with Sidak's post test for the brain. *N* = 8/7 (small infarct/big infarct). **p* < 0.05, ***p* < 0.01, ****p* < 0.001. A list of all analyzed compounds can be found in Table S6.9.

		r (infarct size)	p (correlation)	FC (big/small)	p (big/small)
Plasma	Acetylcarnitine	-0.63	*	0.75	
	PC (32:1)	-0.68	**	0.72	**
	PC (33:1)	-0.57	*	0.70	
	PC (34:1)	-0.55	*	0.83	
	TG (48:3)	-0.71	**	0.34	***
	TG (49:3)	-0.73	**	0.55	***
	TG (50:3)	-0.64	*	0.59	**
	TG (50:4)	-0.73	**	0.53	***
	TG (51:2)	-0.69	**	0.65	***
	TG (51:3)	-0.72	**	0.69	**
	TG (51:4)	-0.67	**	0.67	*
	TG (52:5)	-0.57	*	0.67	*
	TG (53:2)	-0.60	*	0.68	**
	TG (53:5)	-0.69	**	0.69	*
	TG (58:3)	-0.62	*	0.47	*
	TG (58:5)	-0.57	*	0.59	*
	TG (58:6)	-0.58	*	0.65	**
Brain (ispi)	Palmitoyl-EA	0.52	*	1.49	**
	Oleoyl-EA	0.53	*	1.55	**
	Citrulline	0.69	**	1.34	*
Liver	PC (36:2)	0.54	*	1.15	
	PC (38:4)	0.54	*	1.16	
	PC (36:2)/PE (36:2)	0.59	*	1.14	
	PC (38:6)/PE (38:6)	0.62	*	1.13	**
	PC (40:6)/PE (40:6)	0.57	*	1.22	
	PC (40:7)/PE (40:7)	0.61	*	1.19	*
	PC (40:8)/PE (40:8)	0.64	*	1.23	*

Different metabolites and lipids were associated with the infarct size 24 hours after MCAO in plasma, brain and liver (**Table 4.12**). Acetylcarnitine in the plasma was negatively correlated with the infarct size and showed a trend towards a downregulation in the big infarct group (**Figure 4.33**). Compared to the 2 hours cohort (**Table 4.11**), negatively correlated PCs contained shorter and more saturated FAs. Numerous TG species with different chain lengths and saturation degrees decreased with increasing infarct size. The strong correlation of TGs was emphasized by a

Results

significant difference between plasma levels of animals with a small and a big infarct size (**Figure 4.33 B**).

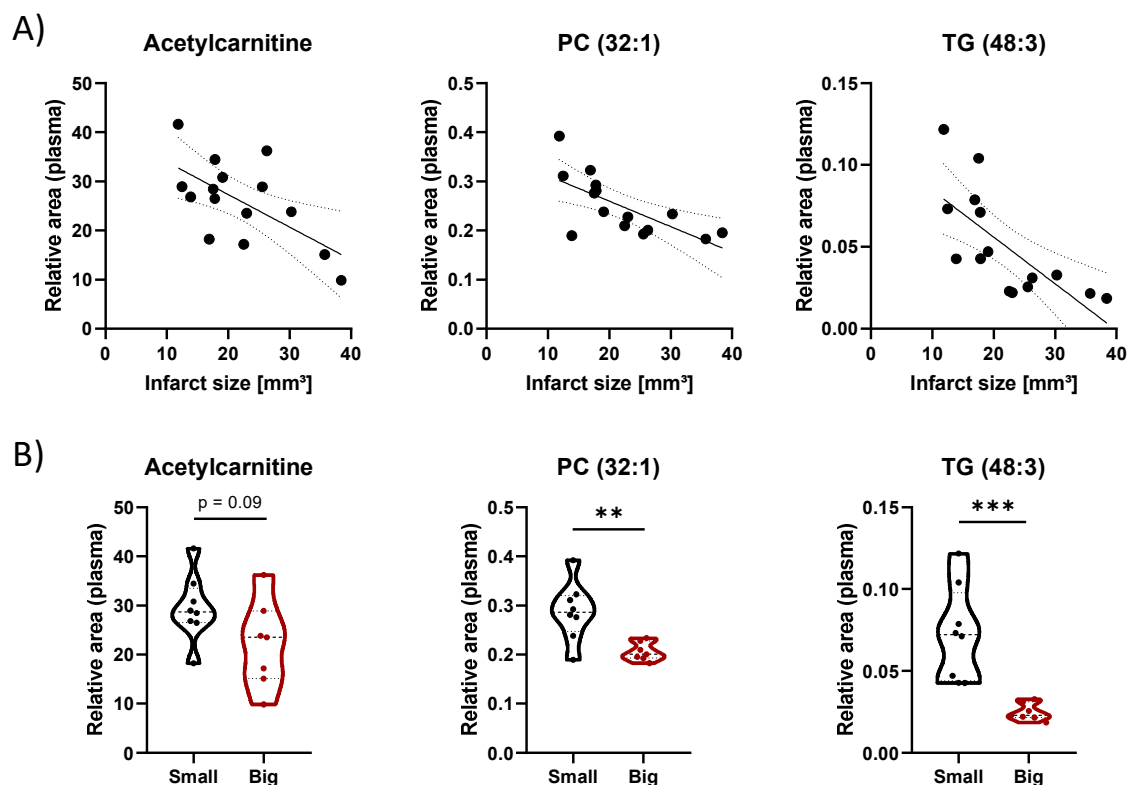


Figure 4.33 Changed lipids (representative) and metabolites in plasma in relation to the infarct size after 24 hours. (A) Correlation with the infarct size, correlation coefficient (r) and p-value according to Table 4.12. Linear regression lines and 95% confidence intervals (CI) are shown. (B) Comparison of animals with a small infarct and a big infarct. Fold changes (FC) and p-values according to Table 4.12. Student's t-test, $n = 8/7$ (small infarct/big infarct). Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Two PCs and five PC/PE ratios in the liver were positively correlated with the infarct size (**Table 4.12**). Although fold changes were relatively small, PC/PE ratios were elevated in the big infarct group compared to animals with a small infarct (**Figure 4.34 A, B**). Levels of corresponding PCs and PEs reflected in the ratios were marginally changed, but elevated PC and decreased PE levels resulted in altered PC/PE ratios (**Figure 4.34 C**).

Results

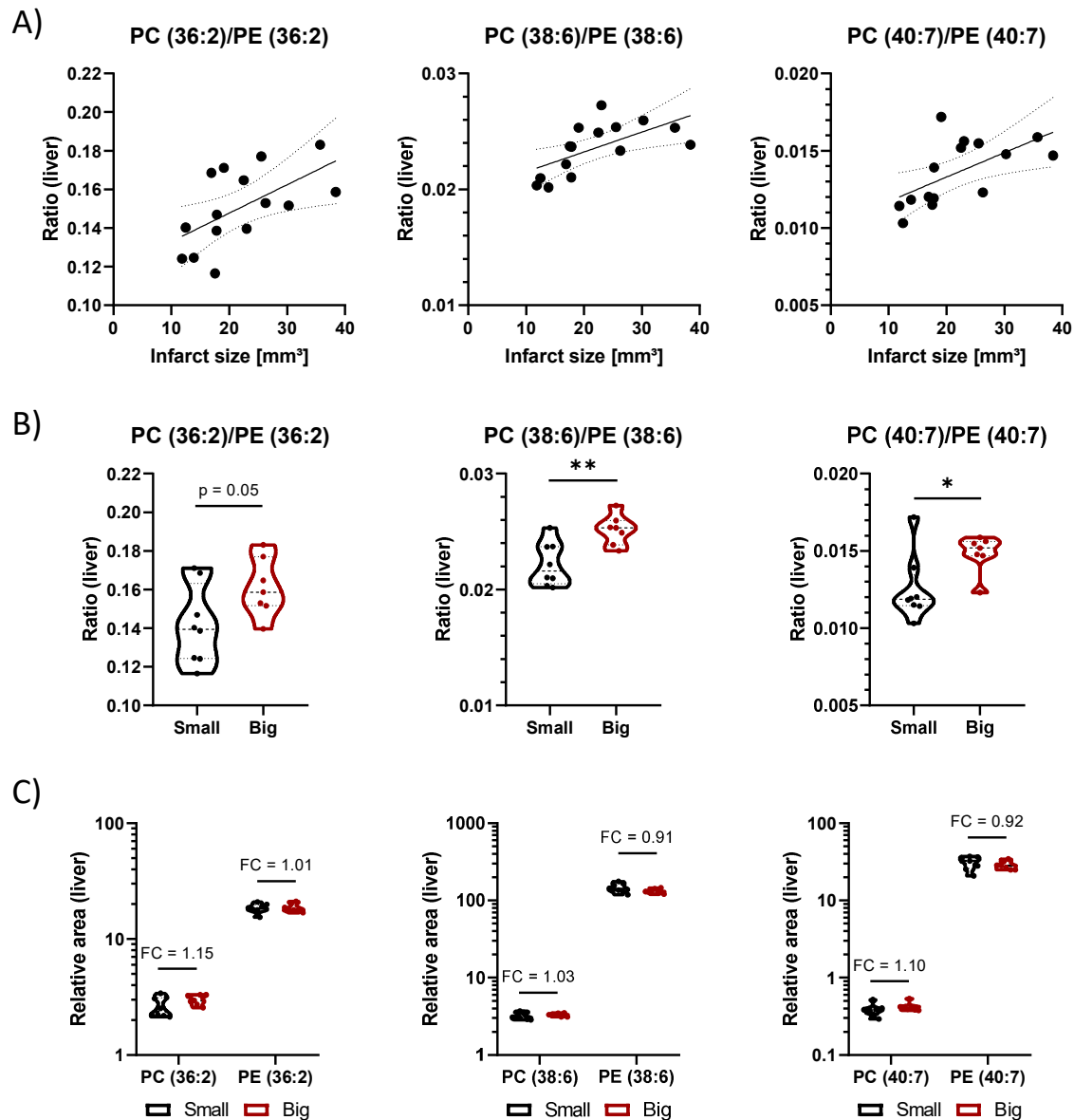


Figure 4.34 Changed PC/PE ratios (representative) in the liver in relation to the infarct size after 24 hours. (A) Correlation with the infarct size, correlation coefficient (r) and p -value according to Table 4.12. Linear regression lines and 95% confidence intervals (CI) are shown. (B) Comparison of animals with a small infarct and animals with a big infarct. Fold changes (FC) and p -values according to Table 4.12. (C) Changes of PCs and PEs contributing to the PC/PE ratios shown in (A) and (B). Student's t -test, $n = 8/7$ (small infarct/big infarct). Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Palmitoyl-EA, oleoyl-EA and citrulline on the ipsilateral side of the brain were positively correlated with the infarct size (Table 4.12, Figure 4.35 A). A dramatic increase was observed compared to the contralateral side for both infarct size groups (Figure 4.35 B). However, the increase was more pronounced for animals with a big infarct, reflected by the significant difference on the ipsilateral side compared to animals with a small infarct.

Results

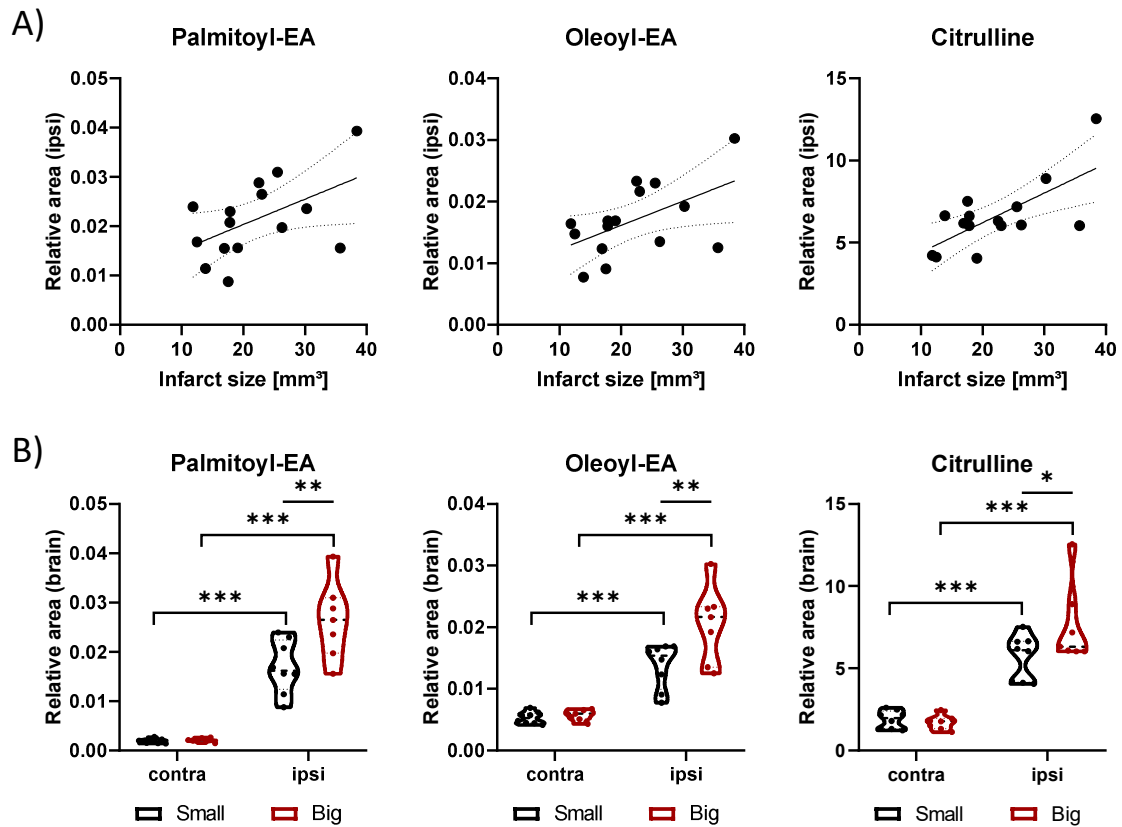


Figure 4.35 Changed lipids and metabolites in the brain in relation to the infarct size after 24 hours. (A) Correlation with the infarct size, correlation coefficient (r) and p -value according to Table 4.12. Linear regression lines and 95% confidence intervals (CI) are shown. (B) Comparison of animals with a small infarct and animals with a big infarct for contra- and ipsilateral side of the brain. Fold changes (FC) and p -values for the ipsilateral side according to Table 4.12. Two-way repeated measures ANOVA with Sidak's post test. $N = 8/7$ (small infarct/big infarct). Violin plots show the distribution of data points. Graphs extend from minimum to maximum. The dashed line represents the median; dotted lines the quartiles. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

5 Discussion

5.1 Metabolomics screen

5.1.1 Generation of an in-house MS² library

The use of experimental MS² libraries is beneficial for reliable compound identification. Ideally, spectra are generated under identical conditions [7, 32-34]. Therefore, we developed a method to create in-house MS² libraries in a fast and reproducible manner. A flow injection with a low flow rate was chosen to avoid manual injection of standards and to generate a broad signal providing enough time for data acquisition. Separate instrument methods for positive and negative ionization mode resulted in a shorter cycle time and the possibility to investigate 6 different NCEs at a time. The t-SIM/dd-MS² experiment offers both distinct MS¹ and MS² spectra compared to AIF, where all detected masses get fragmented simultaneously or PRM, where only MS² scans are generated without the possibility to control the precursor mass spectrum on the MS¹ level. A mass inclusion list for the t-SIM/dd-MS² is mandatory. However, in contrast to PRM, not all listed masses get fragmented, but only masses reaching a certain intensity threshold. Therefore, dd-MS² scans are primarily triggered by the injected metabolite's mass. Random triggering by intense background signals, as in a Full MS/dd-MS² experiment, is avoided by a narrow t-SIM isolation window of 0.4 m/z. Including 10 masses in the inclusion list simplified the method setup, but the acquisition of 10 t-SIM scans increased the cycle time to 20 s. Nevertheless, our approach resulted in the required quantity of high-quality MS² spectra of single standard metabolites to build our in-house library. For about 50 metabolites a higher concentration was necessary to obtain reliable spectra. Sugar-like compounds required adjusted ionization settings. Interfering noise levels, especially for very small masses, or insufficient ionization of non-polar compounds prohibited the inclusion of some metabolites. The application of atmospheric-pressure chemical ionization (APCI), a more effective ionization technique for non-polar compounds that can also reduce the background interference at low mass ranges, could be an option to enlarge our library [32]. It might also be superior for highly polar compounds like sugars [121]. However, as APCI is not suitable for thermally labile compounds, ESI is still the ionization technique of choice for metabolomics. In general, more standard metabolites can be investigated to expand our in-house MS² library.

Due to the immense number of potential metabolites in the metabolome, all spectral databases are bound to be incomplete. Therefore, generating additional data is beneficial for the metabolomics community [7, 122]. The generated MS² library can be used to directly compare single spectra or be included in TraceFinder or Compound Discoverer workflows for data

processing. In-house libraries not only improve the identification confidence in untargeted metabolomics, but the range of standard compounds is adaptable to any research interest, e.g. drugs, food components and contaminants, or analytes, that have not been studied so far. Additionally, standard MS² spectra are essential for developing targeted approaches, where the detection of compounds is usually based on the precursor mass and one or more intense fragments. As fragment intensity and distribution depend on both the chemical structure and the applied CE, spectra at different NCEs should be examined. By incorporating the MS² library, it is possible to generate a targeted MS method without new experimental data.

5.1.2 LC library

Chromatographic separation of metabolites prior to MS analysis offers increased selectivity and reduced complexity at the moment of detection [9]. Additionally, it reduces ion-suppression effects and adds the retention time as an orthogonal factor for metabolite identification. We generated a comprehensive LC library, including scores that assess the chromatographic behavior and retention of 294 metabolites under 57 LC-MS conditions.

Even though 506 compounds in equimolar concentrations were present in the investigated MSM, only 294 could be included in the library, including 46 isomers. Other isomers could not be distinguished and were therefore not included. Some compounds were not detected at all, probably due to an insufficient ionization, others did not show an integrable LC peak or were not fragmented. Because the Full scan/dd-MS² (Top 10) approach selects precursor masses for fragmentation based on signal intensity in the full scan, only the 10 most intense masses will get fragmented. By setting an exclusion time, the user can avoid a fragmentation of the same 10 masses in every cycle. Therefore, more co-eluting compounds are getting fragmented. The stepped NCE (10/50/100 %) enables an informative fragmentation of a large group of chemically different metabolites, for which the formation of fragments needs different levels of CE. Nevertheless, some signals are still too low to be selected or their fragmentation did not provide an MS² spectrum that fulfilled the quality criteria.

From 294 metabolites measured, only a subset (66 – 193) was confirmed under each LC condition, reflecting the coverage bias of untargeted metabolomics. The most influential factor in this study was the column chemistry. Retention of compounds in RP-LC is mainly based on hydrophobic interactions. Under starting conditions, a non-polar analyte ($\log P > 0$) binds to the non-polar stationary phase. With decreasing polarity of the mobile phase, analytes will gradually elute from the stationary phase. More polar compounds interact stronger with the mobile phase and are

therefore not or poorly retained under RP conditions. Consequently, many compounds elute at the same time. As only a limited number of MS² spectra is triggered, some of these compounds will be missed and not confirmed in our approach. Ion-suppression by co-eluting compounds and matrix components additionally impacts non-retained metabolites. The competition for ionization in the ESI source decreases signal intensity, especially at the beginning of a gradient. Particularly for HILIC, it is important to consider not only the log P value, but also other chemical properties of metabolites like charged functional groups or acid-base characteristics. Retention mechanisms are complex and hydrophilic partitioning, hydrogen bonding and electrostatic interactions influence the interaction of metabolites with the polar stationary phase [23]. Predicting the elution order is therefore difficult. The LC library provides information on the behavior of certain metabolites under different conditions and highlights the importance of advised method development. Depending on the focus of the study, the LC parameters can be modified. The focus may be on a particular group of metabolites or specific pathways. While the predictions made here are limited to the tested compounds and analytical conditions, it underlines the importance of considering the effect of analysis parameters on the coverage of the metabolome. The LC library can also be used to develop a metabolomics screen that covers as many metabolites as possible.

5.1.3 Method development

Untargeted metabolomics approaches aim for global coverage of the metabolome. Based on the information summarized in our LC library, we tried to find the best method for an untargeted metabolomics screen with maximum metabolome coverage.

First, six methods with a high number of confirmed compounds and/or low mean total score were selected for RP and HILIC. Comparing the individual scores for sensitivity, peak width, peak asymmetry and retention showed that a high flow rate is beneficial for a narrow peak, but not the retention. However, a certain peak width and retention can be beneficial for data-dependent approaches, as it provides more time for fragmentation and data point acquisition per chromatographic peak. As the sensitivity was not compromised, a lower flow rate for RP conditions was therefore preferred. Besides, it also leads to less backpressure in the system and less consumption of solvents. The examined RP conditions A and B only differed in their column chemistry. While the Ascentis® Express F5 column (condition A) has a pentafluorophenylpropyl (PFP) stationary phase, the Cortecs T3 is a more common C18 column (**Figure 5.1 A and B**). For HILIC, all three conditions were based on the SeQuant® ZIC HILIC column, differing only in the buffer concentration and the pH value (**Figure 5.1 C**).

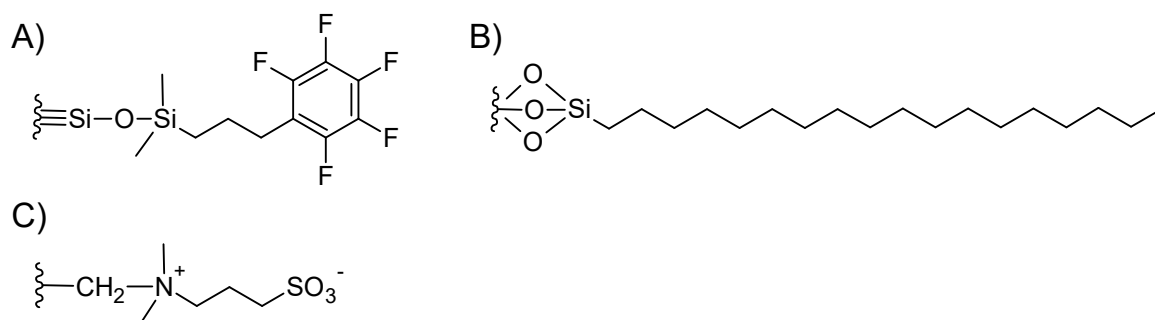


Figure 5.1 Column chemistry. (A) Ascentis® Express F5 (B) Cortecs T3 (C) SeQuant® ZIC HILIC column.

We selected conditions A, B and E to investigate how the different retention mechanisms influence the coverage of metabolites. Compounds with a retention factor $k < 1$ were excluded. They were expected to suffer severely from ion suppression due to co-eluting matrix compounds in biological samples and therefore a lack of detection [123, 124]. Surprisingly, even after the exclusion, the two RP conditions overlapped by 80 %. As the F5 column additionally offers e.g. π - π interactions and polar interactions, a more distinct differentiation from the Cortecs T3 was expected. In contrast, the zwitterionic ZIC HILIC column showed a different metabolite profile. Additionally covered metabolites were mainly polar compounds, whereas non-polar compounds were preferably detected using the RP methods. Isomers play a significant role in metabolomics. Many small metabolites are isomers, showing identical m/z ratios and often similar fragmentation. Nevertheless, a distinction is possible if they differ in their retention time. Regarding the separation of tested isomers, the ZIC HILIC performed best.

Sample volume and measurement time are often limited. Therefore, the MS method was adjusted to apply both positive and negative ionization mode in one run. For the analysis of biological samples, the extraction method impacts the metabolite coverage as well [9]. Multiple protocols for the extraction of plasma and serum have been described and reviewed [125-129]. Simple organic solvent-based protein precipitation is widely used and recommended to obtain a broad metabolite profile. However, the choice of solvent influences that profile. Here, we worked with a solvent mixture of acetone, acetonitrile and methanol for all samples, as published before [25, 111]. More elaborate extraction techniques, like solid-phase extraction (SPE), can increase the purity of extracts, but also affect the metabolite profile and impair reproducibility. The number of 76 and 90 confirmed compounds for conditions B and E in the MSM spiked plasma was lower compared to the pure MSM (169 (B)/120 (E)). In general, combining positive and negative ionization mode can compromise the number of confirmed compounds as there is less time for

fragmentation in each mode. However, the more critical factor for the decreased number of confirmed compounds were matrix effects, especially for the RP condition B. Non-retained compounds are strongly affected by ion-suppression effects due to co-eluting matrix components [123, 124]. Still, RP chromatography is more popular for metabolomics studies than HILIC. It is considered more robust, although the capacity to retain small hydrophilic or ionic metabolites is limited [25, 26]. In this study, the zwitterionic ZIC HILIC column operated at neutral pH showed the best performance when it comes to coverage and peak quality, as reported before [25]. However, one must be aware of the loss of uncharged and hydrophobic compounds.

5.1.4 Method adjustment and validation

High currents between the ESI needle and the source inlet can cause a corona discharge, which should be avoided to not damage the needle. When working with the ZIC HILIC column and a buffer concentration of 10 mM ammonium acetate in both eluents, we observed a spray current pattern that reached 50 μ A and did not fit the applied gradient. The spray current should increase in parallel with an increasing amount of water in the mobile phase. High amounts of acetonitrile should result in a low spray current. However, the buffer ions might interact with the zwitterionic ZIC HILIC column. When a high percentage of acetonitrile is applied, ions bind to the column until it is saturated. After that, more ions reach the MS, resulting in a higher spray current at the beginning of the gradient, where 95 % acetonitrile has been applied for several minutes, compared to the same conditions at the end of the gradient. Hence, we shortened the exposure time of the column to 95 % acetonitrile containing buffer ions. The spray current peaked at approximately 12 min, when the amount of water in the mobile phase increased. The retained buffer ions eluted and caused the extremely high current, which was disproportional to the amount of water in the mobile phase. Consequently, we limited the buffer presence to the water phase only to receive a normal spray current pattern.

The decrease in signal intensity over time can be explained by several factors [130]. First, the degradation of compounds has to be considered. However, the internal standard, consisting of several deuterium-labeled amino acids, is not expected to degrade within 48 h at 4° C. Another factor could be the matrix of extracted plasma samples. As a signal drift was also observed when the internal standard was measured in solvents only, the matrix might not be the only factor. In general, we assume that the signal loss is caused by contamination or dirtying of the LC column and/or the ion source components [130]. Therefore, we established a comprehensive cleaning protocol, implemented every 24 hours of run time.

Discussion

The identification and confirmation of 179 metabolites in human serum samples was dependent on the fragmentation pattern. As reference, we used mzCloud and our in-house MS² library. Of 179 confirmed metabolites, 34 % had a match within the MS² library. For 82 % of these metabolites, the identification based on the MS² library was superior over mzCloud by means of match scores provided by Compound Discoverer. This highlights the value of an in-house library. Standard fragmentation spectra acquired under identical conditions showed the highest resemblance to experimental spectra. However, the limitation of our in-house MS² library is the small number of included metabolites in comparison to online libraries like mzCloud.

Normalized areas of QC samples were used to ensure data quality. In accordance with similar approaches, overall and intra-batch variability were < 20 % in QC samples for 80 % of 94 final compounds [25, 131]. The intra-batch variability refers to a technical replicate. In contrast, overall variability comprises the sample preparation, which was already accounted for by normalization to the internal standard. Therefore, both values reflect the technical variability over all batches and within one batch, respectively. The overall variability is higher, though, since the internal standards are not perfectly representative for all metabolites. To ensure repeatability, overall variability of diluted QCs (0.8:1, v/v) was also monitored (repeatability A and B). The variability was higher when QCs were diluted before the extraction (repeatability A) compared to QCs diluted after the extraction (repeatability B). The linearity of a signal is crucial for the interpretation of metabolomics studies as it has to correlate with the concentration of the detected metabolite. Here, all selected compounds showed linearity over a relative concentration range of at least 0.2 to 1.

The MS²-based identification of 94 compounds is comparable to similar studies working with in-house libraries [132, 133]. Compared to solely mzCloud-based approaches, our strategy provides a higher number of metabolites and the advantage of high-confidence identification based on the in-house MS²-library [10]. Alternatively, some metabolomics studies have reported the detected features or m/z values [10, 134]. However, depending on the data processing, a feature list can be inflated as multiple features often represent the same metabolite [135]. Additionally, biological interpretation of these results is difficult without further data analysis and unambiguous peak assignment.

5.2 Metabolomics and lipidomics analysis of ischemic stroke patients

Focal cerebral ischemia characterizes an ischemic stroke. The subsequent detrimental processes, like inflammation and oxidative stress, cause a peripheral stress response. Additionally, a compromised BBB leads to increased trafficking of metabolites between blood and brain. Therefore, serum lipids and metabolites can reflect the events in the brain. They are potential biomarkers for the ischemia itself and the long-term outcome of patients [92, 136].

5.2.1 Changed metabolite levels in serum after ischemic stroke

Numerous significant changes in lipid and metabolite levels could be observed in the serum of ischemic stroke patients within the first 30 hours after symptom onset.

Interestingly, AcCas were both increased and decreased in the stroke group, depending on the chain length of the FA residue. The increase in even-chain AcCa (14:1), AcCa (14:2), AcCa (16:1) and AcCa (18:1) indicates a mitochondrial dysfunction and compromised β -oxidation (**Figure 5.2**) [137]. The transport of FAs into mitochondria is facilitated by converting acyl-coenzyme A (acyl-CoA) to the corresponding acylcarnitine. AcCas can then enter the mitochondrial matrix where FA breakdown and generation of acetyl-CoA takes place (β -oxidation). Mitochondrial dysfunction is closely related to the deprivation of energy substrates and oxidative stress in stroke [138, 139]. Additionally, it has been shown that AcCas can activate pro-inflammatory signaling [140]. In general, elevated plasma or serum AcCas are also associated with a higher risk of cardiovascular diseases like stroke [141]. Thus, it has to be considered that changed AcCa levels may represent pre-existing differences between stroke and control patients prior to the ischemic attack. Odd-chain AcCas can originate from dairy food intake and lower levels in the serum of ischemic stroke patients might reflect differences in nutrition [142]. However, odd-chain FAs are also a product of peroxisomal α -oxidation of 2-hydroxylated even-chain FAs [143]. As oxidative stress alters the activity of peroxisomes, decreased odd-chain AcCa levels might be the result of an impaired metabolism of 2-hydroxylated FAs [144]. β -Oxidation of odd-chain FAs, but also amino acid catabolism, produce propionyl-CoA. Lower levels of propionylcarnitine (AcCa (3:0)) might therefore be a consequence of lower odd-chain AcCas and less amino acid catabolism. However, as amino acids were downregulated in our study, a higher degradation rate is likely. Decreases in AcCa (3:0) can also originate from increased propionyl-CoA metabolism to succinyl-CoA, which enters the citric acid cycle for energy generation. AcCa (3:0) has also been described as an antioxidant and decreased levels might reflect oxidative stress after ischemic stroke [145].

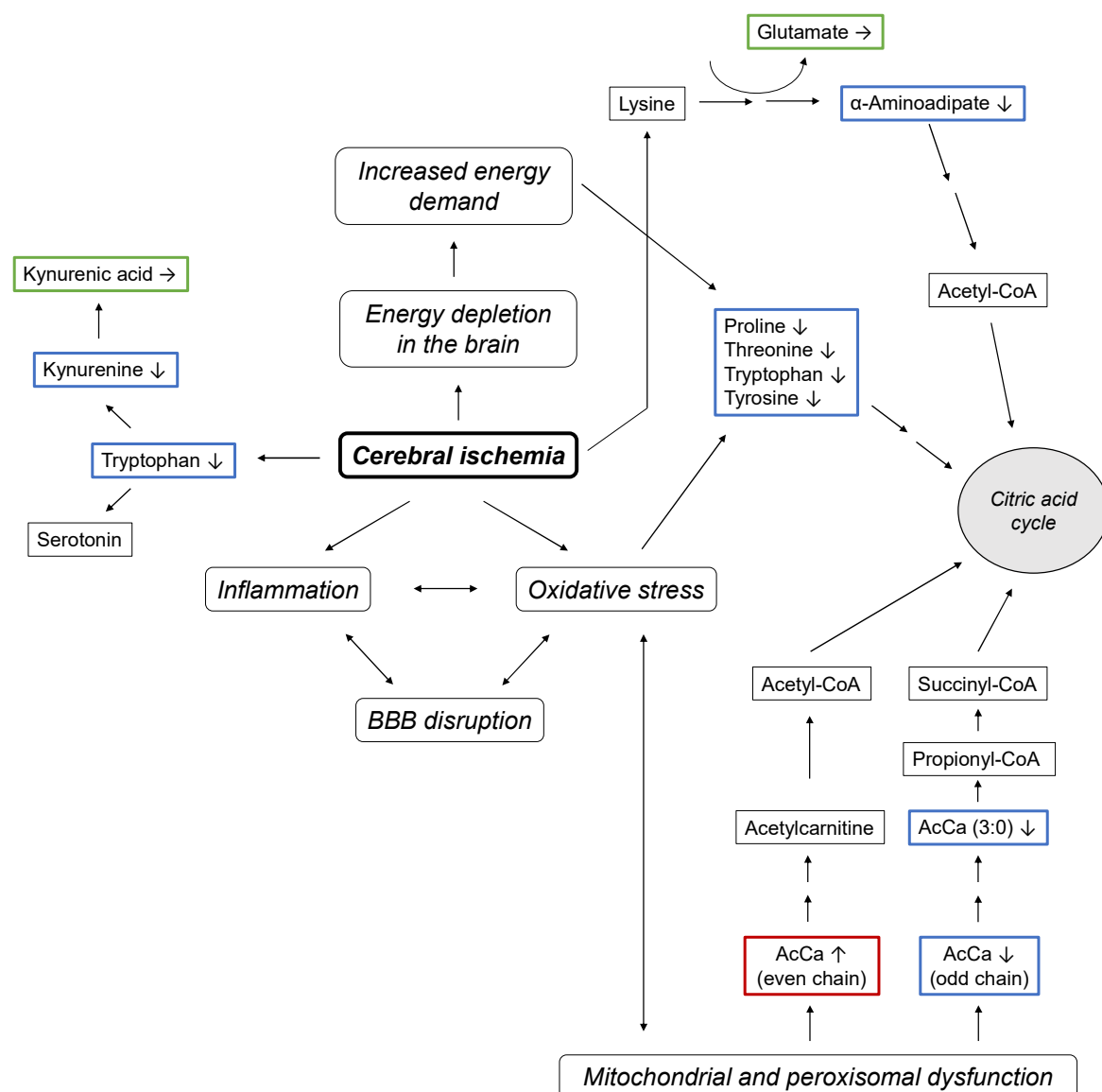


Figure 5.2 Scheme of metabolic changes in relation to ischemic stroke (1). Metabolites highlighted in red or blue were up- and downregulated in the serum of ischemic stroke patients, respectively. Changes are indicated by arrows. Metabolites highlighted in green were measured but not changed in our study. AcCa = acylcarnitine, BBB = blood-brain barrier, CoA = coenzyme A.

Amino acids (proline, threonine, tryptophan and tyrosine) were decreased in the stroke group, which has been reported before [93-96]. Oxidative stress and inflammation can lead to irreversible protein oxidation [146, 147]. One type of oxidation is tyrosine nitration, where RNS react with tyrosine. Another type is protein carbonylation, a non-enzymatic addition of aldehydes and ketones. Modification of certain amino acid residues like tyrosine, proline and threonine makes proteins more prone to degradation. Moreover, the oxidation of free amino acids might result in lower levels in the stroke group (**Figure 5.2**). Additionally, an increased amino acid catabolism could address the high energy demand after ischemia [93]. Decreased levels of tryptophan might

also indicate an activation of the kynurenine pathway, which has been reported in stroke before [148]. A positive association between inflammation, oxidative stress and the tryptophan metabolite kynurenine has been described [149]. In contrast, kynurenine was significantly decreased in our study and the downstream metabolite kynurenic acid was not changed between groups. Less serum tryptophan could also limit serotonin production in the brain, a neurotransmitter necessary for numerous brain functions [150]. Serotonin has been described as an antioxidant and it plays a role in regulating the immune response in the periphery [151, 152]. Several animal studies show that selective serotonin reuptake inhibitors are beneficial in stroke models, although mechanisms are still unknown [153].

α -Aminoadipate is a metabolite of the lysine pathway and has been postulated to be a biomarker for oxidative stress and protein carbonylation [154, 155]. Unexpectedly, our study showed decreased levels in the ischemic stroke group (**Figure 5.2**). Lysine catabolism is assumed to be upregulated after stroke, favouring glutamate synthesis [94]. In our study, glutamate serum levels were not changed, but possible changes in lysine could also influence α -aminoadipate. Metabolization of the amino acid to acetyl-CoA could be a reaction to an increased energy demand after ischemic stroke [156]. Therefore, the flux towards the citric acid cycle might decrease serum levels of α -aminoadipate.

Trigonelline is a degradation product of the nicotinamide metabolism and is generated e.g. from niacin. As it is also a biomarker for the consumption of coffee, the lower serum levels in the stroke group might be due to a restricted coffee intake [157].

4-Guanidinobutanoate is an intermediate in the arginine oxidase/dehydrogenase pathway for the generation of succinate (**Figure 5.3**). As a response to an inflammatory event like cerebral ischemia, the amino acid arginine is increasingly converted to NO [76, 158]. The NOS pathway does not only lead to the generation of RNS, but might be responsible for the decrease in 4-guanidinobutanoate by competing for the substrate arginine. Additionally, it has been proposed that NO inhibits the aldehyde dehydrogenase (AldDH) needed for the conversion of 4-guanidionobutanal to 4-guanidinobutanoate [158]. Alternatively, a shift towards succinate from arginine for energy generation could also influence 4-guanidinobutanoate levels.

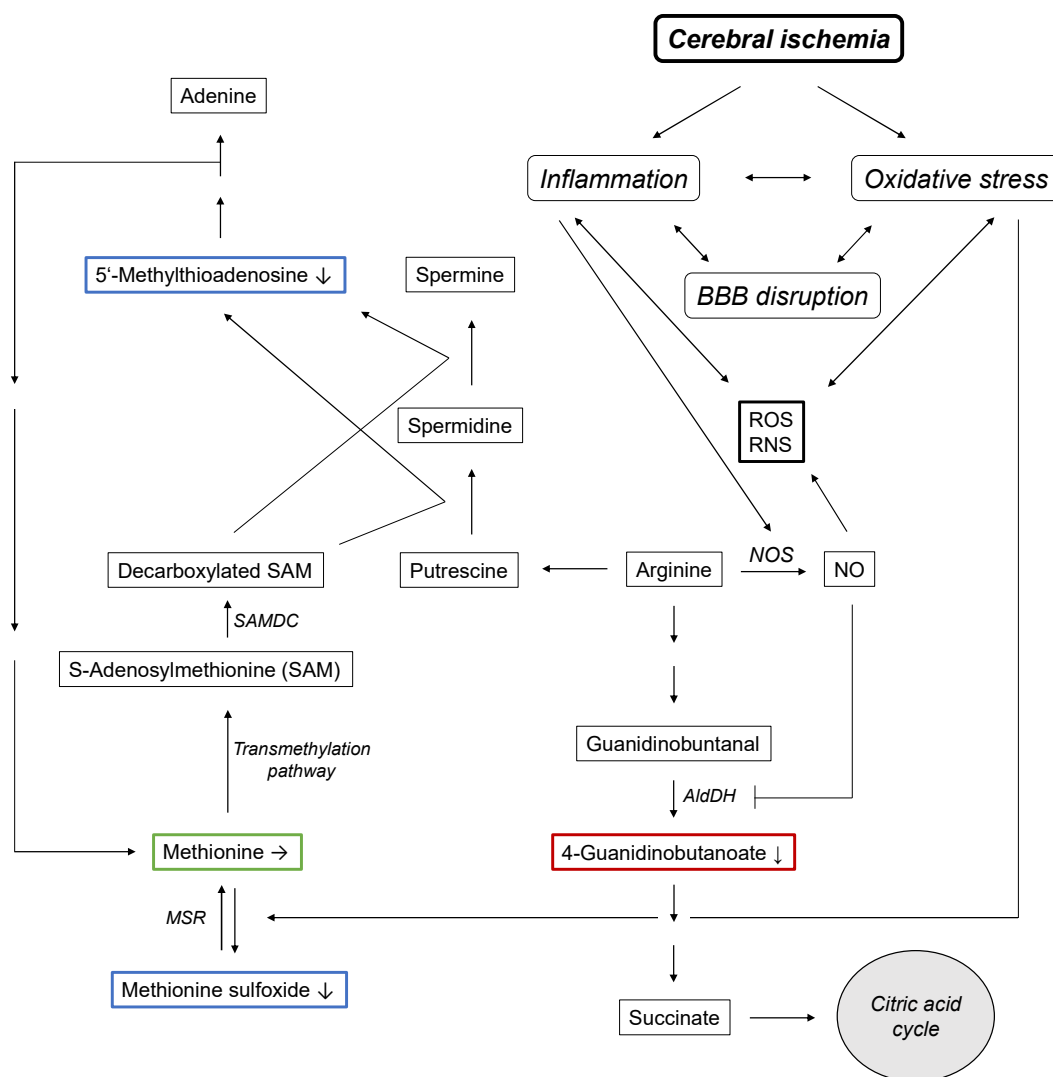


Figure 5.3 Scheme of metabolic changes in relation to ischemic stroke (2). Metabolites highlighted in red or blue were up- and downregulated in the serum of ischemic stroke patients, respectively. Changes are indicated by arrows. Metabolites highlighted in green were measured but not changed in our study. BBB = blood-brain barrier, ROS = reactive oxygen species, RNS = reactive nitrogen species, NO = nitric oxide, NOS = nitric oxide synthase, AldDH = aldehyde dehydrogenase, SAMDC = S-adenosylmethionine decarboxylase, MSR = methionine sulfoxide reductase.

5'-Methylthioadenosine (MTA), a sulfur-containing adenine nucleoside, is produced from its precursor S-adenosylmethionine (SAM) during the biosynthesis of polyamines spermidine and spermine from putrescine (**Figure 5.3**) [159]. An accumulation of the arginine metabolite putrescine has been observed in rat brains after MCAO as well as an inhibition of SAM decarboxylase (SAMDC) after brain injury [160, 161]. Less utilization of SAM in the polyamine pathway, also in the periphery, could lead to lower serum levels of MTA. Reduced availability of SAM, an important methyl donor, could affect MTA levels as well. MTA has been described as neuroprotective *in vitro* but supplementation had no influence on infarct size in an MCAO model [162]. MTA is metabolized to methionine and adenine and plays an essential role in the salvage

pathway of these metabolites [159]. Consequently, a decrease in MTA could also be caused by a higher demand for methionine or adenine derivatives. Methionine is highly susceptible to oxidation by ROS and has been reported to be decreased in stroke [95, 163]. However, its direct oxidation product methionine sulfoxide was reduced in our study and methionine levels were not changed in the ischemic stroke group. As methionine sulfoxide has been shown to be elevated in oxidative stress before, it is likely that the methionine sulfoxide reductase (MSR) is involved in the restoration of methionine levels and the observed decrease in methionine sulfoxide. Enzyme activity could be driven by an increased flux of methionine into the transmethylation pathway, where it acts as a precursor of SAM (**Figure 5.4**).

Strikingly, almost all measured TGs were downregulated in the serum of ischemic stroke patients, even though hypertriglyceridemia is a major risk factor for stroke and increased plasma levels have been described before [164, 165]. Interestingly, several studies show an association between low serum triglycerides and worse stroke severity and stroke outcome [166, 167]. Nutritional deficits have been postulated to be the connection. Interestingly, our data showed a correlation between TGs and NIHSS at admission, but not with the mRS90 (**Figure S6.2 A, B**). There was also no correlation between the last meal intake and serum TG levels, neither in the stroke nor in the control group (**Figure S6.2 C, D**). Nevertheless, lower levels of several TGs in our study might reflect a combination of malnutrition, elevated energy demand and increased lipid peroxidation after ischemic stroke (**Figure 5.4**).

Prominent changes could also be observed for several glycerophospholipid classes and species. Contradictory findings have been reported in the literature about phospholipid changes in stroke [93, 100, 168, 169]. However, a comparison between studies can be difficult, due to differences in detected phospholipid classes and species. Here, significantly decreased PCs and their cleavage products LPCs had medium-chain, saturated or monounsaturated FA residues. Decreased PEs and LPEs had slightly longer FAs bound. The hydrolysis of PCs and PEs to their lyso-counterparts is mediated by phospholipases, including phospholipase A2 (PLA2), which has been associated with inflammation and oxidative stress (**Figure 5.4**) [170-173]. The cleavage at the sn-2 position of glycerophospholipids releases FAs, including inflammatory intermediates like arachidonic acid. As LPCs and LPEs were downregulated in the serum of ischemic stroke patients, they likely undergo degradation as well. Lyso-phospholipases like autotaxin remove the phospho-containing headgroup of LPCs and LPEs, resulting in lysophosphatidic acid (LPA), another inflammatory mediator that has been shown to be elevated in plasma of MCAO mice [174-176]. Decreases in serum PCs and PEs might also be caused by the elevation of the fatty acyl ethanolamides palmitoyl-EA (PEA) and oleoyl-EA (OEA) in stroke patients (**Figure 5.4**). The synthesis of PEA and

Discussion

OEA from PE and PC is also mediated by phospholipase activity. The lipid mediators are generated in response to tissue damage, including ischemia and stroke, and are postulated to have anti-inflammatory and neuroprotective properties [177, 178]. Alternatively, higher EA serum levels could also originate directly from the brain by passing the disrupted BBB. It has been shown that the administration of PEA shortly before or after stroke can reduce the infarct size and neurological phenotype in a rat model [179]. However, there were no significant differences in PEA and OEA between patients with good and bad outcomes in our study.

Both lipid peroxidation and inflammation can add to decreased levels of phospholipids. Especially long-chain polyunsaturated FA moieties are prone to be modified by ROS or RNS. PLA-mediated generation of PUFAs and subsequent synthesis of eicosanoids is also initiated by the degradation of long-chain, polyunsaturated phospholipids. Surprisingly, only short and medium-chain PCs and PEs were significantly downregulated in our study. Taking the ratio of two metabolites or lipids can reveal changes by decreasing inter-individual variability. Interestingly, several ratios of PCs to PEs with the same chain length were significantly increased in the serum of stroke patients, due to the more pronounced decrease in PEs than in the corresponding PCs. An activated PEMT pathway, converting PEs directly to PCs, might explain the changes in the PC/PE ratios. The pathway includes the methylation of PE, with SAM as methyl donor (**Figure 5.4**). As discussed above, a metabolite of SAM - MTA - was decreased in the stroke group. Additionally, methionine sulfoxide as an oxidation product of methionine was downregulated as well. The consumption of SAM for PE methylation might lead to a reduced availability for the generation of MTA and increased flux of methionine towards the transmethylation pathway. However, a change in methionine or SAH serum levels was not detected.

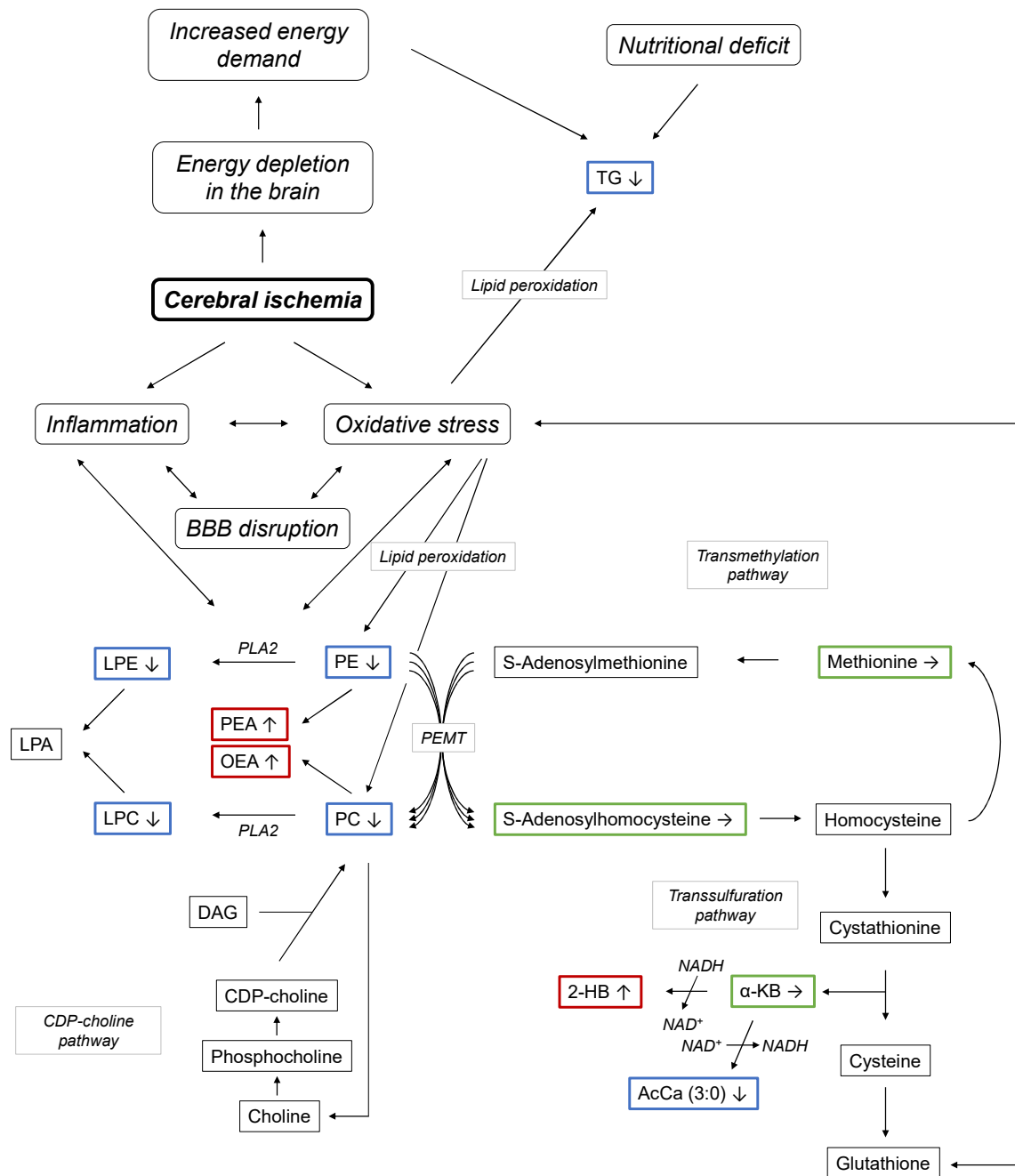


Figure 5.4 Scheme of metabolic changes in relation to ischemic stroke (3). Metabolites highlighted in red or blue were up- and downregulated in the serum of ischemic stroke patients, respectively. Changes are indicated by arrows. Metabolites highlighted in green were measured but not changed in our study. BBB = blood-brain barrier, TG = triacylglycerides, DAG = diacylglycerides, PC = phosphatidylcholine, LPC = lysophosphatidylcholine, PE = phosphatidylethanolamine, LPE = lysophosphatidylethanolamine, LPA = lysophosphatidic acid, PLA2 = phospholipase A2, PEA = palmitoylethanolamide, OEA = oleoylethanolamide, PEPT = phosphatidylethanolamine N-methyltransferase, CDP = cytidine diphosphate, α-KG = α-ketoglutarate, 2-HB = 2-hydroxyglutarate, AcCa = acylcarnitine, NAD⁺/NADH = nicotinamide adenine dinucleotide.

2-Hydroxybutyrate (2-HB) was the only small, hydrophilic compound significantly increased in the serum of stroke patients and has been shown to be upregulated in human plasma after tissue

ischemia before [180]. 2-HB is a byproduct of the transsulfuration pathway and glutathione biosynthesis (**Figure 5.4**). When cystathionine is converted to cysteine, the formed α -ketobutyrate (α -KB) is reduced by NADH to 2-HB. Cysteine is incorporated into glutathione. Oxidative stress can dramatically increase the need for the antioxidant glutathione. However, when cysteine levels become limiting, homocysteine is diverted from the transmethylation pathway. Increased levels of 2-HB might therefore represent highly oxidative conditions and increased production of glutathione [181]. The utilization of homocysteine from the transmethylation pathway fits the assumption of an activated PEMT pathway. As PE methylation is the main consumer of SAM, it also facilitates the turnover to SAH for the generation of homocysteine, cysteine and glutathione through transsulfuration [182]. Increased levels of 2-HB can also reflect an elevated NADH/NAD⁺ ratio during hypoxia, due to impaired oxidative phosphorylation [180, 183]. A shift towards the reduction of α -KB to 2-HB would come with less production of AcCa (3:0), a metabolite of α -KB, fitting the decreased serum levels in our study. 2-HB has also been suggested as an early marker for insulin resistance [183]. In the control group of this study, a significant difference in serum 2-HB levels between patients with and without diabetes could be detected, but not in the stroke patients (**Figure S6.3**). 2-HB also belongs to the group of ketone bodies that are related to fasting [184]. Indeed, the 2-HB levels correlated with the time since the last meal in all subjects included in our study (**Figure S6.4**). Therefore, the interpretation of elevated 2-HB levels in relation to stroke is complex.

Activation of the PEMT pathway after an ischemic attack could be induced by oxidation of long-chain, polyunsaturated phospholipids. Compared to PEs, PCs are more abundant in lipoproteins and cell membranes. They are mainly located in the outer leaflet, whereas PEs are found predominantly in the inner leaflet [185, 186]. As long-chain polyunsaturated PCs were not significantly downregulated in the serum of ischemic stroke patients, the generation from PEs via the PEMT pathway might be a counteracting mechanism to maintain membrane integrity in the periphery and the brain. Increased PC/PE ratios indicate an augmented conversion. Furthermore, PCs generated by the PEMT pathway are the only source of *de novo* generated choline [187]. The small metabolite is essential for synthesizing PCs via the CDP-choline pathway and a strong decrease in medium-chain PCs after stroke could reflect the need for choline (**Figure 5.4**). It has been shown that the PEMT pathway is essential to maintain PC synthesis and choline generation in times of choline deficiency [188]. Choline is known to be beneficial in stroke and has been in the focus of stroke treatment research. It has been suggested that choline promotes brain angiogenesis, improves neurological deficits and decreases the infarct volume [189]. CDP-choline

(citicoline) has been investigated extensively as a treatment option for ischemic stroke [106-108]. As essential intermediate in the synthesis of PCs via the CDP-choline pathway, it is also a choline donor, e.g. for the neurotransmitter acetylcholine. The administration of citicoline has been shown to be beneficial in stroke animal models [190, 191]. Activation of the PEMT pathway, subsequent PC synthesis and *de novo* generation of choline could have similar effects and could therefore be neuroprotective.

5.2.2 Time-dependent development of serum levels after ischemic stroke

Time is an essential factor for the recovery of ischemic stroke patients. Immediate treatment is often not possible, due to a delayed diagnosis or admission to the hospital. Therefore, it is important to consider changes of lipids and metabolites over time. We analyzed serum samples of patients on days 1, 2 and 3 after the ischemic attack to see the time-dependent development of lipid and metabolite levels in the patients. The analysis revealed several changes over three days organized in clusters, representing correlated changes in serum levels of compounds. Interestingly, the altered lipid species clustered according to their lipid classes.

PC/PE ratios were increased on day 1 compared to the control group and decreased again over time. Changes might reflect a temporary activation of the PEMT pathway soon after the ischemic attack to counteract the depletion of PCs. On day 2 after stroke, PC/PE ratios decreased, consistent with elevated serum levels of polyunsaturated, long-chain PEs. Interestingly, PCs with the same chain lengths as PEs were not significantly changed over time, indicating compensation by generating these PCs via the PEMT pathway. However, other PCs were decreased on day 2 and day 3. The fact that medium-chain, saturated or monounsaturated PCs organized in a sub-cluster might reflect the specificity of the CDP-choline and PEMT pathway, depending on chain length and degree of saturation.

Next to PEs, methionine sulfoxide and LPE (16:0) were included in the same cluster. Compared to the control group, these two metabolites were decreased on day 1 and increased on day 2 and day 3. Recovery of LPE (16:0) levels corresponds to increased PE levels in serum. However, it might also reflect decreased inflammation and lysophospholipase activity in stroke patients on day 2 and day 3. We hypothesized that the decrease in methionine sulfoxide on day 1 reflects the flux of methionine into the transmethylation pathway consistent with a temporary activation of the PEMT pathway. Like the substrates of PEMT, long-chain, polyunsaturated PEs, methionine sulfoxide levels were restored over time. PE and methionine sulfoxide levels showed a reversed pattern compared to PC/PE ratios, supporting our hypothesis.

SMs were not significantly changed between control and stroke group on day 1, but they increased on day 2 and day 3. SM generation from ceramides is mediated by sphingomyelin synthases (SMS) and involves the transfer of a choline head group from PCs. Therefore, an increase in SM serum levels could contribute to decreased PC levels on days 2 and 3. Ceramides are known to be inflammatory mediators, whereas the metabolites ceramide-1-phosphate and sphingosine-1-phosphate also show effects [192]. Upregulated SM generation after two days could therefore reflect the declining inflammatory response after the ischemic stroke. Sphingolipids are very abundant in brain tissue and SMs are particularly enriched in the myelin sheath of nerve cells. Consequently, increased SM levels in the serum could also originate from damaged brain tissue by passing a disrupted BBB. Consistently, increased SM plasma levels have been proposed as a biomarker for cerebral microvascular disease [193]. SM (d30:1) was sorted in a different cluster and its levels were directed in the opposite direction, indicating other mechanisms behind the generation or degradation of this lipid.

5.2.3 Early prediction of post-stroke impairment

5.2.3.1 Serum lipid levels of patients with different outcome

Post-stroke impairment can diminish independence and quality of life for patients. To determine early biomarkers for the long-term outcome of patients, we investigated differences in serum lipid levels between patients with a good and a bad outcome. Categorization was based on the mRS90. An $mRS90 \leq 2$ represented a good outcome, whereas an $mRS90 \geq 3$ represented a bad outcome or death ($mRS90 = 6$). We included additional patients from a second ischemic stroke cohort to increase the statistical power. Due to the study design, patients were not matched between the two groups. Therefore, group size, age, gender, NIHSS at admission and diabetic patients are not equally distributed.

Significant changes could be observed for polyunsaturated long-chain PCs. These phospholipids were downregulated in patients with a bad outcome. As discussed in chapter 5.2.1, inflammatory processes and oxidative stress can lead to the degradation of PCs. Especially long-chain polyunsaturated lipids are prone to lipid oxidation due to the double bonds of the FA residues. Interestingly, in comparison to the control group, polyunsaturated PCs were only slightly reduced in the good outcome group, whereas patients with a bad outcome showed significantly downregulated serum levels compared to both the control and the good outcome group. This effect could be explained by elevated oxidative stress and PLA activity and consequently a worse outcome for patients with a severe stroke. However, medium-chain and saturated PCs were

strongly downregulated in all stroke patients compared to the control group, without significant differences regarding the outcome. Therefore, the preservation of long-chain polyunsaturated PC levels in stroke patients with a good outcome might originate from an activation of the PEMT pathway. This is supported by the differences in five PC/PE ratios. Ratios were significantly correlated with the mRS90 value and increased in patients with a good outcome. In contrast, patients with a bad outcome showed decreased PC/PE ratios for three consecutive days. Differences in the ratio not only originated from decreased PC levels in the bad outcome group, but also from increased PE levels, indicating an inhibition of the PEMT pathway in these patients. As discussed before, PCs generated from PEMT are the only *de novo* source of choline. Lower plasma levels of choline and its oxidation product betaine have been shown to be associated with the neurological impairment of stroke patients [194].

Saturated, medium- to long-chain LPCs were only downregulated in patients with a bad outcome. That might reflect an increased breakdown of LPCs to LPAs via autotaxin. Lower LPC serum levels have been reported in stroke patients with post-stroke impairment [97]. LPAs were not detected in our study, but elevated autotaxin expression and activity, as well as increased LPA plasma levels have been shown in an MCAO mouse model [176]. SM (d40:1) was downregulated in patients with a bad outcome, too. Increased inflammation might upregulate the generation of ceramides from SMs, as discussed in chapter 5.2.2.

5.2.3.2 Biomarker-based model for early prediction of post-stroke impairment

Prediction of post-stroke impairment can help optimize treatment options and plan the levels of care. Additionally, early markers for the outcome of patients can help to understand recovery mechanisms that lead to either better or worse outcome. Age and stroke severity, usually represented by the NIHSS, are known to predict the outcome [40, 41]. In our study, we could confirm the correlation by performing multivariate statistical analysis to reduce the complexity of our high dimensional data set. Sparse PLS-DA offers dimension reduction and the selection of variables that contribute most to the supervised discrimination of sample groups. Within the statistical model, age and NIHSS were among the variables, which were most important for the classification of patients with a good or bad outcome. ROC analysis showed that a combination of both factors can predict the outcome of patients with an accuracy of 76 % in our dataset. Another factor contributing to sample discrimination was the PC (38:6)/PE (38:6) ratio. Univariate ROC analysis resulted in a sensitivity (true positive rate) of 70 % at an optimal cutoff of 1.12. However, in combination with the NIHSS and age, there was no additional predictive power of the PC/PE

ratio in the ROC curve-based model. Additionally, the negative correlation of PC (38:6)/PE (38:6) with NIHSS and age was significant, indicating that they predict each other. If a decreased PC/PE ratio is a cause or a consequence of stroke severity cannot be determined based on our data. Theoretically, a more severe ischemic attack leads to more oxidative stress, possibly leading to augmented degradation of long-chain polyunsaturated PCs, as shown for patients with a bad outcome. In contrast, long-chain polyunsaturated PEs were elevated. The involvement of the PEMT pathway was discussed. An activation in patients with a good outcome might counteract the degradation of PCs, whereas in patients with a bad outcome the compensatory mechanism might not be as efficient, especially in older patients. Less generation of PCs and *de novo* choline could in turn lead to a more severe stroke. Supporting the generation of PCs has been a strategy in stroke treatment before. Citicoline (CDP-choline), however, is targeting the CDP-choline pathway, which mainly generates medium-chain PCs. Therefore, additional or alternative intervention with long-chain, polyunsaturated PC synthesis via the PEMT pathway is a promising new option. Nevertheless, further investigations are necessary to confirm the involvement of PEMT and the origin of lipid changes in the serum.

5.3 Changed metabolite levels in a mouse model of ischemic stroke

The final goal of translational research is the implementation of findings in the clinical setting. Investigating conditions or diseases within human cohorts enables a direct transfer of findings without considering interspecies differences. In ischemic stroke research, a lot of work has been done on plasma or serum samples due to the accessibility of biological material [95, 97, 169]. For obvious reasons, studies working with brain tissue or CSF are less common but promising [195, 196]. However, human cohorts have their limitations. Recruiting a sufficient number of patients can be challenging and a standardized sample collection is not always ensured. Especially when it comes to lipid and metabolite profiles, high inter-individual variability is expected. Additionally, in a medical emergency like stroke, the exact time point of sample collection or the patient's nutritional status cannot always be controlled. Therefore, animal models offer the possibility of a more standardized and better-controlled investigation of different diseases. Using inbred animals within the experiment eliminates the factor of genetic variation. Controlled experimental conditions ensure lower variability and matched control groups enable the direct connection of findings with the disease model. Follow-up studies with different interventions or treatments are possible to confirm hypotheses generated from both human cohorts or mouse models.

Discussion

One of the most common models for ischemic stroke is MCAO in mice or rats [197]. While the transient MCAO resembles an ischemia-reperfusion injury and is usually performed by inserting and removing a filament, the here used permanent MCAO (pMCAO) focuses more on the ischemia itself. We used distal electrocoagulation, including a craniotomy, to occlude the middle cerebral artery. The distal pMCAO results in a high survival rate and reproducible infarct size. However, a certain variation was expected and intended in this study to determine metabolic differences depending on the infarct size.

5.3.1 Changed metabolite levels in the brain of MCAO animals

By occluding the middle cerebral artery, cerebral ischemia is induced on the ipsilateral side of the brain and the tissue is deprived of oxygen and nutrients. To investigate the immediate effects of the ischemia, changed metabolite and lipid levels after 2 hours were investigated compared to the contralateral side within animals. The second time point we chose was 24 hours to resemble the “day 1” time point from the human cohort. The ischemic core and the penumbra expand during the acute phase, as reflected by an increased infarct size after 24 hours. Consequently, also metabolic changes were more pronounced.

After 2 and 24 hours, an increase of amino acids was observed on the ipsilateral side of the brain. An accumulation of amino acids in the ischemic area has been reported before [98, 99]. Activated proteolytic cascades, tissue degeneration and impaired protein synthesis can contribute to the increase (**Figure 5.5**). Interestingly, amino acids were downregulated in the serum of ischemic stroke patients, suggesting recruitment of amino acids from blood to brain. However, amino acid levels in the plasma of MCAO mice did not correlate with levels in the brain (**Figure S6.5**). α -Aminoadipate was also increased after MCAO. The metabolite has been proposed as a marker for protein carbonylation and oxidation of lysine residues. Increased proteolysis could consequently lead to increased levels of α -aminoadipate in the ischemic brain. Lysine catabolism for glutamate synthesis could also influence α -aminoadipate abundance. Surprisingly, glutamate levels in the whole brain homogenates were not changed in our study.

Several AcCas were elevated on the ipsilateral side of the brain. Consistent with increased serum levels of AcCas in the human cohort, this probably reflects an impaired mitochondrial function and β -oxidation. Increased carnitine and the pronounced acetyl- and propionylcarnitine (AcCa (3:0)) upregulation might originate from increased utilization of amino acids for energy generation via the citric acid cycle (**Figure 5.5**). Alternatively, impaired oxidative phosphorylation under ischemic conditions might also lead to an accumulation of citric acid metabolites. However, in MCAO mice,

decreased carnitine levels have been reported for the non-functional ischemic core, while levels increased in the peri- and non-infarct areas [98]. Therefore, increased levels of carnitine, acetylcarnitine and AcCa (3:0) in our study probably reflect an increased flux towards the citric acid cycle.

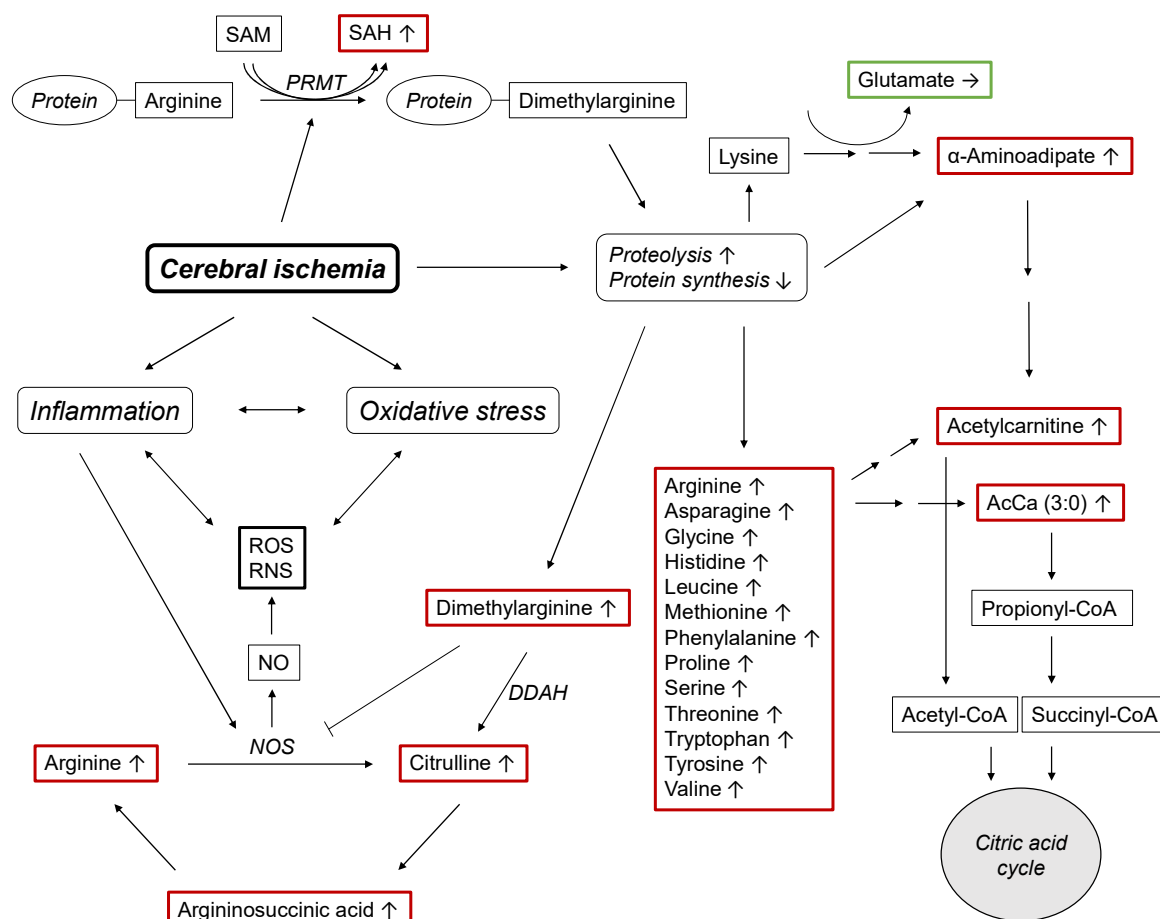


Figure 5.5 Scheme of metabolic changes in the brain after MCAO (1). Metabolites highlighted in red or blue were up- and downregulated on the ipsilateral side, respectively. Changes are indicated by arrows. Metabolites highlighted in green were measured but not changed in our study. SAM = S-adenosylmethionine, SAH = S-adenosylhomocysteine, PRMT = protein arginine methyltransferase, ROS = reactive oxygen species, RNS = reactive nitrogen species, NO = nitric oxide, NOS = nitric oxide synthase, DDAH = dimethylarginine dimethylaminohydrolase.

Argininosuccinic acid, dimethylarginine and citrulline are all involved in the arginine pathway and were elevated on the ipsilateral side of the brain. Arginine, as a precursor of NO, was upregulated as well. The increase of these metabolites after MCAO might reflect an activation of NO generating pathways as a reaction to the induced ischemia (**Figure 5.5**). Whereas argininosuccinic acid can induce oxidative stress in the brain, citrulline is thought to attenuate cerebrovascular injury after brain ischemia by increasing eNOS expression [198, 199]. Asymmetric dimethylarginine (ADMA) is

Discussion

generated via methylation of arginine residues by protein arginine methyltransferases (PRMTs), utilizing SAM as methyl donor [200]. Free ADMA can originate from increased protein degradation and acts as an endogenous NOS inhibitor [201]. Metabolization of ADMA to citrulline is mediated by dimethylarginine dimethylaminohydrolases (DDAHs) [202]. Oxidative stress after an ischemic injury increases the expression of PRMTs and decreases DDAH activity, resulting in elevated ADMA levels [203]. Inhibition of NOS could be beneficial or detrimental. Higher concentrations of ADMA in the CSF of stroke patients have been associated with stroke severity and worse outcome [204]. Accumulating evidence from the literature corroborates a negative effect of ADMA by restricting NO synthesis and vasodilation, regulating cerebral blood flow to the penumbra [205]. However, less NOS activity would also attenuate RNS generation. It is important to consider that we were not able to distinguish between ADMA and the symmetric isomer SDMA. Overall, arginine related changes in the brain reflect the effect of ischemia on NO production. The generation of peroxynitrite causes oxidative damage in the brain and the periphery, as observed in the serum of human patients in our study.

Methionine and cystathionine were elevated on the ipsilateral side 2 and 24 hours after MCAO. Additionally, SAH as well as betaine and choline were increased after 2 and 24 hours, respectively. These metabolites are related to the transmethylation and transsulfuration pathways (**Figure 5.6**). Antioxidants like glutathione are essential to manage oxidative stress after ischemia. Glutathione can be synthesized from homocysteine via cystathionine and cysteine. Increased flux through the transsulfuration pathway in human astrocytes has been shown under oxidative conditions [206]. In MCAO rats, cystathionine β -synthase (CBS) activation in the cerebral cortex was observed [207]. In mouse brain slices and *in vivo*, CBS regulated the cerebral microvascular response to hypoxia [208]. Depending on the substrates, CBS can either generate H_2O from homocysteine and serine, or hydrogen sulfide (H_2S) from homocysteine and cysteine. However, a protective, vasodilating role of H_2S after stroke is still under discussion. In our study, cystathionine was increased, whereas glutathione levels were not significantly changed on the ipsilateral side of the brain, but negatively correlated with the infarct size. Lower levels of the antioxidant glutathione have been reported for the infarct and peri-infarct area in animal models [98, 209]. As we analyzed the whole hemisphere, changes in our study might be underestimated.

Increased levels of SAH indicate an elevated turnover of SAM, possibly driven by the flux through the transsulfuration pathway. Additionally, SAM acts as an allosteric inhibitor of CBS and a higher metabolization rate would fit a higher CBS activity. If homocysteine is shifted towards transsulfuration, increased levels in methionine probably originate from increased protein degradation. This assumption is supported by a strong increase in betaine, a metabolite used for

the remethylation of homocysteine to methionine. Betaine is an oxidation product of choline and has been suggested to be neuroprotective [194]. SAM turnover consequently leads to methylation of proteins, nucleic acids and metabolites. As discussed above, generation of ADMA consumes SAM (Figure 5.5) as well as the polyamine pathway (Figure 5.3, Figure 5.6). As MTA levels were decreased on the ipsilateral side of the brain, less utilization of SAM for polyamine synthesis is likely, as discussed in chapter 5.2.1. Another major consumer of SAM is the PEMT pathway [182]. However, PE levels on the ipsilateral side of the brain were not changed and PC levels were inconsistent 24 hours after MCAO. In contrast, phosphoethanolamine was downregulated after 2 hours. It is a precursor of PE via the CDP-ethanolamine pathway and decreased levels could indicate an increased biosynthesis of PEs as a substrate for PEMT. Generated PCs could be further metabolized to provide choline, which was slightly increased on the ipsilateral side.

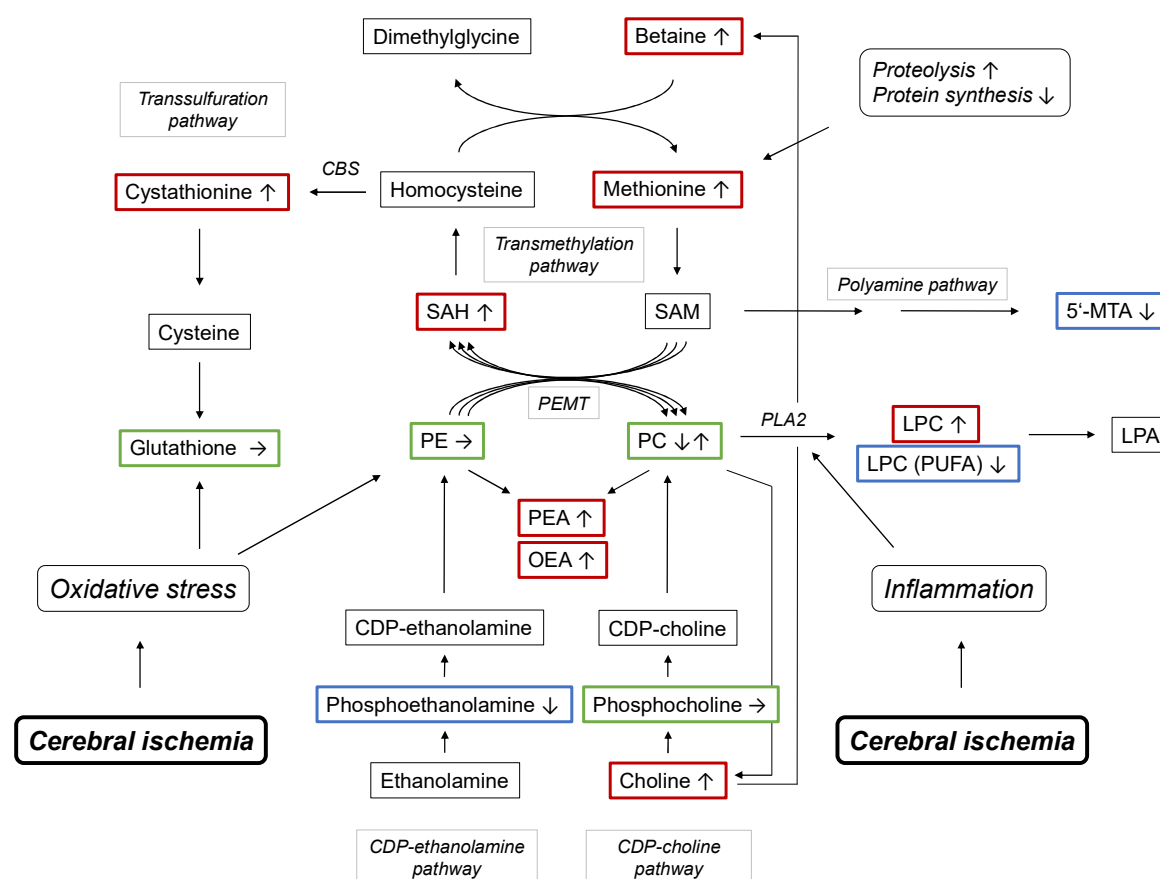


Figure 5.6 Scheme of metabolic changes in the brain after MCAO (2). Metabolites highlighted in red or blue were up- and downregulated on the ipsilateral side, respectively. Changes are indicated by arrows. Metabolites highlighted in green were measured but not changed in our study. CBS = cystathionine β -synthase, SAH = S-adenosylhomocysteine, SAM = S-adenosylmethionine, PE = phosphatidylethanolamine, PC = phosphatidylcholine, LPC = lysophosphatidylcholine, LPA = lysophosphatidic acid, PLA2 = phospholipase A2, PEA = palmitoylethanolamide, OEA = oleoylethanolamide, PEMT = phosphatidylethanolamine N-methyltransferase, CDP = cytidine diphosphate, 5'-MTA = 5'-methylthioadenosine.

Lipids in general, were only significantly changed after 24 hours, indicating delayed alterations in the lipid metabolism after ischemic stroke compared to other metabolites. PEA and OEA were severely increased on the ipsilateral side. An upregulation might be a protective mechanism to counteract the ischemic injury. However, the generation of EAs was not reflected in decreased PE and PC levels in the MCAO brain. Increased levels of PEA and OEA could also be observed in human serum, probably originating from the brain of ischemic stroke patients. Unfortunately, PEA and OEA could not be detected in the plasma of MCAO animals.

Interestingly, saturated LPCs showed an increase, whereas poly-unsaturated LPCs showed a decrease on the ipsilateral side of the brain. Increased LPC levels after MCAO have been visualized with MS imaging before and can be derived from neurons and astrocytes and are involved in microglial activation [210, 211]. PLA2 activity generates LPCs and FAs. Arachidonic acid (FA (20:4)) is a major inflammatory intermediate and cleavage of arachidonic acid-containing PCs, like PC (18:0/20:4), would result in LPCs with saturated FA residues. Contrary decreases in LPCs containing poly-unsaturated FAs indicate lipid peroxidation of LPCs and upstream PCs.

5.3.2 Changed metabolite levels in relation to the infarct size

The infarct size is one of the main parameters investigated after MCAO. It displays stroke severity and the amount of damaged tissue. The neurological deficit 24 hours after MCAO was analyzed based on a latency to move test. However, a correlation with the infarct size was not observed. A missing causality for this behavioral test has been reported before [212]. The advantages of a latency to move test display its weak points at the same time. The simplicity and short observation time only allow a vague estimation of the impairment. As there is no training phase, anxiety-like behavior could influence the results. Additionally, locomotion might be impaired depending on the affected brain area, but independent of the infarct size. Therefore, more complex tests are better suited for assessing impairment in combination with the infarct size. Compared to the evaluated parameters in the human study, the neurological deficit in mice corresponds to the NIHSS at admission to the hospital. As a score for stroke severity, it reflects, among others, motor or sensory impairment. In humans, the lesion volume determined by magnetic resonance imaging (MRI) has been shown to correlate with the NIHSS after 24 hours [213]. Moreover, the NIHSS is strongly associated with the recovery of patients [41, 42]. Consequently, the infarct size of MCAO animals can be considered a predictor of the outcome.

The infarct size had an impact on plasma lipid levels, already 2 hours after MCAO. LPC (22:0) and LPC (26:1) decreased with an increasing infarct. That fits the downregulation of LPC (22:0) and

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other LPCs in the serum of patients with a bad outcome, probably mediated by autotaxin activity. Also medium- to long-chain PCs and two PC/PE ratios showed a negative correlation with the infarct size. Interestingly, the same pattern was observed in the human serum. PCs and PC/PE ratios, like PC (38:6)/PE (38:6), were negatively correlated with the mRS90 score and the NIHSS. Lower PCs and PC/PE ratios might indicate less compensation for PC degradation via the PEMT pathway. However, PE levels in plasma did not show an upwards trend in animals with a big infarct in contrast to the human data. Additionally, there was no correlation of PC/PE ratios in the plasma after 24 hours.

Immediate effects on the ipsilateral brain side could be observed after 2 hours. Interestingly, several long-chain, polyunsaturated PEs showed a negative correlation with the infarct size. Lower PE levels in animals with a big infarct could imply more lipid peroxidation or phospholipase activity. However, other lipid classes, like PCs, were not changed. Therefore, the metabolization of PEs to PCs via the PEMT pathway has to be considered. Changes in brain PE levels with the infarct size might reflect PEMT activation in response to the pronounced tissue damage. Alternatively, the increase in PEA and OEA, detectable after 24 hours in the brain, could influence PE levels already at the 2 hour time point. Lower PE levels on the ipsilateral side of transient MCAO rat brains have been reported [209]. However, no difference compared to the contralateral side was observed in our study. This discrepancy could originate from a generally smaller infarct size in our study or the differences in the stroke animal model.

The negative correlation of glutathione with the infarct size on the ipsilateral side indicates the enhanced consumption of the antioxidant, but changes were not significant compared to the contralateral side. Biosynthesis via the transsulfuration pathway (**Figure 5.6**) could compensate for glutathione depletion under oxidative circumstances. α -Aminoadipate was positively correlated. It was upregulated on both the ipsi- and the contralateral side of the brain in animals with a big infarct. As discussed above, oxidative stress and protein degradation could regulate α -aminoadipate levels, not only on the affected brain side but also globally. Additionally, citrulline showed a positive correlation with the infarct area with a significant increase on the ipsilateral side, especially for big infarcts. Elevated levels might reflect increased metabolization of arginine to NO, aggravating oxidative stress in the brain (**Figure 5.5**). As citrulline can mitigate brain injury by increasing eNOS expression, the association with bigger infarct sizes could also be a counteracting mechanism to contain tissue damage.

The increased citrulline levels on the ipsilateral side and the upregulation in animals with a big infarct remained consistent 24 hours after MCAO. This implies a prolonged shift in the arginine

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metabolism after the induced ischemia. Furthermore, PEA and OEA on the ipsilateral side were positively correlated with the infarct size. A strong upregulation, especially in animals with a big infarct, suggest the activation of neuroprotective mechanisms 24 hours after the MCAO.

In the plasma, associations of lipid levels with the infarct size differed after 24 hours. Whereas long-chain polyunsaturated PCs correlated after 2 hours, only mono-saturated PCs showed a negative correlation after 24 hours. Considering our hypothesis, missing correlations might reflect the recovery of long-chain, polyunsaturated PC levels, mediated by the PEMT pathway, but no complete compensation for PCs with shorter FA residues. A similar pattern was observed for ischemic stroke patients compared to healthy controls, where short- and medium-chain, but not long-chain, PCs were strongly downregulated on day 1. TGs were also decreased in the serum of patients. Similarly, TGs in the plasma of MCAO mice decreased with increasing infarct size, independent of the FA residues. The discussed effect of malnutrition in ischemic stroke patients can likewise be applied to the mouse model. A more pronounced infarct could increase the energy demand and, at the same time, cause a stronger impairment, hindering the animals from eating. Acetylcarnitine was also negatively correlated with the infarct size, implying an increased consumption of acetyl-CoA in the citric acid cycle in response to ischemia.

Strikingly, an association of lipids with the infarct size was detected in the liver after 24 hours. A delayed systemic effect of ischemic stroke on metabolite levels in the liver has been shown in rats before [214]. In our study, two PCs and five PC/PE ratios were positively correlated with the infarct size. Elevated levels in animals with bigger infarcts indicate an activation of the hepatic PEMT pathway. PEMT is highly expressed in the liver and reduced PC/PE ratios have been reported in conditions like non-alcoholic fatty liver disease (NAFLD) [215]. In a mouse model, PEMT deficiency lead to a decreased PC/PE ratio in the liver and impaired membrane integrity [216]. In stroke, stimulation of proteases and phospholipases, as well as lipid peroxidation, lead to cell membrane damage. Activation of the PEMT pathway and following increase of PC/PE ratios might therefore be a counteractive mechanism in the liver as a response to either local events in the brain or systemic inflammation and oxidative stress. In mice, the PC/PE ratio in the liver increased with infarct size after 24 hours, whereas plasma levels were decreased after 2 but not 24 hours. It seems likely that PEMT activity in the liver compensates for decreased plasma ratios. In patients, serum PC/PE levels were increased in patients with a good outcome and decreased in patients with a bad outcome. If PEMT activity in the liver would compensate for decreased ratios after ischemic stroke, this compensatory mechanism might work better in patients with a good outcome. Interestingly, NAFLD has been associated with more severe strokes and a worse outcome [217]. As PEMT-derived PCs are essential for VLDL excretion from the liver, decreased

TG plasma levels could also influence PEMT activation. Increased transfer of VLDL to the periphery could provide the brain and other extrahepatic tissue with phospholipids and TGs.

Smaller infarcts have been observed for young female mice compared to young males, mediated by neuroprotective effects of endogenous estrogen [218, 219]. Interestingly, *PEMT* gene and protein expression is activated by estrogen [220]. If estrogen levels influence the PEMT pathway in a potentially beneficial way after ischemia has to be investigated. We worked with both male and female mice. However, our cohorts were too small to estimate a sex effect on the infarct size. For humans, a systematic literature review describes a higher disability upon admission for female patients. However, a favorable functional outcome at discharge was more likely compared to men [221].

5.4 Summary and outlook

In summary, this study provides a comprehensive metabolomics and lipidomics profile of ischemic stroke.

For the biochemical translation of metabolomics and lipidomics data, it is essential to ensure an unambiguous compound identification. Development of the metabolomics screen and generation of an MS² and LC library improved the identification confidence and the overall quality of the metabolite analysis. However, the coverage bias for both metabolites and lipids has to be considered in the interpretation of results. Untargeted metabolomics and lipidomics measurements provide an enormous amount of data. Our quality and identification criteria limit the number of actually investigated compounds. Guided by advanced statistics, the analysis of the whole data set would be possible. However, the biological significance still depends on the identification of prominent features. For unknown compounds, this can be a time-consuming and challenging process.

The investigation of human serum of ischemic stroke patients showed that several alterations can be observed compared to the healthy control group. Most striking were the changes related to the phospholipid metabolism. Additionally, PC/PE ratios of long-chain polyunsaturated lipid species differed between patients with a good and patients with a bad outcome on the first three days after ischemia. The direct metabolic connection between long-chain polyunsaturated PCs and PEs is the PEMT pathway, which converts PEs to PCs via methylation, using SAM as methyl donor. Our data suggest a positive role of an activated PEMT pathway in the recovery of patients. The incorporation of an MCAO animal model revealed dramatic changes in the brain 2 and

24 hours after the induction of ischemia. Those changes were related to the occurrence of RNS and oxidative stress as well as to the involvement of the transmethylation and transsulfuration pathways. Correlation analysis of plasma lipids with the infarct size supported the results obtained from human serum. Additionally, a systemic reaction related to the PEMT pathway was suggested in the liver. However, our results are limited to relative changes in metabolite and lipid levels. We cannot make definite statements about the actual flux, enzyme activity or gene expression. Certainly, the results obtained in our study have to be confirmed in further experiments and independent cohorts.

Interestingly, a genome-wide association study (GWAS) linked variants in loci overlapping the *PEMT*, *RAI1* and *RASD1* genes to increased susceptibility for ischemic stroke [222]. If a genetic predisposition related to *PEMT* could also influence the outcome of patients has to be investigated. An established risk factor for ischemic stroke is elevated plasma homocysteine, and plasma levels in the acute phase can predict mortality [223]. As a metabolite of the transmethylation and transsulfuration pathway, it might be interesting for future experiments to determine homocysteine levels in patients or mice. In our study, stroke risk factors were not considered in particular. However, taking genetic associations and other predispositions into account could be a promising leverage point in the future.

The MCAO mouse model offers excellent opportunities for follow-up experiments. An untreated control group could extend insights into the general impact of focal ischemia on the brain, plasma and liver. Detailed neurological testing would complement the infarct size measurement. To confirm or falsify our hypothesis of an activated PEMT pathway, enzyme activity and gene expression have to be analyzed. Investigating genetically modified mice or different treatments could deliver further evidence. As global PEMT knockout animals show severe alterations in liver and energy metabolism, a cell type or tissue-specific knockout might be promising [224]. Increasing the supply of substrates like PEs or SAM could influence PEMT activity. Supplementation of the animal's diet or injection at different time points after MCAO are possible. If these interventions had an impact on the infarct size and the neurological deficit of mice, the PEMT pathway would be a very promising target for the treatment of ischemic stroke patients.

6 Supplement

6.1 Supplementary figures

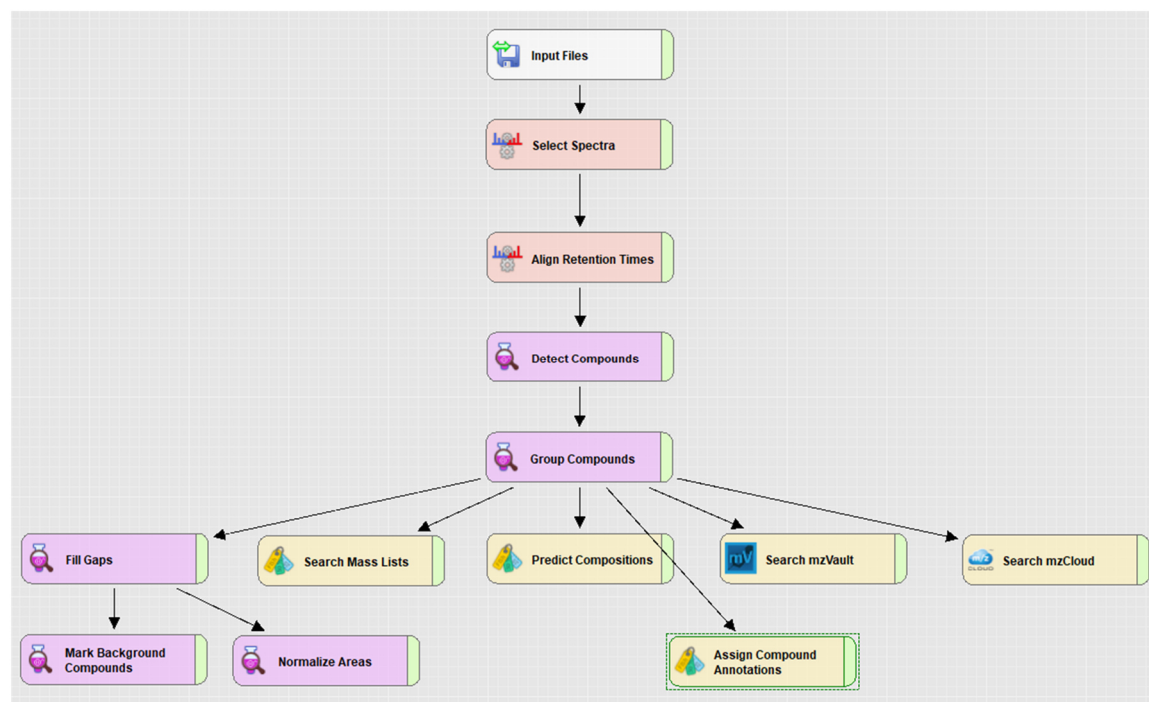


Figure S6.1 Compound Discoverer workflow for data analysis of the metabolomics screen.

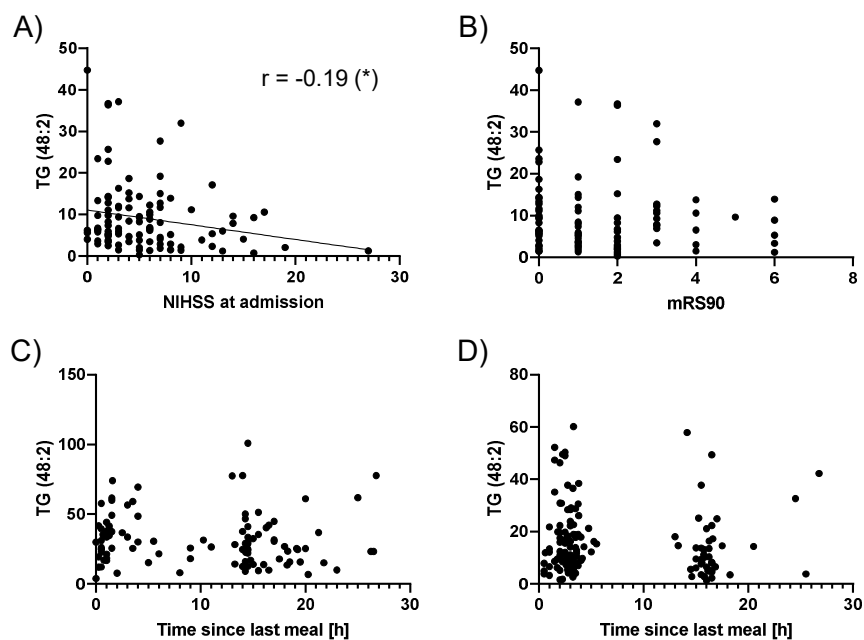


Figure S6.2 Correlation of TG (48:2) with NIHSS, mRS90, and time since last meal. Relative area of TG (48:2) as an example for all TGs. (A) Correlation with NIHSS at admission, $n = 107$, $r =$ Spearman rank correlation coefficient, $*p \leq 0.05$. (B) Correlation with mRS90, $n = 107$. (C) Correlation with time since last meal [hours] in stroke patients, $n = 103$. (D) Correlation with time since last meal [hours] in control patients, $n = 139$.

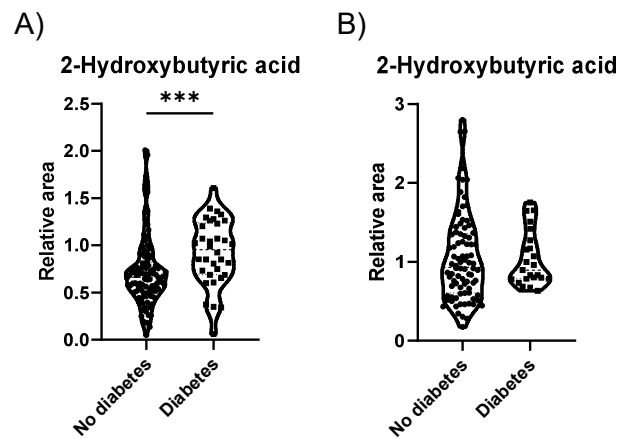


Figure S6.3 2-Hydroxybutyric acid serum levels in patients with and without diabetes. (A) Control patients, $n = 105/34$ (no diabetes/diabetes). (B) Stroke patients, $n = 86/23$ (no diabetes/diabetes). Wilcoxon Mann Whitney test, *** $p \leq 0.001$.

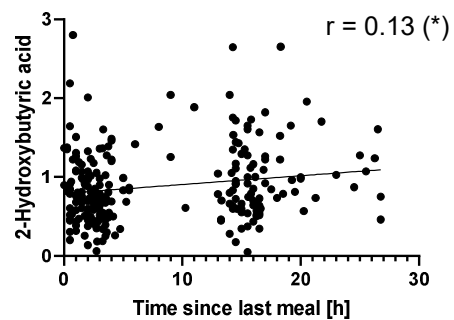


Figure S6.4 Correlation of 2-hydroxybutyric acid with time since last meal in all patients. $N = 242$, $r =$ Spearman rank correlation coefficient, * $p \leq 0.05$.

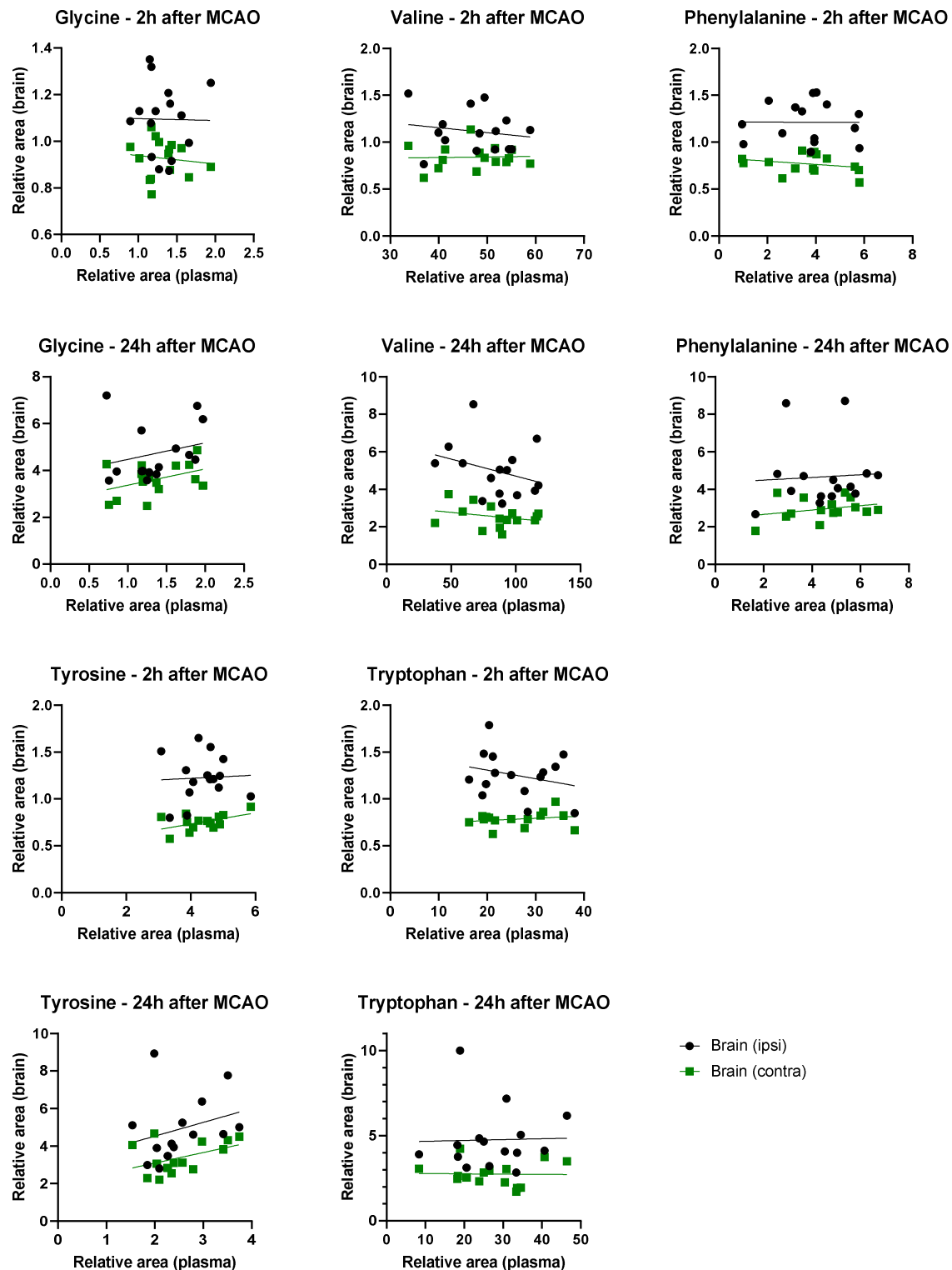


Figure S6.5 Correlation of amino acid levels in the plasma and the brain of MCAO animals. Relative concentrations of amino acids in the plasma, ipsilateral and contralateral side of the brain 2 hours and 24 hours after MCAO. Amino acids that were significantly increased on the ipsilateral side are shown (see Table 4.9 and Table 4.10). None of the amino acids showed a significant correlation between plasma and brain (Pearson correlation). $N = 15$, one outlier was excluded for tyrosine (plasma, 24 hours).

6.2 Supplementary tables

Table S6.1 Overview of tested LC library conditions. All tested conditions with detailed information about LC parameters, confirmed compounds and total score. *additives only in Eluent A **according to Table 6.2 ***according to Figure 5.8.

LC library ID	Column (RP)	Eluents (A/B)	Additives	Concentration	pH
RP1	Accucore C18 (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP2	Accucore C18 (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	water/methanol	Ammonium hydroxide* Formic Acid*	0.15 % 0.05 %	9
RP3	Ascentis Express C18 (Supelco) 5 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP4	Ascentis Express ES-CN (Supelco) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP5	Ascentis Express F5 (Supelco) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP6	Ascentis Express Phenyl-Hexyl (Supelco) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP7	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP8	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP9	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP10	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP11	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP12	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP13	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP14	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP15	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Ammonium acetate* Ammonium hydroxide* Formic Acid*	20 mM 0.055 % 0.025 %	7
RP16	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Ammonium hydroxide* Formic Acid*	0.11 % 0.045 %	7
RP17	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Ammonium hydroxide* Formic Acid*	0.15 % 0.05 %	9
RP18	Acquity UPLC BEH C18 (Waters) 1.7 µm, 100 x 2.1 mm	water/methanol	Ammonium hydroxide*	0.1 %	11
RP19	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP20	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP21	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP22	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP23	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP24	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP25	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP26	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP27	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP28	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP29	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP30	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP31	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP32	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP33	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP34	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP35	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP36	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
RP37	Cortecs T3 (Waters) 2.7 µm, 100 x 2.1 mm	water/methanol	Ammonium hydroxide* Formic Acid*	0.11 % 0.045 %	7
RP38	Hypercarb (Thermo) 5 µm 150 x 2.1 mm	water/methanol	Formic Acid	0.1 %	3
LC library ID	Column (HILIC)	Eluents (A/B)	Additives	Concentration	pH
HILIC1	Accucore HILIC (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium formate Formic Acid	10 mM 0.1 %	3
HILIC2	Accucore HILIC (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate Acetic Acid	10 mM 0.1 %	4
HILIC3	Accucore HILIC (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate	15 mM	6
HILIC4	Accucore HILIC (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate	5 mM	6
HILIC5	Accucore HILIC (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium formate	10 mM	6
HILIC6	Accucore HILIC (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate	10 mM	6
HILIC7	Accucore HILIC (Thermo Scientific) 2.6 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate	10 mM	6
HILIC8	X-Bridge BEH Amide (Waters) 2.5 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium formate Formic Acid	10 mM 0.1 %	3
HILIC9	X-Bridge BEH Amide (Waters) 2.5 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate Acetic Acid	5 mM 0.1 %	4
HILIC10	X-Bridge BEH Amide (Waters) 2.5 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate Acetic Acid	10 mM 0.1 %	4
HILIC11	X-Bridge BEH Amide (Waters) 2.5 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate	5 mM	6
HILIC12	X-Bridge BEH Amide (Waters) 2.5 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium formate	10 mM	6
HILIC13	X-Bridge BEH Amide (Waters) 2.5 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate	10 mM	6
HILIC14	X-Bridge BEH Amide (Waters) 2.5 µm, 100 x 2.1 mm	H2O/95%ACN	Ammonium acetate Ammonium hydroxide	10 mM 0.1 %	9
HILIC15	SeQuant ZIC HILIC (Merck) 5 µm, 150 x 2.1 mm	H2O/95%ACN	Ammonium acetate Acetic Acid	10 mM 0.1 %	4
HILIC16	SeQuant ZIC HILIC (Merck) 5 µm, 150 x 2.1 mm	H2O/95%ACN	Ammonium acetate	5 mM	6
HILIC17	SeQuant ZIC HILIC (Merck) 5 µm, 150 x 2.1 mm	H2O/95%ACN	Ammonium acetate	15 mM	6
HILIC18	SeQuant ZIC HILIC (Merck) 5 µm, 150 x 2.1 mm	H2O/95%ACN	Ammonium formate	10 mM	6
HILIC19	SeQuant ZIC HILIC (Merck) 5 µm, 150 x 2.1 mm	H2O/95%ACN	Ammonium acetate	10 mM	6

Supplement

Continued from Table S6.1 Overview of tested LC library conditions. All tested conditions with detailed information about LC parameters, confirmed compounds and total score. *additives only in Eluent A **according to Table 6.2 ***according to Figure 5.8.

LC library ID	Gradient**	Injection volume [µl]	Flow rate [ml/min]	Metabolite standard Mix - solvent	Scan ranges**	Confirmed compounds	Total score	Condition***
RP1	A	5	0.2	100% MeOH	method 1	99	2.70	
RP2	A	5	0.2	100% MeOH	method 1	66	3.30	
RP3	E	5	0.1	50% MeOH	method 2	162	2.90	
RP4	E	5	0.1	50% MeOH	method 2	167	2.90	
RP5	E	5	0.1	50% MeOH	method 2	167	2.98	A
RP6	E	5	0.1	50% MeOH	method 2	166	2.90	
RP7	B	5	0.1	100% MeOH	method 1	111	2.77	
RP8	B	5	0.1	100% MeOH	method 2	128	2.76	
RP9	B	10	0.1	100% MeOH	method 2	109	3.18	
RP10	B	15	0.1	100% MeOH	method 2	107	3.32	
RP11	B	20	0.1	100% MeOH	method 2	109	3.38	
RP12	A	5	0.1	50% MeOH	method 2	142	2.62	
RP13	E	5	0.1	50% MeOH	method 2	158	2.98	
RP14	E	5	0.2	50% MeOH	method 2	148	2.57	
RP15	E	5	0.2	50% MeOH	method 2	123	2.65	
RP16	B	5	0.1	100% MeOH	method 1	125	2.94	
RP17	B	5	0.1	100% MeOH	method 1	120	2.88	
RP18	B	5	0.1	100% MeOH	method 1	120	2.60	
RP19	E	5	0.1	50% MeOH	method 2	169	2.97	B
RP20	A	5	0.1	50% MeOH	method 2	153	2.69	
RP21	C	5	0.1	50% MeOH	method 2	148	2.65	
RP22	A	5	0.15	50% MeOH	method 2	152	2.59	
RP23	C	5	0.15	50% MeOH	method 2	148	2.55	
RP24	A	5	0.2	100% MeOH	method 1	122	2.73	
RP25	A	5	0.2	100% MeOH	method 2	145	2.90	
RP26	A	10	0.2	100% MeOH	method 2	145	3.22	
RP27	A	15	0.2	100% MeOH	method 2	148	3.35	
RP28	A	20	0.2	100% MeOH	method 2	146	3.43	
RP29	A	5	0.2	50% MeOH	method 2	147	2.55	
RP30	C	5	0.2	50% MeOH	method 2	142	2.63	
RP31	A	5	0.25	50% MeOH	method 2	152	2.56	
RP32	C	5	0.25	50% MeOH	method 2	143	2.57	
RP33	D	5	0.3	50% MeOH	method 2	134	2.58	
RP34	A	5	0.3	50% MeOH	method 2	146	2.56	
RP35	C	5	0.3	50% MeOH	method 2	143	2.58	
RP36	E	5	0.3	50% MeOH	method 2	148	2.49	C
RP37	A	5	0.2	100% MeOH	method 1	91	2.93	
RP38	F	5	0.1	50% MeOH	method 2	88	3.20	
LC library ID	Gradient	Injection volume [µl]	Flow rate [ml/min]	Metabolite standard Mix - solvent	Scan ranges	Confirmed compounds	Total score	Condition***
HILIC1	G	5	0.5	50%MeOH/50%ACN	method 2	91	2.72	
HILIC2	G	5	0.5	50%MeOH/50%ACN	method 2	106	2.59	
HILIC3	G	5	0.5	50%MeOH/50%ACN	method 2	90	2.63	
HILIC4	G	5	0.5	50%MeOH/50%ACN	method 2	99	2.71	
HILIC5	G	5	0.5	50%MeOH/50%ACN	method 2	105	2.74	
HILIC6	G	5	0.5	50%MeOH/50%ACN	method 2	121	2.81	
HILIC7	G	5	0.5	50%MeOH/50%H2O	method 2	94	3.09	
HILIC8	G	5	0.5	50%MeOH/50%ACN	method 2	103	2.53	
HILIC9	G	5	0.5	50%MeOH/50%ACN	method 2	106	2.58	
HILIC10	G	5	0.5	50%MeOH/50%ACN	method 2	102	2.79	
HILIC11	G	5	0.5	50%MeOH/50%ACN	method 2	102	2.66	
HILIC12	G	5	0.5	50%MeOH/50%ACN	method 2	87	2.49	
HILIC13	G	5	0.5	50%MeOH/50%ACN	method 2	105	2.63	
HILIC14	G	5	0.5	50%MeOH/50%ACN	method 2	112	2.61	
HILIC15	G	5	0.75	50%MeOH/50%ACN	method 2	115	2.54	F
HILIC16	G	5	0.75	50%MeOH/50%ACN	method 2	126	2.72	D
HILIC17	G	5	0.75	50%MeOH/50%ACN	method 2	107	2.61	
HILIC18	G	5	0.75	50%MeOH/50%ACN	method 2	95	2.71	
HILIC19	G	5	0.75	50%MeOH/50%ACN	method 2	120	2.57	E

Table S6.2 Instrument settings and gradients for LC library generation and method development.

ESI source	+ve mode	-ve mode
Sheat gas flow rate	30	20
Aux gas flow rate	10	10
Spray voltage [kV]	4.5	3.5
Capillary temp. [°C]	300	320
S-lens RF level	75	75

Full MS	General settings
Resolution	70000
AGC target	3.00E+06
Maximum IT [ms]	200
Scan range	
Method 1 (both scan ranges in one Full MS/dd-MS ² experiment)	50 - 500 m/z 500 - 1500 m/z
Method 2 (two separate Full MS/dd-MS ² experiments for scan ranges)	60 - 150 m/z 150 - 1500 m/z
spiked plasma	60 - 900 m/z
dd-MS ²	
Resolution	17500
AGC target	5.00E+05
Maximum IT [ms]	50
Loop count	10
Isolation window	1 m/z
NCE (eV)	10/50/100
dd-settings	
Minimum AGC target	2.50E+02
Dynamic exclusion [s]	20

Gradient A	
Time	% B
0	5
5	5
25	95
25.1	100 (flow rate = 0.3 ml/min)
35	100 (flow rate = 0.3 ml/min)
35.1	5
45	5
Gradient B	
Time	% B
0	5
5	5
25	95
25.1	100
35	100
35.1	5
45	5
Gradient C	
Time	% B
0	0
1	0
6	5
30	100
35	100
35.1	0
40	0
Gradient D	
Time	% B
0	0
5	0
25	100
30	100
30.1	0
35	0
Gradient E	
Time	% B
0	5
5	5
25	100
30	100
30.1	5
35	5
Gradient F	
Time	% B
0	5
5	5
20	100
35	100
35.1	5
45	5
Gradient G	
Time	% B
0	95
5	95
20	57
25	57
30	95
40	95

Table S6.3 Compound list of the MS² library and LC library.

Adduct (+ve mode)	Adduct (-ve mode)	Compound name	Formula	Neutral mass	HMDB ID	MS ² library (included)	LC library (included)	Comparison with mzCloud (Figure 4.3 B)	Included in Figure 4.5 - 4.7
[M+H] ⁺		TRIMETHYLAMINE	C3H9N	59.07350	HMDB000906	x		not present	
[M+H] ⁺		PYRAZOLE	C3H4N2	68.03690		x		not present	
	[M-H] ⁻	PROPENOATE	C3H4O2	72.02113	HMDB31647	x		present	
[M+H] ⁺		METHYLGUANIDINE	C2H7N3	73.06400	HMDB01522	x	x	not present	x
	[M-H] ⁻	GLYOXYLIC ACID	C2H2O3	74.00039	HMDB000119	x		present	
[M+H] ⁺		1,3-DIAMINOPROPANE	C3H10N2	74.08440	HMDB000002	x	x	present	
[M+H] ⁺	[M-H] ⁻	GLYCINE	C2H5NO2	75.03203	HMDB00123	x	x	only CID for positive mode	
	[M-H] ⁻	GLYCOLATE	C2H4O3	76.01605	HMDB00115	x		present	
	[M-H] ⁻	DIACETYL	C4H6O2	86.03678	HMDB03407	x		present	
	[M-H] ⁻	PYRUVATE	C3H4O3	88.01605	HMDB000243	x	x	present	
[M+H] ⁺		PUTRESCINE	C4H12N2	88.10005	HMDB01414	x		present	
[M+H] ⁺	[M-H] ⁻	BETA-ALANINE	C3H7NO2	89.04768	HMDB000056	x		present	
[M+H] ⁺	[M-H] ⁻	D-ALANINE	C3H7NO2	89.04768	HMDB01310	x		present	
	[M-H] ⁻	L-ALANINE	C3H7NO2	89.04768	HMDB000161	x		present	
[M+H] ⁺	[M-H] ⁻	SARCOSINE	C3H7NO2	89.04768	HMDB000271	x		not present	
	[M-H] ⁻	(S)-LACTATE	C3H6O3	90.03170	HMDB000190	x		present	
[M+H] ⁺		ANILINE	C6H7N	93.05785	HMDB03012	x	x	present	x
[M+H] ⁺		2-HYDROXYPYRIDINE	C5H5NO	95.03711	HMDB13751	x	x	not present	x
[M+H] ⁺		5-VALEROLACTONE	C5H8O2	100.05243		x		not present	
[M+H] ⁺	[M-H] ⁻	1-AMINOCYCLOPROPANE-1-CARBOXYLATE	C4H7NO2	101.04768	HMDB36458	x	x	not present	
	[M-H] ⁻	ACETOACETATE	C4H6O3	102.03169	HMDB000060	x		not present	
[M+H] ⁺		CADAVERINE	C5H14N2	102.11570	HMDB02322	x	x	not present	x
	[M-H] ⁻	N-FORMYLGLYCINE	C3H5NO3	103.02694		x	x	not present	
[M+H] ⁺	[M-H] ⁻	2-AMINO-2-METHYLPROPANOATE	C4H9NO2	103.06333	HMDB01906	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	4-AMINOBUTANOATE	C4H9NO2	103.06333	HMDB000112	x		present	
M+		CHOLINE	C5H14NO	103.09917	HMDB000097	x	x	present	x
	[M-H] ⁻	MALONATE	C3H4O4	104.01096	HMDB000691	x		present	
	[M-H] ⁻	2-HYDROXYBUTYRIC ACID	C4H8O3	104.04734	HMDB000008	x		not present	
	[M-H] ⁻	3-HYDROXYBUTANOIC ACID	C4H8O3	104.04735	HMDB000011	x		present	
	[M-H] ⁻	ALPHA-HYDROXYISOBUTYRIC ACID	C4H8O3	104.04735	HMDB000729	x		not present	
[M+H] ⁺	[M-H] ⁻	L-2,3-DIAMINOPROPIONIC ACID	C3H8N2O2	104.05858	HMDB02006	x	x	present	
[M+H] ⁺		DIETHANOLAMINE	C4H11NO2	105.07898	HMDB04437	x	x	present	x
	[M-H] ⁻	GLYCERATE	C3H6O4	106.02661	HMDB000139	x		present	
	[M-H] ⁻	D-GLYCERIC ACID	C3H6O4	106.02661	HMDB000139	x		present	
[M+H] ⁺	[M-H] ⁻	HYPOTAURINE	C2H7NO2S	109.01975	HMDB000965	x	x	present	x
[M+H] ⁺	[M-H] ⁻	2-AMINOPHENOL	C6H7NO	109.05276		x	x	only positive mode available	
	[M-H] ⁻	CATECHOL	C6H6O2	110.03678	HMDB000957	x		present	
[M+H] ⁺	[M-H] ⁻	PYRROLE-2-CARBOXYLATE	C5H5NO2	111.03202	HMDB04230	x		present	
[M+H] ⁺	[M-H] ⁻	CYTOSINE	C4H5N3O	111.04326	HMDB000630	x	x	present	x
[M+H] ⁺		HISTAMINE	C5H9N3	111.07965	HMDB000870	x	x	present	x
[M+H] ⁺	[M-H] ⁻	URACIL	C4H4N2O2	112.02728	HMDB000300	x	x	present	x
[M+H] ⁺		SORBATE	C6H8O2	112.05243	HMDB29581	x	x	present	x
[M+H] ⁺	[M-H] ⁻	CREATININE	C4H7N3O	113.05891	HMDB000562	x	x	only positive mode available	x
[M+H] ⁺		5,6-DIHYDROURACIL	C4H6N2O2	114.04293	HMDB000076	x	x	not present	
[M+H] ⁺		L-PROLINE	C5H9NO2	115.06333	HMDB000162	x		present	
	[M-H] ⁻	FUMARATE	C4H4O4	116.01096	HMDB000134	x		present	
	[M-H] ⁻	MALEIC ACID	C4H4O4	116.01096	HMDB000176	x	x	present	
[M+H] ⁺		L-HOMOCYSTEINE THIOLACTONE	C4H7NO5	117.02483	HMDB02287	x	x	not present	
	[M-H] ⁻	N-ACETYLGLYCINE	C4H7NO3	117.04259	HMDB000532	x	x	present	x
[M+H] ⁺	[M-H] ⁻	GUANIDINOACETATE	C3H7N3O2	117.05383	HMDB000128	x	x	present	x
[M+H] ⁺		INDOLE	C8H7N	117.05785	HMDB000738	x	x	present	
[M+H] ⁺	[M-H] ⁻	5-AMINOPENTANOATE	C5H11NO2	117.07898	HMDB03355	x		present	
[M+H] ⁺		BETAINE	C5H11NO2	117.07898	HMDB000043	x		present	
[M+H] ⁺	[M-H] ⁻	L-NORVALINE	C5H11NO2	117.07898	HMDB13716	x		not present	
[M+H] ⁺	[M-H] ⁻	L-VALINE	C5H11NO2	117.07898	HMDB000883	x		only positive mode available	
	[M-H] ⁻	METHYLMALONATE	C4H6O4	118.02661	HMDB00202	x		present	
	[M-H] ⁻	SUCCINATE	C4H6O4	118.02661	HMDB000254	x	x	present	
[M+H] ⁺	[M-H] ⁻	HOMOSERINE	C4H9NO3	119.05824	HMDB000719	x		present	
[M+H] ⁺	[M-H] ⁻	L-ALLOTHREONINE	C4H9NO3	119.05824	HMDB04041	x		present	
[M+H] ⁺	[M-H] ⁻	L-THREONINE	C4H9NO3	119.05824	HMDB000167	x		present	
[M+H] ⁺	[M-H] ⁻	PURINE	C5H4N4	120.04360	HMDB01366	x	x	present	x
[M+H] ⁺		L-CYSTEINE	C3H7NO2S	121.01975	HMDB000574	x		present	
[M+H] ⁺		PHENETHYLAMINE	C8H11N	121.08915	HMDB12275	x	x	present	x
	[M-H] ⁻	3-HYDROXYBENZALDEHYDE	C7H6O2	122.03678		x		not present	
[M+H] ⁺		4-HYDROXYBENZALDEHYDE	C7H6O2	122.03678	HMDB11718	x		present	
	[M-H] ⁻	BENZOATE	C7H6O2	122.03678	HMDB01870	x		present	
[M+H] ⁺		NICOTINAMIDE	C6H6N2O	122.04801	HMDB01406	x	x	present	x
[M+H] ⁺	[M-H] ⁻	NICOTINATE	C6H5NO2	123.03203	HMDB01488	x		present	
[M+H] ⁺		PICOLINIC ACID	C6H5NO2	123.03203	HMDB02243	x		present	
	[M-H] ⁻	3-HYDROXYBENZYL ALCOHOL	C7H8O2	124.05243	HMDB59712	x	x	present	
	[M-H] ⁻	4-METHYLCAECOL	C7H8O2	124.05243	HMDB000873	x	x	present	
[M+H] ⁺	[M-H] ⁻	TAURINE	C2H7NO3S	125.01467	HMDB000251	x	x	present	x
[M+H] ⁺	[M-H] ⁻	(2-AMINOETHYL)PHOSPHONATE	C2H8NO3P	125.02418	HMDB11747	x	x	only negative mode available	
[M+H] ⁺	[M-H] ⁻	5-METHYLCYTOSINE	C5H7N3O	125.05891	HMDB02894	x		present	x
[M+H] ⁺		3-METHYLHISTAMINE	C6H11N3	125.09530	HMDB01861	x	x	present	x
[M+H] ⁺		4-IMIDAZOLEACETIC ACID	C5H6N2O2	126.04293	HMDB02024	x	x	present	
[M+H] ⁺	[M-H] ⁻	THYMINE	C5H6N2O2	126.04293	HMDB000262	x	x	only positive mode available	
[M+H] ⁺	[M-H] ⁻	5-OXO-D-PROLINE	C5H7NO3	129.04259	HMDB000805	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	5-OXO-L-PROLINE	C5H7NO3	129.04259	HMDB000267	x		only positive mode available	
[M+H] ⁺		QUINOLINE	C9H7N	129.05785	HMDB33731	x	x	present	x
[M+H] ⁺	[M-H] ⁻	L-PIPECOLIC ACID	C6H11NO2	129.07898	HMDB000716	x		present	
[M+H] ⁺	[M-H] ⁻	PIPECOLATE	C6H11NO2	129.07898	HMDB000070	x		present	
	[M-H] ⁻	2-METHYLMALATE	C5H6O4	130.02661	HMDB000634	x		present	
	[M-H] ⁻	ITACONATE	C5H6O4	130.02661	HMDB02092	x		present	
	[M-H] ⁻	4-METHYL-2-OXO-PENTANOIC ACID	C6H10O3	130.06299	HMDB000695	x		not present	
[M+H] ⁺		MEVALOLACTONE	C6H10O3	130.06300	HMDB006024	x	x	present	
[M+H] ⁺		N-ACETYLPUTRESCINE	C6H14N2O	130.11061	HMDB02064	x	x	present	x
[M+H] ⁺		AGMATINE	C5H14N4	130.12180	HMDB01432	x	x	present	
[M+H] ⁺	[M-H] ⁻	4-HYDROXY-L-PROLINE	C5H9NO3	131.05824	HMDB06055	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	5-AMINOLEVULINIC ACID	C5H9NO3	131.05824	HMDB01149	x		present	
[M+H] ⁺	[M-H] ⁻	CIS-4-HYDROXY-D-PROLINE	C5H9NO3	131.05824	HMDB06040	x		present	
[M+H] ⁺	[M-H] ⁻	N-ACETYL-L-ALANINE	C5H9NO3	131.05824	HMDB000766	x	x	only negative mode available	
[M+H] ⁺	[M-H] ⁻	TRANS-4-HYDROXYPROLINE	C5H9NO3	131.05824	HMDB000725	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	CREATINE	C4H9N3O2	131.06948	HMDB000064	x	x	present	x
[M+H] ⁺	[M-H] ⁻	LEUCINE	C6H13NO2	131.09463	HMDB000687	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	L-ISOLEUCINE	C6H13NO2	131.09463	HMDB000172	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	NORLEUCINE	C6H13NO2	131.09463	HMDB01645	x		only positive mode available	
[M+H] ⁺		BIS(3-AMINOPROPYL)AMINE	C6H17N3	131.14225	HMDB11634	x	x	not present	x
	[M-H] ⁻	ETHYLMALONIC ACID	C5H8O4	132.04226	HMDB000622	x	x	present	
	[M-H] ⁻	GLUTARATE	C5H8O4	132.04226	HMDB000661	x	x	present	
[M-H2O+H] ⁺	[M-H] ⁻	3-UREIDOPROPIONATE	C4H8N2O3	132.05349	HMDB000026	x		present	
[M+H] ⁺	[M-H] ⁻	D-ORNITHINE	C5H12N2O2	132.08987	HMDB03374	x		present	

Continued from Table S6.3 Compound list of MS² library and LC library.

Adduct (+ve mode)	Adduct (-ve mode)	Compound name	Formula	Neutral mass	HMDB ID	MS ² library (included)	LC library (included)	Comparison with mzCloud (Figure 4.3 B)	Included in Figure 4.5 - 4.7
[M+H] ⁺	[M-H] ⁻	L-ORNITHINE	C5H12N2O2	132.0897	HMDB00214	x		present	
[M+H] ⁺	[M-H] ⁻	D-ASPARTATE	C4H7NO2	133.03751	HMDB006483	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	L-ASPARTATE	C4H7NO2	133.03751	HMDB00191	x		present	
[M+H] ⁺	[M-H] ⁻	(R)-MALATE	C4H6O5	134.02153	HMDB031518	x		present	
[M+H] ⁺	[M-H] ⁻	HOMOCYSTEINE	C4H9NO2S	135.03540	HMDB00742	x	x	present	
[M+H] ⁺	[M-H] ⁻	ADENINE	C5H5N5	135.05450	HMDB00034	x	x	present	x
[M+H] ⁺	[M-H] ⁻	HYPOXANTHINE	C5H4N4O2	136.03851	HMDB00157	x	x	present	x
[M+H] ⁺	[M-H] ⁻	1-METHYLNICOTINAMIDE	C7H9N2O	136.06421	HMDB00699	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N,N-DIMETHYL-1,4-PHENYLENEDIAMINE	C8H12N2	136.10005		x		not present	
[M+H] ⁺	[M-H] ⁻	4-AMINOBENZOATE	C7H7NO2	137.04768	HMDB01392	x	x	present	
[M+H] ⁺	[M-H] ⁻	ANTHRANILATE	C7H7NO2	137.04768	HMDB01123	x	x	present	
[M+H] ⁺	[M-H] ⁻	SALICYLAMIDE	C7H7NO2	137.04768	HMDB15687	x	x	present	
[M+H] ⁺	[M-H] ⁻	TRIGONELLINE	C7H7NO2	137.04768	HMDB00875	x	x	present	
[M+H] ⁺	[M-H] ⁻	PHENYLETHANOLAMINE	C8H11NO	137.08406	HMDB01065	x	x	present	
[M+H] ⁺	[M-H] ⁻	TYRAMINE	C8H11NO	137.08406	HMDB00306	x	x	present	
[M+H] ⁺	[M-H] ⁻	3-HYDROXYBENZOATE	C7H6O3	138.03170	HMDB02466	x	x	present	
[M+H] ⁺	[M-H] ⁻	4-HYDROXYBENZOATE	C7H6O3	138.03170	HMDB00500	x	x	present	
[M+H] ⁺	[M-H] ⁻	SALICYLATE	C7H6O3	138.03170	HMDB01895	x	x	present	
[M+H] ⁺	[M-H] ⁻	UROCANATE	C6H6N2O2	138.04293	HMDB00301	x	x	present	x
[M+H] ⁺	[M-H] ⁻	6-HYDROXYNICOTINATE	C6H5NO3	139.02694	HMDB02658	x	x	present	x
[M+H] ⁺	[M-H] ⁻	PHOSPHONOCETATE	C2H5O5P	139.98746	HMDB04110	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	2-AMINOETHYL DIHYDROGEN PHOSPHATE	C2H8NO4P	141.01910	HMDB00224	x	x	only negative mode available	
[M+H] ⁺	[M-H] ⁻	L-HISTIDINOL	C6H11NO3	141.09021	HMDB03431	x	x	present	x
[M+H] ⁺	[M-H] ⁻	5-HYDROXYMETHYLURACIL	C5H6N2O3	142.03784	HMDB00469	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	1-NAPHTHYLAMINE	C10H9N	143.07350		x	x	not present	
[M+H] ⁺	[M-H] ⁻	4-ACETAMIDOBUTANOATE	C6H11NO3	145.07389	HMDB03681	x	x	present	x
[M+H] ⁺	[M-H] ⁻	4-GUANIDINOBTANOATE	C5H11N3O2	145.08513	HMDB03464	x	x	present	x
[M+H] ⁺	[M-H] ⁻	ACETYLCHOLINE	C7H16NO2	145.10973	HMDB00895	x	x	present	
[M+H] ⁺	[M-H] ⁻	DEOXYCARITINE	C7H15NO2	145.11028	HMDB06831	x	x	not present	
[M+H] ⁺	[M-H] ⁻	SPERMIDINE	C7H19N3	145.15790	HMDB01257	x	x	present	x
[M+H] ⁺	[M-H] ⁻	ALPHA-KETOGLUTARIC ACID	C5H6O5	146.02153	HMDB00208	x		present	
[M+H] ⁺	[M-H] ⁻	2-METHYLGLUTARIC ACID	C6H10O4	146.05791	HMDB00422	x		present	
[M+H] ⁺	[M-H] ⁻	3-METHYLGLUTARIC ACID	C6H10O4	146.05791	HMDB00752	x		present	
[M+H] ⁺	[M-H] ⁻	ADIPIC ACID	C6H10O4	146.05791	HMDB00448	x		present	
[M+H] ⁺	[M-H] ⁻	MONO-METHYL GLUTARATE	C6H10O4	146.05791	HMDB00858	x		not present	
[M+H] ⁺	[M-H] ⁻	L-GLUTAMINE	C5H10N2O3	146.06914	HMDB00641	x	x	only positive mode available	x
[M+H] ⁺	[M-H] ⁻	DIMETHYLBENZIMIDAZOLE	C9H10N2	146.08440	HMDB03701	x	x	only positive mode available	x
[M+H] ⁺	[M-H] ⁻	L-LYSINE	C6H14N2O2	146.10553	HMDB00182	x	x	only positive mode available	x
[M+H] ⁺	[M-H] ⁻	L-GLUTAMIC ACID	C5H9NO4	147.05316	HMDB00148	x		present	
[M+H] ⁺	[M-H] ⁻	N-ACETYL-DL-SERINE	C5H9NO4	147.05316	HMDB02931	x		present	
[M+H] ⁺	[M-H] ⁻	N-METHYL-D-ASPARTIC ACID	C5H9NO4	147.05316	HMDB02393	x		present	
[M+H] ⁺	[M-H] ⁻	O-ACETYL-L-SERINE	C5H9NO4	147.05316	HMDB03011	x		present	
[M+H] ⁺	[M-H] ⁻	3-METHYL-2-OXINDOLE	C9H9NO	147.06841		x	x	not present	
[M+H] ⁺	[M-H] ⁻	CITRAMALATE	C5H8O5	148.03700	HMDB00426	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	TRANS-CINNAMATE	C9H8O2	148.05243	HMDB00930	x	x	present	x
[M+H] ⁺	[M-H] ⁻	(RS)-MEVALONIC ACID	C6H12O4	148.07360	HMDB00227	x	x	present	x
[M+H] ⁺	[M-H] ⁻	3-METHYLADENINE	C6H7N5	149.07015	HMDB01600	x	x	present	x
[M+H] ⁺	[M-H] ⁻	MESO-TARTARIC ACID	C4H6O6	150.01589		x		present	
[M+H] ⁺	[M-H] ⁻	(R,R)-TARTARIC ACID	C4H6O6	150.01644	HMDB00956	x		present	
[M+H] ⁺	[M-H] ⁻	(S,S)-TARTARIC ACID	C4H6O6	150.01644	HMDB29878	x		present	
[M+H] ⁺	[M-H] ⁻	2-HYDROXY-4-(METHYLTHIO)BUTYRIC ACID	C5H10O3S	150.03452	HMDB37115	x	x	present	
[M+H] ⁺	[M-H] ⁻	D-(-)-ARABINOSE	C5H10O5	150.05282	HMDB29942	x		present	
[M+H] ⁺	[M-H] ⁻	D-LYXOSE	C5H10O5	150.05282		x		present	
[M+H] ⁺	[M-H] ⁻	ARABINOSE	C5H10O5	150.05283	HMDB00646	x		present	
[M+H] ⁺	[M-H] ⁻	D-RIBOSE	C5H10O5	150.05283	HMDB00283	x		present	
[M+H] ⁺	[M-H] ⁻	D-XYLOSE	C5H10O5	150.05283	HMDB00098	x		present	
[M+H] ⁺	[M-H] ⁻	GUANINE	C5H5N5O2	151.04941	HMDB00132	x	x	present	x
[M+H] ⁺	[M-H] ⁻	XANTHINE	C5H4N4O2	152.03343	HMDB00292	x	x	only CID for positive mode	x
[M+H] ⁺	[M-H] ⁻	2-HYDROXYPHENYLACETIC ACID	C8H8O3	152.04734	HMDB00703	x	x	present	
[M+H] ⁺	[M-H] ⁻	MANDELIC ACID	C8H8O3	152.04734	HMDB00703	x		present	
[M+H] ⁺	[M-H] ⁻	2',4'-DIHYDROXYACETOPHENONE	C8H8O3	152.04735	HMDB29659	x	x	not present	
[M+H] ⁺	[M-H] ⁻	3-HYDROXYPHENYLACETATE	C8H8O3	152.04735	HMDB00440	x		present	
[M+H] ⁺	[M-H] ⁻	4-HYDROXYPHENYLACETATE	C8H8O3	152.04735	HMDB00020	x		present	
[M+H] ⁺	[M-H] ⁻	RESORCINOL MONOACETATE	C8H8O3	152.04735		x		present	
[M+H] ⁺	[M-H] ⁻	L-ARABITOL	C5H12O5	152.06848	HMDB01851	x		present	
[M+H] ⁺	[M-H] ⁻	RIBITOL	C5H12O5	152.06848	HMDB00508	x		not present	
[M+H] ⁺	[M-H] ⁻	XYLITOL	C5H12O5	152.06848	HMDB02917	x		present	
[M+H] ⁺	[M-H] ⁻	3-SULFINO-L-ALANINE	C3H7NO4S	153.00958	HMDB60179	x		present	
[M+H] ⁺	[M-H] ⁻	3-AMINO-4-HYDROXYBENZOIC ACID	C7H7NO3	153.04259		x		not present	
[M+H] ⁺	[M-H] ⁻	3-HYDROXYANTHRANILATE	C7H7NO3	153.04259	HMDB01476	x	x	present	
[M+H] ⁺	[M-H] ⁻	3-AMINO-5-HYDROXYBENZOIC ACID	C7H7NO3	153.04260		x		not present	
[M+H] ⁺	[M-H] ⁻	OCTOPAMINE	C8H11NO2	153.07897	HMDB04825	x		not present	
[M+H] ⁺	[M-H] ⁻	DOPAMINE	C8H11NO2	153.07898	HMDB00073	x	x	present	
[M+H] ⁺	[M-H] ⁻	2,3-DIHYDROXYBENZOATE	C7H6O4	154.02661	HMDB00397	x		present	
[M+H] ⁺	[M-H] ⁻	2,5-DIHYDROXYBENZOATE	C7H6O4	154.02661	HMDB00152	x	x	present	
[M+H] ⁺	[M-H] ⁻	3,4-DIHYDROXYBENZOATE	C7H6O4	154.02661	HMDB01856	x	x	present	
[M+H] ⁺	[M-H] ⁻	L-HISTIDINE	C6H9N3O2	155.06948	HMDB00177	x	x	present	x
[M+H] ⁺	[M-H] ⁻	OROTIC ACID	C5H4N2O4	156.01656	HMDB00226	x	x	present	
[M+H] ⁺	[M-H] ⁻	2,4-DIHYDROXYPYRIMIDINE-5-CARBOXYLIC ACID	C5H4N2O4	156.01711		x	x	not present	
[M+H] ⁺	[M-H] ⁻	(S)-DIHYDROOROTATE	C5H6N2O4	158.03276	HMDB03349	x	x	present	x
[M+H] ⁺	[M-H] ⁻	ALLANTOIN	C4H6N4O3	158.04399	HMDB00462	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	NONANOATE	C9H18O2	158.13068	HMDB00847	x		only negative mode available	
[M+H] ⁺	[M-H] ⁻	INDOLE-3-ACETALDEHYDE	C10H9NO	159.06841	HMDB01190	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	2-OXOADIPATE	C6H8O5	160.03718	HMDB00225	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	6-CARBOXYHEXANOATE	C7H12O4	160.07356	HMDB00857	x	x	present	x
[M+H] ⁺	[M-H] ⁻	TRYPTAMINE	C10H12N2	160.10005	HMDB00303	x	x	present	
[M+H] ⁺	[M-H] ⁻	ALPHA-AMINOADIPATE	C6H11NO4	161.06881	HMDB00510	x		present	
[M+H] ⁺	[M-H] ⁻	N-METHYL-L-GLUTAMATE	C6H11NO4	161.06881		x		not present	
[M+H] ⁺	[M-H] ⁻	INDOLE-3-ETHANOL	C10H11NO	161.08406	HMDB03447	x	x	present	x
[M+H] ⁺	[M-H] ⁻	L-CARNITINE	C7H15NO3	161.10519	HMDB00062	x	x	present	x
[M+H] ⁺	[M-H] ⁻	3-HYDROXY-3-METHYLGLUTARATE	C6H10O5	162.05283	HMDB00355	x	x	present	x
[M+H] ⁺	[M-H] ⁻	DL-5-HYDROXYLYSINE	C6H14N2O3	162.10044	HMDB00450	x		present	
[M+H] ⁺	[M-H] ⁻	(S)-NICOTINE	C10H14N2	162.11570	HMDB01934	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N-ACETYL-L-CYSTEINE	C5H9NO3S	163.03031	HMDB01890	x		present	
[M+H] ⁺	[M-H] ⁻	PTERIN	C6H6N5O	163.04941	HMDB00802	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	2,4-DIHYDROXYPTERIDINE	C6H4N4O2	164.03343		x	x	not present	
[M+H] ⁺	[M-H] ⁻	4-COUMARATE	C9H8O3	164.04735	HMDB02035	x	x	present	
[M+H] ⁺	[M-H] ⁻	L-RHAMNOSE	C6H12O5	164.06792	HMDB00849	x		not present	
[M+H] ⁺	[M-H] ⁻	6-DEOXY-L-GALACTOSE	C6H12O5	164.06848	HMDB00174	x		present	
[M+H] ⁺	[M-H] ⁻	L-PHENYLALANINE	C9H11NO2	165.07898	HMDB00159	x	x	present	
[M+H] ⁺	[M-H] ⁻	THIOPURINE S-METHYLETHYLER	C6G6N4S	166.03132	HMDB060412	x	x	present	x
[M+H] ⁺	[M-H] ⁻	3-(2-HYDROXYPHENYL)PROPANOATE	C9H10O3	166.06300	HMDB33752	x	x	not present	x

Continued from Table S6.3 Compound list of MS² library and LC library.

Adduct (+ve mode)	Adduct (-ve mode)	Compound name	Formula	Neutral mass	HMDB ID	MS ² library (included)	LC library (included)	Comparison with mzCloud (Figure 4.3 B)	Included in Figure 4.5 - 4.7
	[M-H] ⁻	PYRIDINE-2,3-DICARBOXYLATE	C7H5NO4	167.02186	HMDB00232	x	x	present	
[M+H] ⁺		4-HYDROXY-L-PHENYLGLYCINE	C8H9NO3	167.05824		x		not present	
[M+H] ⁺	[M-H] ⁻	PYRIDOXAL	C8H9NO3	167.05824	HMDB01545	x	x	only positive mode available	
[M+H] ⁺		3-METHOXYTYRAMINE	C9H13NO2	167.09464	HMDB00022	x	x	present	x
	[M-H] ⁻	PHOSPHO(ENOL)PYRUVIC ACID	C3H5O6P	167.98238	HMDB00263	x		present	
[M+H] ⁺	[M-H] ⁻	URATE	C5H4N4O3	168.02834	HMDB00289	x	x	present	x
	[M-H] ⁻	3,4-DIHYDROXYPHENYLACETATE	C8H8O4	168.04226	HMDB01336	x		present	
[M+H] ⁺	[M-H] ⁻	HOMOGENISATE	C8H8O4	168.04226	HMDB00130	x	x	present	
[M+H] ⁺	[M-H] ⁻	PYRIDOXAMINE	C8H12N2O2	168.08988	HMDB01431	x	x	present	x
[M+H] ⁺	[M-H] ⁻	L-CYSTEIC ACID	C3H7NO5S	169.00449	HMDB02757	x	x	present	x
	[M-H] ⁻	NORADRENALINE	C8H11NO3	169.07389	HMDB00216	x	x	present	
[M+H] ⁺	[M-H] ⁻	PYRIDOXINE	C8H11NO3	169.07389	HMDB00239	x	x	present	
[M+H] ⁺	[M-H] ⁻	N(PAI)-METHYL-L-HISTIDINE	C7H11N3O2	169.08513	HMDB00001	x	x	present	x
	[M-H] ⁻	DIHYDROXYACETONE PHOSPHATE	C3H7O6P	169.99748	HMDB01473	x		not present	
	[M-H] ⁻	3,4-DIHYDROXYPHENYL GLYCOL	C8H10O4	170.05791	HMDB00318	x	x	only CID for negative mode	
	[M-H] ⁻	SN-GLYCEROL 3-PHOSPHATE	C3H9O6P	172.01313	HMDB00126	x		present	
	[M-H] ⁻	GLYCEROL 2-PHOSPHATE	C3H9O6P	172.01367	HMDB02520	x		not present	
	[M-H] ⁻	3-DEHYDROSHIKIMATE	C7H8O5	172.03717		x	x	not present	
[M+H] ⁺	[M-H] ⁻	DECANOATE	C10H20O2	172.14633	HMDB00511	x		only negative mode available	
[M+H] ⁺	[M-H] ⁻	ANILINE-2-SULFONIC ACID	C6H7NO3S	173.01467		x	x	not present	
[M+H] ⁺	[M-H] ⁻	2-QUINOLINECARBOXYLIC ACID	C10H7NO2	173.04768	HMDB00842	x	x	only negative mode available	
[M+H] ⁺	[M-H] ⁻	4-QUINOLINECARBOXYLIC ACID	C10H7NO2	173.04768		x	x	not present	
[M+H] ⁺	[M-H] ⁻	N-ACETYL-L-LEUCINE	C8H15NO3	173.10519	HMDB11756	x	x	present	x
	[M-H] ⁻	TRANS-ACONITATE	C6H6O6	174.01644	HMDB00958	x		present	
	[M-H] ⁻	SHIKIMATE	C7H10O5	174.05283	HMDB03070	x	x	not present	x
	[M-H] ⁻	N-ALPHA-ACETYL-L-ASPARAGINE	C6H10N2O4	174.06406	HMDB06028	x	x	present	x
[M+H] ⁺	[M-H] ⁻	INDOLE-3-ACETAMIDE	C10H10N2O	174.07931	HMDB29739	x	x	not present	x
	[M-H] ⁻	SUBERIC ACID	C8H14O4	174.08921	HMDB00893	x	x	present	x
[M+H] ⁺	[M-H] ⁻	L-ARGININE	C6H14N4O2	174.11168	HMDB00517	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N-METHYLTRYPTAMINE	C11H14N2	174.11515	HMDB04370	x	x	present	x
	[M-H] ⁻	N-ACETYL-L-ASPARTIC ACID	C6H9NO5	175.04807	HMDB00812	x	x	present	
[M+H] ⁺	[M-H] ⁻	N-AMIDINO-L-ASPARTATE	C5H9N3O4	175.05931	HMDB03157	x	x	present	x
[M+H] ⁺	[M-H] ⁻	INDOLE-3-ACETATE	C10H9NO2	175.06333	HMDB00197	x	x	present	
	[M-H] ⁻	D-GLUCURONOLACTONE	C6H8O6	176.03209	HMDB06355	x		present	
[M+H] ⁺		SEROTONIN	C10H12N2O	176.09496	HMDB00259	x	x	present	x
[M+H] ⁺	[M-H] ⁻	FORMYL-L-METHIONYL PEPTIDE	C6H11NO3S	177.04597	HMDB01015	x	x	only negative mode available	x
	[M-H] ⁻	CYS-GLY	C5H10N2O3S	178.04122	HMDB00078	x		present	
	[M-H] ⁻	D-GLUCONIC ACID GAMA-LACTONE	C6H10O6	178.04774	HMDB03466	x		not present	
	[M-H] ⁻	D-GLUCONO-1,5-LACTONE	C6H10O6	178.04774	HMDB00150	x		present	
[M+H] ⁺		S-CARBOXYMETHYL-L-CYSTEINE	C5H9NO4S	179.02523	HMDB29415	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	HIPPURATE	C9H9NO3	179.05824	HMDB00714	x	x	present	x
[M+H] ⁺		D-(+)-GALACTOSAMINE	C6H13NO5	179.07938		x		present	
[M+H] ⁺		D-(+)-GLUCOSAMINE	C6H13NO5	179.07938	HMDB01514	x		present	
[M+H] ⁺		D-MANNOSAMINE	C6H13NO5	179.07938		x		not present	
[M+H] ⁺	[M-H] ⁻	1-METHYL-6,7-DIHYDROXY-1,2,3,4-TETRAHYDROISOQUINOLINE	C10H13NO2	179.09464	HMDB42012	x	x	present	x
[M+H] ⁺	[M-H] ⁻	CAFFEATE	C9H8O4	180.04226	HMDB01964	x		present	
[M+H] ⁺	[M-H] ⁻	L-METHIONINE SULFOXIMINE	C5H12N2O3S	180.05687	HMDB29430	x	x	not present	x
	[M-H] ⁻	ALLOSE	C6H12O6	180.06339	HMDB01151	x		not present	
	[M-H] ⁻	ALPHA-D-GLUCOSE	C6H12O6	180.06339	HMDB03345	x		present	
	[M-H] ⁻	D-GALACTOSE	C6H12O6	180.06339	HMDB00143	x		present	
	[M-H] ⁻	D-PSICOSE	C6H12O6	180.06339		x		not present	
	[M-H] ⁻	D-TAGATOSE	C6H12O6	180.06339	HMDB03418	x		not present	
	[M-H] ⁻	L-SORBOSE	C6H12O6	180.06339	HMDB01266	x		not present	
	[M-H] ⁻	MANNNOSE	C6H12O6	180.06339	HMDB00169	x		present	
	[M-H] ⁻	MYO-INOSITOL	C6H12O6	180.06339	HMDB00211	x		not present	
[M+H] ⁺	[M-H] ⁻	PARAXANTHINE	C7H8N4O2	180.06473	HMDB01860	x		only positive mode available	
[M+H] ⁺		THEOBROMINE	C7H8N4O2	180.06473	HMDB02825	x	x	present	
[M+H] ⁺	[M-H] ⁻	THEOPHYLLINE	C7H8N4O2	180.06473	HMDB01889	x		present	
[M+H] ⁺	[M-H] ⁻	L-TYROSINE	C9H11NO3	181.07389	HMDB00158	x	x	present	x
	[M-H] ⁻	3-(4-HYDROXYPHENYL) LACTATE	C9H10O4	182.05791	HMDB00755	x	x	present	
	[M-H] ⁻	HOMOVANILLATE	C9H10O4	182.05791	HMDB00118	x	x	present	
[M+H] ⁺	[M-H] ⁻	METHYL VANILLATE	C9H10O4	182.05791		x		not present	
	[M-H] ⁻	D-SORBITOL	C6H14O6	182.07849	HMDB00247	x		not present	
	[M-H] ⁻	GALACTITOL	C6H14O6	182.07904	HMDB00107	x		present	
	[M-H] ⁻	MANNITOL	C6H14O6	182.07904	HMDB00765	x		present	
	[M-H] ⁻	4-PYRIDOXATE	C8H9NO4	183.05316	HMDB00017	x	x	present	x
[M+H] ⁺		PHOSPHOCHOLINE	C5H14NO4P	183.06604	HMDB01565	x	x	not present	x
[M+H] ⁺		DL-NORMETANEPHRINE	C9H13NO3	183.08954	HMDB00819	x		not present	
[M+H] ⁺	[M-H] ⁻	EPINEPHRINE	C9H13NO3	183.08954	HMDB00068	x		present	
	[M-H] ⁻	DIHYDROXYMANDELIC ACID	C8H8O5	184.03718	HMDB01866	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	4-HYDROXY-3-METHOXYPHENYLGLYCOL	C9H12O4	184.07301	HMDB01490	x		not present	
[M+H] ⁺	[M-H] ⁻	O-PHOSPHO-DL-SERINE	C3H8NO6P	185.00893	HMDB01721	x		not present	
[M+H] ⁺	[M-H] ⁻	O-PHOSPHO-L-SERINE	C3H8NO6P	185.00893	HMDB00272	x		not present	
	[M-H] ⁻	L-2-PHOSPHOGLYCERIC ACID	C3H7O7P	185.99239	HMDB00362	x		not present	
	[M-H] ⁻	D-(-)-3-PHOSPHOGLYCERIC ACID	C3H7O7P	185.99294	HMDB00807	x		present	
[M-H2O+H] ⁺	[M-H2O-H] ⁻	1-HYDROXY-2-NAPHTHOATE	C11H8O3	188.04735		x	x	not present	
	[M-H] ⁻	AZELAIC ACID	C9H16O4	188.10486	HMDB00784	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N-ALPHA-ACETYL-L-LYSINE	C8H16N2O3	188.11609	HMDB00446	x	x	not present	x
	[M-H] ⁻	10-HYDROXYDECANOATE	C10H20O3	188.14125		x	x	present	
[M+H] ⁺		N-EPSILON,N-EPSILON,N-EPSILON-TRIMETHYLLYSINE	C9H20N2O2	188.15248	HMDB01325	x	x	present	x
[M+H] ⁺	[M-H] ⁻	4-HYDROXY-2-QUINOLINECARBOXYLIC ACID	C10H7NO3	189.04259	HMDB00715	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N-ACETYL-DL-GLUTAMIC ACID	C7H11NO5	189.06372	HMDB01138	x	x	only CID for positive mode	x
[M+H] ⁺		METHYL INDOLE-3-ACETATE	C11H11NO2	189.07898	HMDB29738	x	x	present	x
[M+H] ⁺	[M-H] ⁻	LL-2,6-DIAMINOHEPTANEDIOATE	C7H14N2O4	190.09536	HMDB01370	x	x	only positive mode available	x
[M+H] ⁺	[M-H] ⁻	5-HYDROXYINDOLEACETATE	C10H9NO3	191.05824	HMDB00763	x	x	present	x
	[M-H] ⁻	N-ACETYL-DL-METHIONINE	C7H13NO3S	191.06162	HMDB11745	x	x	present	x
	[M-H] ⁻	CITRATE	C6H8O7	192.02701	HMDB00094	x		present	
	[M-H] ⁻	ISOCITRIC ACID	C6H8O7	192.02701	HMDB00193	x		present	
	[M-H] ⁻	QUINATE	C7H12O6	192.06339	HMDB03072	x	x	present	x
	[M-H] ⁻	D-(+)-GALACTURONIC ACID	C6H10O7	194.04210	HMDB02545	x		present	
	[M-H] ⁻	D-GLUCURONIC ACID	C6H10O7	194.04265	HMDB00127	x		present	
[M+H] ⁺	[M-H] ⁻	FERULATE	C10H10O4	194.05791	HMDB00954	x	x	present	x
	[M-H] ⁻	METHYL BETA-D-GALACTOSIDE	C7H14O6	194.07904	HMDB29965	x	x	not present	x
[M+H] ⁺		CAFFEINE	C8H10N4O2	194.08038	HMDB01847	x	x	present	x
[M+H] ⁺	[M-H] ⁻	GLUCOSAMINATE	C6H13NO6	195.07429		x	x	not present	
	[M-H] ⁻	D-GLUCONATE	C6H12O7	196.05775	HMDB00625	x	x	present	x
[M+H] ⁺	[M-H] ⁻	SELENOMETHIONINE	C5H11NO2Se	196.99550	HMDB03966	x	x	only positive mode available	x
[M+H] ⁺	[M-H] ⁻	3,4-DIHYDROXY-L-PHENYLALANINE	C9H11NO4	197.06881	HMDB00181	x	x	present	
	[M-H] ⁻	3-METHOXY-4-HYDROXYMANDELATE	C9H10O5	198.05283	HMDB00291	x	x	only CID for negative mode	x
[M+H] ⁺	[M-H] ⁻	LAURIC ACID	C12H24O2	200.17763	HMDB00638	x	x	only negative mode available	
[M+H] ⁺		SPERMINE	C10H26N4	202.21575	HMDB01256	x	x	present	x

Continued from Table S6.3 Compound list of MS² library and LC library.

Adduct (+ve mode)	Adduct (-ve mode)	Compound name	Formula	Neutral mass	HMDB ID	MS ² library (included)	LC library (included)	Comparison with mzCloud (Figure 4.3 B)	Included in Figure 4.5 - 4.7
[M+H] ⁺	[M-H] ⁻	ETHYL 3-INDOLEACETATE	C12H13NO2	203.09463		x	x	not present	
[M+H] ⁺	[M-H] ⁻	L-TRYPTOPHANAMIDE	C11H13N3O	203.10586	HMDB13318	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	N6-(DELTA2-ISOPENTENYL)-ADENINE	C10H13N5	203.11710		x	x	not present	
[M+H] ⁺	[M-H] ⁻	D-TRYPTOPHAN	C11H12N2O2	204.08988	HMDB13609	x		present	
[M+H] ⁺	[M-H] ⁻	XANTHURENIC ACID	C10H7NO4	205.03751	HMDB00881	x	x	present	x
[M+H] ⁺		LIPOAMIDE	C8H15NO52	205.05951	HMDB00962	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	N-ACETYL-L-PHENYLALANINE	C11H13NO3	207.08954	HMDB00512	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	KYNURENINE	C10H12N2O3	208.08479	HMDB00684	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	L-KYNURENINE	C10H12N2O3	208.08479	HMDB00684	x		only positive mode available	
	[M-H] ⁻	D-SACCHARIC ACID	C6H10O8	210.03757	HMDB00663	x		present	
	[M-H] ⁻	GALACTARATE	C6H10O8	210.03757	HMDB00639	x		not present	
[M+H] ⁺	[M-H] ⁻	CREATINE PHOSPHATE	C4H10N3O5P	211.03581	HMDB01511	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	3-METHOXY-L-TYROSINE	C10H13NO4	211.08446	HMDB01434	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	INDOXYL SULFATE	C8H7NO4S	213.00903	HMDB00682	x	x	present	x
[M+H] ⁺	[M-H] ⁻	DETHIOBIOTIN	C10H18N2O3	214.13174	HMDB03581	x	x	present	x
	[M-H] ⁻	OMEGA-HYDROXYDODECANOIC ACID	C12H24O3	216.17255	HMDB02059	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N-ACETYL SEROTONIN	C12H14N2O2	218.10553	HMDB01238	x	x	present	x
	[M-H] ⁻	O-SUCCINYL-L-HOMOSERINE	C9H13NO6	219.07429		x	x	not present	
[M+H] ⁺	[M-H] ⁻	D-PANTOTHENIC ACID	C9H17NO5	219.11067	HMDB00210	x	x	only CID for positive mode	x
[M+H] ⁺	[M-H] ⁻	5-HYDROXY-L-TRYPTOPHAN	C11H12N2O3	220.08479	HMDB00472	x	x	present	x
[M+H] ⁺		2-ACETAMIDO-2-DEOXY-BETA-D-GLUCOSYLAMINE	C8H16N2O5	220.10592	HMDB01104	x		not present	
[M+H] ⁺		N-ACETYL-D-GALACTOSAMINE	C8H15NO6	221.08994	HMDB00212	x		present	
[M+H] ⁺		N-ACETYL-D-MANNOSAMINE	C8H15NO6	221.08994	HMDB01129	x		not present	
[M+H] ⁺	[M-H] ⁻	L-CYSTATHIONINE	C7H14N2O4S	222.06743	HMDB00099	x	x	present	
[M+H] ⁺	[M-H] ⁻	3-HYDROXYKYNURENINE	C10H12N2O4	224.07971	HMDB00732	x	x	only negative mode available	
[M+H] ⁺		METHYLJASMONATE	C13H20O3	224.14125	HMDB36583	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	3-NITRO-L-TYROSINE	C9H10N2O5	226.05897	HMDB01904	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	CARNOSINE	C9H14N4O3	226.10659	HMDB00033	x	x	only CID for negative mode	x
[M+H] ⁺	[M-H] ⁻	DEOXYCYTIDINE	C9H13N3O4	227.09061	HMDB00014	x	x	only negative mode available	x
	[M-H] ⁻	DEOXYURIDINE	C9H12N2O5	228.07462	HMDB00012	x	x	present	
[M+H] ⁺	[M-H] ⁻	MYRISTIC ACID	C14H28O2	228.20893	HMDB00806	x		only negative mode available	
	[M-H] ⁻	D-RIBOSE 5-PHOSPHATE	C5H11O8P	230.01916	HMDB01548	x	x	present	
[M+H] ⁺	[M-H] ⁻	MELATONIN	C13H16N2O2	232.12118	HMDB01389	x	x	present	x
[M+H] ⁺	[M-H] ⁻	L-CYSTINE	C6H12N2O4S2	240.02385	HMDB00192	x	x	present	
[M+H] ⁺	[M-H] ⁻	L-ANSERINE	C10H16N4O3	240.12169	HMDB00194	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	LUMICHRONE	C12H10O4O2	242.08038		x	x	not present	
[M+H] ⁺	[M-H] ⁻	THYMIDINE	C10H14N2O5	242.09027	HMDB00273	x	x	present	
[M+H] ⁺	[M-H] ⁻	CYTIDINE	C9H13N3O5	243.08552	HMDB00089	x	x	present	x
[M+H] ⁺	[M-H] ⁻	URIDINE	C9H12N2O6	244.06954	HMDB00296	x	x	only negative mode available	x
	[M-H] ⁻	GLYCERALDEHYDE 3-PHOSPHATE DIETHYL ACETAL	C17H17O7P	244.07064		x	x	not present	
[M+H] ⁺	[M-H] ⁻	BIOTIN	C10H16N2O3S	244.08817	HMDB00030	x	x	present	x
[M+H] ⁺		N1-ACETYLSPERMINE	C12H28N4O	244.22631	HMDB01186	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N-ACETYL-D-TRYPTOPHAN	C13H14N3O3	246.10044	HMDB13713	x	x	present	x
[M+H] ⁺	[M-H] ⁻	PYRIDOXAL 5'-PHOSPHATE	C8H10NO6P	247.02402	HMDB01491	x		only positive mode available	
[M+H] ⁺		SELENOCYSTAMINE	C4H12N2Se2	247.93309				not present	
[M+H] ⁺		2'-DEOXYADENOSINE	C10H13N5O3	251.10129	HMDB00101	x	x	present	
[M+H] ⁺	[M-H] ⁻	5'-DEOXYADENOSINE	C10H13N5O3	251.10184	HMDB01983	x	x	not present	
[M+H] ⁺	[M-H] ⁻	PALMITOLEIC ACID	C16H30O2	254.22458	HMDB03229	x	x	present	
[M+H] ⁺	[M-H] ⁻	PALMITATE	C16H32O2	256.24023	HMDB00220	x	x	only negative mode available	
[M+H] ⁺		SN-GLYCERO-3-PHOSPHOCHOLINE	C8H20NO6P	257.10228	HMDB00086	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	D-GLUCOSAMINE 6-SULFATE	C6H13NO8S	259.03619	HMDB00592	x	x	only CID for positive mode	x
[M+H] ⁺	[M-H] ⁻	D-GLUCOSAMINE 6-PHOSPHATE	C6H14NO8P	259.04571	HMDB01254	x	x	only negative mode available	
	[M-H] ⁻	ALPHA-D-GALACTOSE 1-PHOSPHATE	C6H13O9P	260.02971	HMDB00645	x		only CID for negative mode	
	[M-H] ⁻	ALPHA-D-GLUCOSE 1-PHOSPHATE	C6H13O9P	260.02972	HMDB01586	x		present	
	[M-H] ⁻	D-FRUCTOSE 6-PHOSPHATE	C6H13O9P	260.02972	HMDB00124	x		present	
[M+H] ⁺	[M-H] ⁻	D-GLUCOSE 6-PHOSPHATE	C6H13O9P	260.02972	HMDB01401	x		present	
	[M-H] ⁻	D-MANNOSE 6-PHOSPHATE	C6H13O9P	260.02972	HMDB01078	x		present	
M+	[M-2H] ⁻	THIAMINE	C12H17N4OS	264.10503	HMDB00235	x	x	only positive mode available	x
	[M-H] ⁻	2,3-DIPHOSPHO-D-GLYCERIC ACID	C3H8O10P2	265.95872	HMDB01294	x		present	
[M+H] ⁺	[M-H] ⁻	2'-DEOXYGUANOSINE	C10H13N5O4	267.09621	HMDB00085	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	ADENOSINE	C10H13N5O4	267.09676	HMDB00050	x		present	
[M+H] ⁺	[M-H] ⁻	HOMOCYSTINE	C8H16N2O4S2	268.05515	HMDB00575	x	x	only CID for positive mode	x
[M+H] ⁺	[M-H] ⁻	INOSINE	C10H12N4O5	268.08077	HMDB00195	x	x	only positive mode available	x
[M+H] ⁺	[M-H] ⁻	HEPTADECANOATE	C17H34O2	270.25588	HMDB02259	x		not present	
[M+H] ⁺		ESTRADIOL-17ALPHA	C18H24O2	272.17763	HMDB00429	x		only CID for positive mode	
	[M-H] ⁻	6-PHOSPHOGLUCONIC ACID	C6H13O10P	276.02464	HMDB01316	x	x	not present	
[M+H] ⁺	[M-H] ⁻	1-METHYLADENOSINE	C11H15N5O4	281.11241	HMDB03331	x	x	only positive mode available	x
	[M-H] ⁻	ELAIDIC ACID	C18H34O2	282.25588	HMDB00573	x		only CID for negative mode	
	[M-H] ⁻	OLEATE	C18H34O2	282.25588	HMDB00207	x		present	
	[M-H] ⁻	PETROSELENIC ACID	C18H34O2	282.25588	HMDB02080	x		present	
[M+H] ⁺	[M-H] ⁻	GUANOSINE	C10H13N5O5	283.09167	HMDB00133	x	x	only negative mode available	x
	[M-H] ⁻	XANTHOSINE	C10H12N4O6	284.07569	HMDB00299	x	x	present	x
[M+H] ⁺	[M-H] ⁻	STEARATE	C18H36O2	284.27153	HMDB00827	x	x	present	
[M+H] ⁺	[M-H] ⁻	OPHTHALMIC ACID	C11H19N3O6	289.12739	HMDB05765	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	5'-METHYLTHIOADENOSINE	C11H15N5O3S	297.08956	HMDB01173	x	x	present	x
	[M-H] ⁻	RETINOATE	C20H28O2	300.20893	HMDB01852	x	x	present	x
[M+H] ⁺		SPHINGANINE	C18H39NO2	301.29808	HMDB00269	x	x	only CID for positive mode	x
[M+H] ⁺		RAC-GLYCEROL 1-MYRISTATE	C17H34O4	302.24571	HMDB11561	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	CYTIDINE 2',3'-CYCLIC MONOPHOSPHATE	C9H12N3O7P	305.04129	HMDB11691	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	2'-DEOXYCYTIDINE 5'-MONOPHOSPHATE	C9H14N3O7P	307.05694	HMDB01202	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	GLUTATHIONE	C10H17N3O6S	307.08381	HMDB00125	x	x	present	
	[M-H] ⁻	2'-DEOXYURIDINE 5'-MONOPHOSPHATE	C9H13N2O8P	308.04096	HMDB01409	x	x	present	x
[M+H] ⁺	[M-H] ⁻	N-ACETYLNEURAMINATE	C11H19NO9	309.10598	HMDB00230	x	x	present	x
	[M-H] ⁻	D-RIBULOSE 1,5-BISPHOSPHATE	C5H12O11P2	309.98548	HMDB11688	x		not present	
[M+H] ⁺	[M-H] ⁻	ARACHIDIC ACID	C20H40O2	312.30283	HMDB02212	x		only negative mode available	
	[M-HPO3-H] ⁻	GERANYL PYROPHOSPHATE	C10H20O7P2	314.06788	HMDB01285	x		not present	
[M+H] ⁺	[M-H] ⁻	THYMIDINE 5'-MONOPHOSPHATE	C10H15N2O8P	322.05661	HMDB01227	x	x	present	x
[M+H] ⁺	[M-H] ⁻	3'-CMP	C9H14N3O8P	323.05186		x		not present	
[M+H] ⁺	[M-H] ⁻	CMP	C9H14N3O8P	323.05186	HMDB00095	x		only positive mode available	
[M+H] ⁺	[M-H] ⁻	URIDINE 5-MONOPHOSPHATE	C9H13N2O9P	324.03587	HMDB00288	x	x	only negative mode available	x
	[M-H] ⁻	DCCOSAHEXAENOIC ACID	C22H32O2	328.24023	HMDB02183	x		present	
[M+H] ⁺	[M-H] ⁻	ADENOSINE 2',3'-CYCLIC MONOPHOSPHATE	C10H12N5O6P	329.05197	HMDB11616	x	x	not present	
[M+H] ⁺	[M-H] ⁻	3',5'-CYCLIC AMP	C10H12N5O6P	329.05252	HMDB00058	x	x	present	
[M+H] ⁺	[M-H] ⁻	DAMP	C10H14N5O6P	331.06817	HMDB00905	x	x	only negative mode available	x
[M+H] ⁺		NICOTINAMIDE MONONUCLEOTIDE	C11H15N2O8P	334.05661	HMDB00229	x	x	present	x
[M+H] ⁺	[M-H] ⁻	5-AMINOIMIDAZOLE-4-CARBOXYAMIDE-1-BETA-D-RIBOFURANOSYL 5'-MONOPHOSPHATE	C9H15N4O8P	338.06275	HMDB01517	x	x	present	x
	[M-H] ⁻	ERICIC ACID	C22H42O2	338.31848	HMDB02068	x		present	
	[M-H] ⁻	FRUCTOSE 1,6-BIPHOSPHATE	C6H14O12P2	339.99606	HMDB01058	x		not present	
	[M-H] ⁻	D-(+)-TREHALOSE	C12H22O11	342.11566	HMDB00975	x		present	
[M+H] ⁺		D-LACTOSE	C12H22O11	342.11566	HMDB00186	x		present	
	[M-H] ⁻	PALATINOSE	C12H22O11	342.11621		x		not present	

Continued from Table S6.3 Compound list of MS² library and LC library.

Adduct (+ve mode)	Adduct (-ve mode)	Compound name	Formula	Neutral mass	HMDB ID	MS ² library (included)	LC library (included)	Comparison with mzCloud (Figure 4.3 B)	Included in Figure 4.5 - 4.7
[M-H2O+H] ⁺	[M-H] ⁻	MALTOSE	C12H22O11	342.11622	HMDB00163	x		present	
	[M-H] ⁻	D-(+)-CELLULOSE	C12H22O11	342.11622	HMDB00055	x		not present	
	[M-H] ⁻	MELIBIOSE	C12H22O11	342.11622	HMDB00048	x		not present	
	[M-H] ⁻	SUCROSE	C12H22O11	342.11622	HMDB000258	x		present	
[M+H] ⁺		LAUROYL-CARNITINE	C19H37NO4	343.27225	HMDB002250	x	x	not present	x
[M+H] ⁺		THIAMINE MONOPHOSPHATE	C12H17N4O4P5	344.07081	HMDB02666	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE	C10H12N5O7P	345.04744	HMDB01314	x	x	not present	x
[M+H] ⁺		CORTICOSTERONE	C21H30O4	346.21441	HMDB01547	x		present	
[M+H] ⁺		REICHSTEINS SUBSTANCE S	C21H30O4	346.21441	HMDB00015	x		not present	
[M+H] ⁺	[M-H] ⁻	2'-DEOXYGUANOSINE 5'-MONOPHOSPHATE	C10H14N5O7P	347.06309	HMDB01044	x	x	only negative mode available	
[M+H] ⁺	[M-H] ⁻	ADENOSINE 5'-MONOPHOSPHATE	C10H14N5O7P	347.06309	HMDB00045	x	x	present	
[M+H] ⁺	[M-H] ⁻	INOSINE 5'-MONOPHOSPHATE	C10H13N4O8P	348.04710	HMDB00175	x	x	present	x
[M+H] ⁺	[M-H] ⁻	TRICOSANOIC ACID	C23H46O2	354.34978	HMDB01160	x		not present	
[M+H] ⁺		1-OLEOYL-RAC-GLYCEROL	C21H40O4	356.29266	HMDB11567	x	x	present	x
[M+H] ⁺	[M-H] ⁻	ROSAMARINIC ACID	C18H16O8	360.08452	HMDB03572	x		only positive mode available	
[M+H] ⁺		CORTISONE	C21H28O5	360.19368	HMDB02802	x	x	present	x
[M+H] ⁺	[M-H] ⁻	THYROTROPIN RELEASING HORMONE	C16H22N6O4	362.17025	HMDB06080	x	x	only positive mode available	x
[M+H] ⁺		CORTISOL	C21H30O5	362.20933	HMDB00063	x	x	present	x
[M+H] ⁺	[M-H] ⁻	GUANOSINE 5'-MONOPHOSPHATE	C10H14N5O8P	363.05800	HMDB01397	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	XANTHOSINE 5'-MONOPHOSPHATE	C10H13N4O9P	364.04202	HMDB01554	x	x	not present	x
	[M-H] ⁻	NERVONIC ACID	C24H46O2	366.34978	HMDB02368	x		present	
[M+H] ⁺		DEOXYCORTICOSTERONE ACETATE	C23H32O4	372.23006		x	x	present	
[M+H] ⁺	[M-H] ⁻	RIBOFLAVIN	C17H20N4O6	376.13829	HMDB00244	x	x	only CID for positive mode	x
	[M-H] ⁻	3-ALPHA-HYDROXY-5BETA-CHOLANATE	C24H40O3	376.29775	HMDB000761	x		present	
[M+H] ⁺	[M-H] ⁻	5-(5'-ADENOSYL)-L-HOMOCYSTEINE	C14H20N6O5S	384.12159	HMDB00039	x	x	present	x
[M+H] ⁺		CHOLESTA-5,7-DIEN-3BETA-OL	C27H44O	384.33922	HMDB00032	x		not present	
	[M-H] ⁻	2'-DEOXYCYTIDINE 5'-DIPHOSPHATE	C9H15N3O10P2	387.02327	HMDB01245	x	x	present	
	[M-H] ⁻	5-PHOSPHO-D-RIBOSE 1'-DIPHOSPHATE	C5H13O14P3	389.95182	HMDB000280	x		present	
[M+H] ⁺		BIS(2-ETHYLHEXYL)PHTHALATE	C24H38O4	390.27701		x	x	present	
[M+H] ⁺	[M-H] ⁻	S-HEXYL-GLUTATHIONE	C16H29N3O6S	391.17771		x	x	not present	
	[M-H] ⁻	CHENODEOXYCHOLATE	C24H40O4	392.29266	HMDB000518	x		present	
	[M-H] ⁻	DEOXYCHOLATE	C24H40O4	392.29266	HMDB000626	x		present	
[M+H] ⁺		VITAMIN D2	C28H44O	396.33922	HMDB000900	x	x	not present	x
M+		5-(5'-ADENOSYL)-L-METHIONINE	C15H23N6O5S	398.13669	HMDB01185	x		present	
[M+H] ⁺	[M-H] ⁻	CYTIDINE 5'-DIPHOSPHATE	C9H15N3O11P2	403.01819	HMDB01546	x	x	not present	
	[M-H] ⁻	URIDINE 5'-DIPHOSPHATE	C9H14N2O12P2	404.00220	HMDB000295	x		present	
[M+H] ⁺	[M-H] ⁻	CORTISOL 21-ACETATE	C23H32O6	404.21989		x	x	not present	
	[M-H] ⁻	CHOLATE	C24H40O5	408.28758	HMDB000619	x	x	present	x
[M+H] ⁺		SQUALENE	C30H50	410.39125	HMDB000256	x		not present	
[M+H] ⁺	[M-H] ⁻	2'-DEOXYADENOSINE 5'-DIPHOSPHATE	C10H15N5O9P2	411.03451	HMDB01508	x		present	
[M+H] ⁺	[M-H] ⁻	THIAMINE PYROPHOSPHATE	C12H18N4O7P2S	424.03659	HMDB01372	x	x	not present	
[M+H] ⁺		LANOSTEROL	C30H50O	426.38617	HMDB01251	x	x	not present	
	[M-H] ⁻	2'-DEOXYGUANOSINE 5'-DIPHOSPHATE	C10H15N5O10P2	427.02941	HMDB000960	x		present	
[M+H] ⁺	[M-H] ⁻	ADENOSINE 3',5'-DIPHOSPHATE	C10H15N5O10P2	427.02942	HMDB000061	x		not present	
[M+H] ⁺	[M-H] ⁻	ADENOSINE 5'-DIPHOSPHATE	C10H15N5O10P2	427.02942	HMDB01341	x		present	
	[M-H] ⁻	INOSINE 5'-DIPHOSPHATE	C10H14N4O11P2	428.01344	HMDB03335	x		not present	
[M+H] ⁺		ALPHA-TOCOPHEROL	C29H50O2	430.38108	HMDB01893	x	x	present	
[M+H] ⁺	[M-H] ⁻	3,5-DIODO-L-TYROSINE	C9H9I2NO3	432.86718	HMDB03474	x	x	present	x
[M+H] ⁺	[M-H] ⁻	FOLIC ACID	C19H19N7O6	441.13968	HMDB001212	x	x	present	
[M+H] ⁺	[M-H] ⁻	GUANOSINE 5'-DIPHOSPHATE	C10H15N5O11P2	443.02432	HMDB01201	x	x	present	x
[M+H] ⁺	[M-H] ⁻	DIHYDROFOLATE	C19H21N7O6	443.15533	HMDB01056	x	x	only negative mode available	x
[M+H] ⁺		MENADIOL	C19H21N7O6	443.15533	HMDB01056	x	x	only negative mode available	x
	[M-2H] ²⁻	TETRAHYDROFOLATE	C19H23N7O6	445.17098	HMDB01846	x	x	not present	
[M+H] ⁺		PHYLOQUINONE	C31H46O2	450.34978	HMDB03555	x		not present	
[M+H] ⁺	[M-H] ⁻	GLYCOCHOLATE	C26H43NO6	465.30904	HMDB00138	x	x	only CID for positive mode	x
	[M-H] ⁻	2'-DEOXYURIDINE 5'-TRIPHOSPHATE	C9H15N3O14P3	467.97362	HMDB01191	x		present	
[M+H] ⁺	[M-H] ⁻	CYTIDINE 5'-TRIPHOSPHATE	C9H16N3O14P3	482.98452	HMDB00082	x	x	not present	
	[M-H] ⁻	TAUROTHIOCHOLATE	C26H45NO5S	483.30185	HMDB00722	x	x	not present	x
	[M-H] ⁻	URIDINE 5'-TRIPHOSPHATE	C9H15N3O15P3	483.96854	HMDB00285	x	x	not present	
[M+H] ⁺	[M-H] ⁻	CYTIDINE 5'-DIPHOSPHOCHOLINE	C14H26N4O11P2	488.10678	HMDB01413	x	x	only positive mode available	x
	[M-H] ⁻	2'-DEOXYADENOSINE 5'-TRIPHOSPHATE	C10H16N5O12P3	491.00084	HMDB01532	x		present	
	[M-H] ⁻	D-(+)-RAFFINOSE	C18H32O16	504.16849	HMDB03213	x	x	present	
	[M-H] ⁻	2'-DEOXYGUANOSINE 5'-TRIPHOSPHATE	C10H16N5O13P3	506.99575	HMDB01440	x		present	
	[M-H] ⁻	ADENOSINE 5'-TRIPHOSPHATE	C10H16N5O13P3	506.99575	HMDB00538	x		present	
	[M-H] ⁻	INOSINE 5'-TRIPHOSPHATE	C10H15N4O14P3	507.97977	HMDB00189	x	x	present	
	[M-H] ⁻	GUANOSINE 5'-TRIPHOSPHATE	C10H16N5O14P3	522.99067	HMDB01273	x	x	present	
[M+H] ⁺	[M-H] ⁻	3,5-DIODO-L-THYRONINE	C15H13I2NO4	524.89341	HMDB00582	x	x	not present	x
[M+H] ⁺		BETA-CAROTENE	C40H56	536.43820	HMDB00561	x		not present	
[M+H] ⁺	[M-H] ⁻	ADENOSINE 5'-DIPHOSPHORIBOSE	C15H23N5O14P2	559.07168	HMDB01178	x	x	only negative mode available	
[M+H] ⁺	[M-H] ⁻	PROTOPORPHYRIN	C34H34N4O4	562.25801	HMDB00241	x	x	only negative mode available	x
	[M-H] ⁻	THYMIDINE 5'-DIPHOSPHO-ALPHA-D-GLUCOSE	C16H26N2O16P2	564.07576	HMDB01328	x	x	not present	x
[M+H] ⁺		1,2-DIODECANOYL-SN-GLYCERO-3-PHOSPHOCHOLINE	C28H56NO8P	565.37436		x	x	not present	
	[M-H] ⁻	URIDINE 5'-DIPHOSPHOGLACTOSE	C15H24N2O17P2	566.05503	HMDB00302	x		present	
	[M-H] ⁻	URIDINE 5'-DIPHOSPHOGLUCOSE	C15H24N2O17P2	566.05503	HMDB00286	x		not present	
	[M-H] ⁻	URIDINE 5'-DIPHOSPHOGLUCURONIC ACID	C15H22N2O18P2	580.03430	HMDB000935	x	x	present	
[M+H] ⁺	[M-H] ⁻	BILIVERDIN	C33H34N4O6	582.24784	HMDB01008	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	BILIRUBIN	C33H36N4O6	584.26349	HMDB00054	x	x	present	x
[M+H] ⁺	[M-H] ⁻	ADENOSINE 5'-DIPHOSPHOGLUCOSE	C16H25N5O15P2	589.08169	HMDB06557	x	x	only negative mode available	x
[M+H] ⁺	[M-H] ⁻	GUANOSINE 5'-DIPHOSPHOGLUCOSE	C16H25N5O16P2	605.07660	HMDB03351	x		only negative mode available	
[M+H] ⁺	[M-H] ⁻	GUANOSINE 5'-DIPHOSPHO-D-MANNONE	C16H25N5O16P2	605.07716	HMDB01163	x		only negative mode available	
	[M-H] ⁻	URIDINE 5'-DIPHOSPHO-N-ACETYLGLACTOSAMINE	C17H27N3O17P2	607.08154	HMDB00304	x		not present	
	[M-H] ⁻	URIDINE 5'-DIPHOSPHO-N-ACETYLGLUCOSAMINE	C17H27N3O17P2	607.08158	HMDB00290	x		present	
[M+H] ⁺	[M-H] ⁻	3,3',5'-TRIODOETHYRONE	C15H12I3NO4	650.79007		x	x	present	
	[M-2H] ²⁻	PHYTIC ACID	C6H18O24P6	659.86139	HMDB03502	x		present	
[M+H] ⁺	[M-H] ⁻	NAD	C21H27N7O14P2	663.10912	HMDB00902	x	x	only positive mode available	
[M+H] ⁺	[M-H] ⁻	NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	C21H26N6O15P2	664.09369		x	x	not present	
	[M-H] ⁻	STACHYOSE	C24H42O21	666.22186	HMDB03553	x	x	not present	
[M+H] ⁺	[M-H] ⁻	1,2-DIPALMITOYL-RAC-GLYCERO-3-PHOSPHOETHANOLAMINE	C37H74NO8P	691.51521	HMDB08923	x	x	not present	x
[M+H] ⁺		SPHINGOMYELIN (d36:2)	C41H83N2O6P	730.59887	HMDB01348	x	x	not present	x
[M+H] ⁺	[M-H] ⁻	BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE	C21H28N7O17P3	743.07545	HMDB00217	x	x	only positive mode available	
[M+H] ⁺	[M-H] ⁻	NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE	C21H27N6O18P3	744.05892		x		not present	
	[M-H] ⁻	BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE 2'-PHOSPHATE REDUCED	C21H30N7O17P3	745.09055	HMDB00221	x		present	
[M+H] ⁺		L-ALPHA-PHOSPHATIDYLCHOLINE (34:3)	C42H80NO8P	757.56161		x	x	not present	
	[M-H] ⁻	COENZYME A	C21H36N7O16P3S	767.11522	HMDB01423	x		present	
	[M-2H] ²⁻	COENZYME A	C21H36N7O16P3S	767.11522	HMDB01423	x		present	
[M+H] ⁺	[M-H] ⁻	THYROXINE	C15H11I4NO4	776.68672	HMDB00248	x	x	present	x
[M+H] ⁺	[M-H] ⁻	FLAVIN ADENINE DINUCLEOTIDE	C27H33N9O15P2	785.15714	HMDB01248	x	x	only CID for positive mode	x
	[M-2H] ²⁻	3-METHYLCROTONYL-COA	C26H42N7O17P3S	849.15708	HMDB01493	x	x	not present	
[M+H] ⁺		COENZYME Q10	C59H90O4	862.68336	HMDB01072	x	x	not present	
	[M-2H] ²⁻	DL-3-HYDROXY-3-METHYLGUTARYL COENZYME A	C27H44N7O20P3S	911.15692	HMDB01375	x		not present	
[M+H] ⁺		VITAMIN B12	C63H88CoN14O14P	1354.56741	HMDB00607	x		not present	

Table S6.4 List of tested isomeric compounds. Red = no separation, green = separate peaks. LC methods according to Table S6.1.

Selected isomeric compounds	Formula	Neutral mass	k value				
			RP 5 condition A	RP 19 condition B	HILIC 19 condition E	RP 4	RP 6
BETA-ALANINE	C3H7NO2	89.04768			19.9		
SARCOSINE	C3H7NO2	89.04768			18.1		
L-ALANINE	C3H7NO2	89.04768			18.2		
D-ALANINE	C3H7NO2	89.04768			18.2		
4-AMINOBUTANOATE	C4H9NO2	103.06333			20.4		
2-AMINO-2-METHYLPROPANOATE	C4H9NO2	103.06333			17.0		
2-HYDROXYBUTYRIC ACID	C4H8O3	104.04735	1.2	1.6	8.9		
ALPHA-HYDROXYISOBUTYRIC ACID	C4H8O3	104.04735	1.2	1.5	7.9		
3-HYDROXYBUTANOIC ACID	C4H8O3	104.04735	0.9	1.2	11.7		
MALEIC ACID	C4H4O4	116.01096	3.1	2.6	2.9	2.6	
FUMARATE	C4H4O4	116.01096	1.3	1.5	20.9	2.1	
L-VALINE	C5H11NO2	117.07898	0.6		15.7		
BETAINE	C5H11NO2	117.07898	0.2		14.6		
L-NORVALINE	C5H11NO2	117.07898	0.7		15.3		
5-AMINOPENTANOATE	C5H11NO2	117.07898	0.6		20.9		
SUCCINATE	C4H6O4	118.02661	0.9	1.1	20.1		
METHYLMALONATE	C4H6O4	118.02661	1.4	1.7	16.5		
3-HYDROXYBENZALDEHYDE	C7H6O2	122.03678		5.8	0.1		7.6
BENZOATE	C7H6O2	122.03678		7.2	3.5		8.9
4-HYDROXYBENZALDEHYDE	C7H6O2	122.03678		5.5	0.1		7.3
3-HYDROXYBENZYL ALCOHOL	C7H8O2	124.05243	4.2	4.5		3.8	5.4
4-METHYLCATECHOL	C7H8O2	124.05243	6.1	6.0		6.0	7.6
THYMINE	C5H6N2O2	126.04293	1.3	1.5	0.7	2.1	2.4
4-IMIDAZOLEACETIC ACID	C5H6N2O2	126.04293	0.4	0.2	18.2	1.3	1.2
2-METHYLMALATE	C5H6O4	130.02661	3.8	3.9	2.9		2.6
ITACONATE	C5H6O4	130.02661	2.3	3.2	18.2		3.4
4-METHYL-2-OXO-PENTANOIC ACID	C6H10O3	130.063	6.5	7.5		4.3	6.4
MEVALOLACTONE	C6H10O3	130.063	1.7	1.6		1.9	2.7
TRANS-4-HYDROXYPROLINE	C5H9NO3	131.05824	0.1	0.1	18.2	1.3	1.2
4-HYDROXY-L-PROLINE	C5H9NO3	131.05824	0.1	0.1	18.2	1.3	1.2
5-AMINOLEVULINIC ACID	C5H9NO3	131.05824	0.3	0.1	21.3	1.2	1.2
N-ACETYL-L-ALANINE	C5H9NO3	131.05824	1.1	1.2	13.8	1.8	2.0
CIS-4-HYDROXY-D-PROLINE	C5H9NO3	131.05824	0.1	0.1	19.4	1.3	1.2
L-ISOLEUCINE	C6H13NO2	131.09463	2.0				
LEUCINE	C6H13NO2	131.09463	2.4				
NORLEUCINE	C6H13NO2	131.09463	2.5				
GLUTARATE	C5H8O4	132.04226	1.9	2.4	20.7		2.9
ETHYLMALONIC ACID	C5H8O4	132.04226	3.4	4.1	9.6		4.0
TRIGONELLINE	C7H7NO2	137.04768	0.3	0.2	14.9	1.4	1.3
4-AMINOBENZOATE	C7H7NO2	137.04768	3.6	3.5	4.8	4.2	4.8
ANTHRANILATE	C7H7NO2	137.04768	6.1	5.8	3.5	5.6	7.6
SALICYLAMIDE	C7H7NO2	137.04768	6.5	5.8	0.1	6.8	7.5
TYRAMINE	C8H11NO	137.08406	2.9	0.9	11.2		
PHENYLETHANOLAMINE	C8H11NO	137.08406	5.0	1.5	4.3		
4-HYDROXYBENZOATE	C7H6O3	138.0317	5.5	5.1	12.0	6.4	6.3
3-HYDROXYBENZOATE	C7H6O3	138.0317	6.0	5.7	12.8	6.5	7.1
SALICYLATE	C7H6O3	138.0317	8.2	7.8	1.5	8.1	9.2
ACETYLCHOLINE	C7H16NO2	145.10973	3.0		4.5		
DEOXYCARNITINE	C7H15NO2	145.10973	0.8		21.8		
3-METHYLGLUTARIC ACID	C6H10O4	146.05791			19.6		
MONO-METHYL GLUTARATE	C6H10O4	146.05791			2.4		
2-METHYLGLUTARIC ACID	C6H10O4	146.05791			19.5		
ADIPIC ACID	C6H10O4	146.05791			20.4		
L-GLUTAMIC ACID	C5H9NO4	147.05316			21.0		
N-METHYL-D-ASPARTIC ACID	C5H9NO4	147.05316			19.9		
O-ACETYL-L-SERINE	C5H9NO4	147.05316			16.3		
N-ACETYL-DL-SERINE	C5H9NO4	147.05316			16.3		
MESO-TARTARIC ACID	C4H6O6	150.01589		0.5			
(R,R)-TARTARIC ACID	C4H6O6	150.01589		0.9			
(S,S)-TARTARIC ACID	C4H6O6	150.01589		0.9			
MANDELIC ACID	C8H8O3	152.04734	4.5	4.9	4.8	3.4	6.3
2-HYDROXYPHENYLACETIC ACID	C8H8O3	152.04734	5.9	5.9	2.3	5.7	7.4
4-HYDROXYPHENYLACETATE	C8H8O3	152.04735	5.5	5.4	12.4	5.6	6.7
2',4'-DIHYDROXYACETOPHENONE	C8H8O3	152.04735	7.9	6.8	-	8.2	8.9
3-HYDROXYPHENYLACETATE	C8H8O3	152.04735	5.7	5.6	12.6	5.8	7.1
RESORCINOL MONOACETATE	C8H8O3	152.04735	9.1	7.5	-	8.8	9.6
3-HYDROXYANTHRANILATE	C7H7NO3	153.0426	4.2	3.7	12.4	3.8	4.3
3-AMINO-4-HYDROXYBENZOIC ACID	C7H7NO3	153.0426	0.9	0.6	15.8	1.6	1.5
3-AMINO-5-HYDROXYBENZOIC ACID	C7H7NO3	153.0426	1.0	0.7	17.0	1.8	1.6
OCTOPAMINE	C8H11NO2	153.07897	0.6				
DOPAMINE	C8H11NO2	153.07897	1.7				

Continued from Table S6.4 List of tested isomeric compounds. Red = no separation, green = separate peaks. LC methods according to Table S6.1.

Selected isomeric compounds	Formula	Neutral mass	k value				
			RP 5 condition A	RP 19 condition B	HILIC 19 condition E	RP 4	RP 6
2,3-DIHYDROXYBENZOATE	C7H6O4	154.02661	6.5	6.1	5.9	6.7	7.2
3,4-DIHYDROXYBENZOATE	C7H6O4	154.02661	4.5	4.0	16.3	5.2	4.6
2,5-DIHYDROXYBENZOATE	C7H6O4	154.02661	6.3	5.5	10.6	6.6	6.3
OROTIC ACID	C5H4N2O4	156.01656		1.7	13.8		1.4
2,4-DIHYDROXYPYRIMIDINE-5-CARBOXYLIC ACID	C5H4N2O4	156.01656		0.9	16.7		2.0
ALPHA-AMINOADIPATE	C6H11NO4	161.06881			21.8		
N-METHYL-L-GLUTAMATE	C6H11NO4	161.06881			19.6		
4-COUMARATE	C9H8O3	164.04735		6.5	10.7		8.1
PHENYLPYRUVATE	C9H8O3	164.04735		8.1	2.6		7.5
4-HYDROXY-L-PHENYLGLYCINE	C8H9NO3	167.05824	0.2		18.1		
PYRIDOXAL	C8H9NO3	167.05824	1.2		0.9		
3,4-DIHYDROXYPHENYLACETATE	C8H8O4	168.04226	4.6	4.5	2.1	4.1	5.5
HOMOGENISATE	C8H8O4	168.04226	3.4	3.0	12.4	3.2	3.4
PYRIDOXINE	C8H11NO3	169.07389	2.0				
NORADRENALINE	C8H11NO3	169.07389	0.4				
2-QUINOLINECARBOXYLIC ACID	C10H7NO2	173.04768	5.7	5.2	8.4	5.4	7
4-QUINOLINECARBOXYLIC ACID	C10H7NO2	173.04768	3.4	1.6	6.0	2.3	2.7
THEOPHYLLINE	C7H8N4O2	180.06473	5.1	5.0		5.6	6.7
PARAXANTHINE	C7H8N4O2	180.06473	4.9	4.8		5.4	6.6
THEOBROMINE	C7H8N4O2	180.06473	4.2	4.2		4.5	5.9
METHYL VANILLATE	C9H10O4	182.05791	7.7	7.3	0.0	8.5	9.3
HOMOVANILLATE	C9H10O4	182.05791	6.0	5.7	9.6	6.3	7.3
3-(4-HYDROXYPHENYL)LACTATE	C9H10O4	182.05791	4.8	4.8	13.4	4.9	5.7
DL-NORMETANEPHRINE	C9H13NO3	183.08954			11.5		
EPINEPHRINE	C9H13NO3	183.08954			15.1		
2'-DEOXYADENOSINE	C10H13N5O3	251.10129	2.5	1.8			2.5
5'-DEOXYADENOSINE	C10H13N5O3	251.10129	4.3	3.4			3.6
2'-DEOXYGUANOSINE	C10H13N5O4	267.09621			12.4		
ADENOSINE	C10H13N5O4	267.09621			3.2		
ADENOSINE 2',3'-CYCLIC MONOPHOSPHATE	C10H12N5O6P	329.05197		1.7	14.6		
3',5'-CYCLIC AMP	C10H12N5O6P	329.05197		3.6	15.6		
2'-DEOXYGUANOSINE 5'-MONOPHOSPHATE	C10H14N5O7P	347.06309		2.2			
ADENOSINE 5'-MONOPHOSPHATE	C10H14N5O7P	347.06309		0.6			

Table S6.5 List of confirmed compounds found in human serum. Overall and intra-batch variability, repeatability and linearity as shown in Figure 4.13.

Nr	Confirmed compounds in human serum	Overall variability [CV %]	Intra-batch variability [CV %]	Repeatability A [CV %]	Repeatability B [CV %]	Linearity [R ²]
1	1,3-Dimethyluric acid	20.2%	20.3%	21.4%	13.2%	0.981
2	1-Methylguanine	17.4%	15.4%	19.5%	15.6%	0.994
3	2,3-Dihydroxybenzoate	26.1%	23.6%	21.9%	18.9%	0.999
4	2,5-Dihydroxybenzoate	27.1%	26.5%	29.3%	29.1%	1.000
5	2-Aminobutyric acid	9.9%	9.5%	10.8%	7.6%	0.998
6	2'-Deoxyinosine	17.9%	15.8%	23.2%	13.9%	0.996
7	2-Hydroxy(iso)valeric acid	34.9%	33.7%	29.3%	28.4%	0.995
8	2-Methylhippuric acid	32.9%	33.3%	27.0%	25.8%	0.950
9	3-Phenyllactic acid	28.3%	25.7%	26.7%	16.9%	0.997
10	4-Guanidinobutanoate	13.0%	11.0%	9.4%	10.4%	1.000
11	4-Hydroxyproline	9.1%	9.0%	13.3%	7.4%	1.000
12	4-Indolecarbaldehyde	8.6%	8.9%	14.8%	11.1%	1.000
13	5-Hydroxyindoleacetate	12.7%	12.5%	15.0%	12.9%	1.000
14	5'-Methylthioadenosine	16.0%	15.4%	16.3%	10.1%	0.986
15	5-Oxo-proline	12.6%	11.9%	12.3%	8.6%	0.993
16	7-Methylguanine	22.5%	18.7%	21.3%	13.9%	0.996
17	Acetylarginine	13.2%	12.0%	16.8%	14.8%	0.999
18	Acetylcarnitine	14.6%	11.7%	11.5%	12.1%	0.999
19	Adenosine	17.3%	15.1%	21.1%	15.6%	0.998
20	Alanine	7.8%	6.9%	8.6%	5.1%	1.000
21	Alpha-Amino adipate	14.2%	12.4%	17.6%	10.7%	0.990
22	Alpha-ketoglutaric acid	17.0%	16.5%	18.4%	15.6%	0.993
23	Ascorbic acid-2-sulfate	19.9%	17.8%	20.5%	13.0%	0.997
24	Asparagine	9.3%	8.8%	13.6%	9.5%	0.997
25	Aspartate	17.4%	16.8%	20.8%	16.0%	0.930
26	Aspartyl-Phenylalanine	18.4%	16.7%	22.4%	14.8%	1.000
27	C6 sugar alcohol	14.4%	13.3%	14.3%	9.4%	0.998
28	Carnitine	10.3%	9.9%	13.7%	8.6%	0.996
29	Carnosine	33.2%	26.8%	24.6%	20.0%	0.998
30	cis-5,8,11,14,17-Eicosapentaenoic acid	23.1%	23.9%	29.2%	17.5%	0.966

Continued from Table S6.5 List of confirmed compounds found in human serum. Overall and intra-batch variability, repeatability and linearity as shown in Figure 4.13.

Nr	Confirmed compounds in human serum	Overall variability [CV %]	Intra-batch variability [CV %]	Repeatability A [CV %]	Repeatability B [CV %]	Linearity [R ²]
31	Citrulline	16.1%	13.4%	15.6%	11.1%	1.000
32	Coumaric Acid	10.9%	9.7%	13.2%	10.8%	0.998
33	Creatine	7.5%	7.7%	10.1%	6.5%	1.000
34	Creatinine	16.4%	14.8%	15.2%	11.5%	0.999
35	Decanoylcarnitine	14.8%	12.8%	15.0%	11.7%	0.999
36	Deoxycarnitine	18.4%	15.9%	24.4%	18.5%	0.994
37	Disaccharides	22.8%	23.1%	19.3%	21.3%	0.988
38	DL-3-Aminoisobutyric acid	9.0%	8.8%	10.9%	9.4%	0.998
39	DL-Dipalmitoylphosphatidylcholine	18.0%	17.1%	25.1%	17.4%	0.979
40	Ferulate	24.9%	24.5%	25.7%	24.4%	0.997
41	Glutamic Acid	10.7%	9.4%	16.2%	8.3%	0.967
42	Glutamine	8.7%	8.0%	10.2%	8.1%	0.994
43	Glycine	9.1%	8.3%	13.5%	8.8%	1.000
44	Guanidinoacetate	13.6%	12.8%	17.7%	10.8%	1.000
45	Guanidinoethyl sulfonate	19.9%	16.3%	16.7%	17.1%	0.991
46	Guanine	18.3%	14.9%	20.6%	14.6%	0.995
47	Guanosine	13.8%	13.4%	18.7%	14.8%	0.998
48	Heptadecanoate	19.3%	18.1%	20.5%	10.6%	0.987
49	Hydroxybenzaldehyde	10.2%	9.3%	13.9%	11.6%	0.997
50	Hypotaurine	9.9%	8.6%	11.3%	9.3%	0.997
51	Imidazoleacetic Acid	13.6%	12.1%	15.0%	15.6%	0.998
52	Indole	9.5%	8.8%	15.1%	12.3%	0.998
53	Indoleacrylic acid	11.1%	10.9%	11.9%	11.5%	0.998
54	Kynurenic acid	30.0%	28.3%	26.5%	23.4%	0.990
55	Kynurenine	12.9%	13.5%	14.4%	13.9%	0.993
56	L-alpha-phosphatidylcholine	18.6%	16.3%	22.3%	12.5%	1.000
57	L-Ergothioneine	12.6%	11.1%	11.6%	12.5%	0.992
58	Methionine	7.3%	6.8%	9.2%	7.4%	0.999
59	Methionine sulfoxide	9.6%	9.0%	12.3%	9.1%	1.000
60	Methylimidazoleacetic acid	16.7%	14.5%	15.6%	14.1%	0.996
61	Methylnicotinamide	22.5%	20.3%	21.6%	15.4%	0.993
62	N4-Acetylcytidine	15.2%	14.3%	17.8%	11.4%	0.999
63	N6-Acetyl-L-lysine	19.6%	19.4%	19.5%	18.1%	0.986
64	N-Acetyl-DL-glutamic Acid	20.2%	18.2%	23.4%	14.9%	0.989
65	N-Acetylhistamine	22.1%	19.8%	25.5%	19.2%	0.989
66	N-Acetyl-L-carnosine	35.1%	19.4%	17.6%	14.5%	0.998
67	N-Acetylneuraminic acid	24.5%	24.1%	25.9%	17.8%	0.995
68	N-Amidino-L-aspartate	20.6%	15.0%	17.3%	14.5%	0.993
69	Naphthylamine	8.5%	8.5%	15.8%	11.7%	1.000
70	N-Phenylacetylglutamine	11.6%	10.7%	14.2%	8.2%	0.998
71	Oleic acid	24.6%	20.2%	24.0%	14.3%	0.985
72	Palmitoylcarnitine	12.4%	12.1%	17.9%	11.8%	0.990
73	Pantothenic acid	18.0%	16.9%	20.0%	15.4%	1.000
74	Phenylacetic acid/Toluic acid	21.5%	19.8%	20.2%	11.9%	0.989
75	Phenylalanine	12.0%	11.2%	28.3%	16.0%	0.963
76	Proline	6.2%	6.0%	9.0%	6.0%	1.000
77	Propionylcarnitine	16.0%	12.1%	15.9%	12.7%	0.999
78	Pseudouridine	14.9%	13.1%	17.0%	12.2%	0.997
79	Quinine	30.1%	26.0%	28.8%	28.7%	0.983
80	S-Adenosylhomocysteine	18.2%	15.6%	22.1%	15.0%	1.000
81	Serine	7.7%	7.3%	10.0%	8.1%	1.000
82	Sphingomyelin	18.2%	17.6%	23.6%	22.1%	0.933
83	Stachydrine	14.0%	10.5%	14.1%	7.6%	1.000
84	Taurine	10.6%	9.1%	11.4%	7.4%	1.000
85	Threonine/Homoserine	10.1%	9.2%	14.7%	7.0%	0.998
86	Thymine	21.5%	19.5%	19.9%	20.4%	0.999
87	Trigonelline	10.8%	9.1%	8.1%	7.6%	0.998
88	Tryptophan	11.8%	10.9%	16.9%	12.8%	0.995
89	Tyrosine	8.8%	8.2%	9.9%	7.9%	0.999
90	Uracil	22.4%	17.9%	26.2%	15.9%	0.982
91	Uridine	22.1%	21.2%	23.1%	24.3%	0.995
92	Valine/Betaine	6.6%	6.3%	7.2%	5.5%	0.999
93	Xanthine	35.6%	32.7%	28.5%	22.5%	0.973
94	Xanthosine	23.5%	19.6%	18.2%	19.3%	0.998
95	(S)-Nicotine	41.4%	42.2%	40.6%	36.4%	0.964
96	1,7-Dimethyluric acid	56.1%	54.7%	56.8%	46.9%	0.956
97	1-Methyladenosine	21.8%	20.5%	26.1%	33.9%	0.995
98	1-Methylhistidine	75.2%	45.8%	34.2%	32.6%	0.397
99	2-Amino-2-methylpropanoate	33.6%	31.7%	32.1%	33.4%	0.900
100	2-Hydroxyhippuric acid	56.2%	50.3%	73.1%	34.8%	0.999
101	3-(4-Hydroxyphenyl)lactate	47.7%	45.2%	58.7%	49.3%	0.871
102	3-Hydroxybutanoic Acid	46.1%	40.2%	36.8%	31.9%	0.986
103	3-Methyladipic acid/Pimelic acid	43.2%	41.0%	51.6%	53.7%	0.989
104	3-methylhistamine	29.3%	28.0%	54.9%	43.5%	0.913
105	3-Methylhistidine	26.1%	25.7%	45.9%	37.7%	0.952
106	4-Acetamidobutanoic acid	27.8%	23.2%	39.8%	30.5%	0.997
107	4-Aminohippuric acid	34.9%	33.1%	35.9%	20.4%	1.000
108	5-Aminolevulinic acid	27.3%	25.9%	45.3%	70.6%	0.993
109	5-Aminovaleric acid	44.2%	37.4%	106.2%	53.5%	0.059
110	8_11_14-Eicosatrienoic acid	15.9%	16.6%	21.0%	14.0%	0.987

Continued from Table S6.5 List of confirmed compounds found in human serum. Overall and intra-batch variability, repeatability and linearity as shown in Figure 4.13.

Nr	Confirmed compounds in human serum	Overall variability [CV %]	Intra-batch variability [CV %]	Repeatability A [CV %]	Repeatability B [CV %]	Linearity [R ²]
111	Agmatine	78.9%	77.0%	116.3%	151.0%	0.397
112	Arginine	97.4%	103.1%	58.8%	73.0%	0.378
113	Ascorbic acid	266.7%	130.6%	157.9%	148.7%	0.834
114	Beta-Alanine	53.6%	51.0%	119.7%	49.0%	0.972
115	C5 sugar alcohols	544.8%	163.0%	89.3%	129.3%	0.730
116	C6 uronic acids	38.6%	37.1%	36.8%	32.2%	0.985
117	C6H12O5	128.1%	80.3%	76.8%	100.6%	0.375
118	Caprylic acid	16.1%	15.3%	37.6%	24.5%	0.707
119	Cholate	50.3%	35.8%	45.7%	32.9%	0.998
120	Choline	31.8%	31.3%	30.5%	30.7%	0.992
121	Cinnamoylglycine	80.9%	60.8%	58.9%	51.0%	0.908
122	Cystathionine	32.1%	30.0%	57.4%	13.5%	0.856
123	Cysteine-S-sulfate	14.5%	14.3%	32.0%	9.7%	0.590
124	Cytosine	27.9%	23.8%	32.1%	23.9%	0.770
125	Decanoate	12.0%	11.3%	36.6%	20.4%	0.385
126	DL-Lactate	173.0%	131.0%	155.2%	211.8%	0.288
127	Docosahexaenoic Acid	20.0%	18.8%	22.6%	11.3%	0.999
128	Dodecanedioic acid	39.9%	39.3%	60.0%	35.6%	0.649
129	Ethylmalonic acid	14.5%	13.1%	27.9%	17.9%	0.788
130	Formyl-L-methionyl peptide	74.3%	69.9%	82.5%	117.2%	0.748
131	Furoate	41.2%	38.9%	39.1%	36.2%	0.987
132	GABA	21.6%	20.2%	47.4%	35.7%	0.996
133	Glutaric Acid/Methylsuccinic acid	42.1%	41.9%	37.5%	38.1%	0.918
134	Glyceric Acid	39.4%	34.9%	29.5%	34.2%	0.997
135	Glycocholate	28.6%	24.8%	101.1%	37.7%	0.978
136	Glycylglycine	29.2%	27.3%	39.3%	33.5%	0.975
137	Hesperetin	15.2%	16.0%	35.3%	30.5%	#DIV/0!
138	Hexanoylcarnitine	20.0%	17.1%	48.5%	38.3%	0.974
139	Hexoses	36.4%	37.0%	40.7%	30.6%	0.922
140	Hippurate	67.8%	65.6%	80.3%	58.5%	0.884
141	Histidine	37.5%	33.4%	37.5%	48.4%	0.978
142	Hydroxy(iso)butyric Acid	41.1%	40.0%	38.2%	32.9%	0.981
143	Hydroxycaproic acid	37.6%	35.2%	34.1%	26.6%	0.991
144	Hydroxyindole	78.2%	62.0%	55.0%	68.6%	0.866
145	Indole-3-acetaldehyde	349.6%	151.8%	167.6%	193.9%	0.847
146	Indole-3-lactic acid	95.9%	82.3%	104.0%	113.3%	0.928
147	Indole-3-pyruvic acid	69.6%	56.9%	70.5%	40.3%	0.980
148	Indoxylsulfate	43.9%	42.9%	64.4%	81.7%	0.802
149	Isoleucine	24.2%	21.3%	41.9%	35.5%	0.996
150	Lauric Acid	13.7%	13.2%	24.2%	16.5%	0.753
151	Lauroylcarnitine	12.4%	12.2%	32.0%	16.1%	0.995
152	L-Cystine	23.6%	22.4%	32.9%	18.2%	0.678
153	Leucine	75.0%	67.1%	151.5%	105.1%	0.400
154	Linoleic acid	40.9%	33.5%	42.6%	36.1%	0.920
155	Lysine	100.7%	105.7%	65.0%	56.8%	0.396
156	Methyluric acid	22.5%	20.3%	35.3%	35.3%	0.984
157	Methylxanthine	48.2%	43.7%	35.9%	40.7%	0.993
158	Myristic Acid	15.6%	16.1%	22.1%	20.9%	0.988
159	N-Acetylalanine	31.2%	29.7%	32.4%	22.8%	0.951
160	N-acetyl-DL-tryptophan	35.6%	30.6%	45.2%	48.6%	0.978
161	N-Acetylglycine	30.8%	27.5%	39.8%	45.4%	0.999
162	N-Acetyl-L-aspartic Acid	27.7%	26.1%	41.7%	53.1%	0.995
163	N-Acetyl-L-methionine	193.0%	108.5%	195.8%	66.8%	0.845
164	N-Acetyl-L-phenylalanine	47.5%	46.2%	61.7%	41.1%	0.984
165	Nicotinamide	29.4%	27.2%	31.1%	30.4%	1.000
166	Nonanoic acid	15.1%	14.5%	35.9%	26.7%	0.732
167	Ornithine	103.2%	109.5%	87.2%	90.4%	0.394
168	Palmitoleic Acid	38.5%	32.4%	38.9%	44.1%	0.401
169	Pyruvic Acid	101.3%	84.5%	92.2%	92.3%	0.535
170	Quinate	73.6%	53.9%	52.7%	59.3%	0.975
171	Salicylic acid	49.5%	41.8%	45.1%	60.2%	0.630
172	Serotonin	18.9%	17.2%	41.1%	24.1%	0.984
173	SN-Glycero-3-phosphocholine	16.1%	16.2%	45.3%	19.3%	0.984
174	Sphingosine-1-phosphate	20.1%	18.1%	41.0%	20.1%	1.000
175	Symmetric dimethylarginine	34.8%	33.0%	32.1%	27.0%	0.990
176	Tetradecanedioic acid	28.4%	28.4%	30.3%	26.1%	0.966
177	Urate	197.9%	76.5%	1039.0%	181.3%	0.364
178	Valeric acid	37.7%	35.0%	37.5%	38.1%	0.992
179	Xanthurenic Acid	21.1%	19.5%	65.9%	31.5%	0.899

Table S6.6 All compounds analyzed in chapter 4.2.1. Corresponding fold change (stroke/control), FDR adjusted p-value.

Compound	Fold change	p-value
AcCa (8:0)	1.00	4.61E-01
AcCa (8:1)	0.87	6.74E-02
AcCa (10:3)	0.79	3.05E-02
AcCa (11:1)	0.75	1.19E-05
AcCa (12:0)	1.10	2.82E-01
AcCa (13:0)	0.77	6.19E-03
AcCa (13:1)	0.73	1.35E-05
AcCa (14:0)	1.08	4.02E-01
AcCa (14:1)	1.39	1.35E-04
AcCa (14:2)	1.32	2.39E-03
AcCa (16:0)	1.07	2.82E-01
AcCa (16:1)	1.33	1.35E-04
AcCa (18:0)	1.02	7.84E-01
AcCa (18:1)	1.21	2.96E-04
AcCa (24:0)	0.90	1.14E-01
AcCa (26:1)	0.95	4.94E-01
CE(18:2)	1.02	6.74E-01
CE(20:3)	1.07	1.17E-01
CE(20:4)	1.15	6.78E-02
CE(20:5)	1.26	2.96E-01
CE(22:6)	1.08	3.95E-01
Cer (d40:0)	0.91	1.69E-01
Cer (d40:1)	0.92	1.54E-01
Cer (d40:2)	0.92	1.68E-01
Cer (d41:0)	0.83	2.32E-02
Cer (d42:1)	0.89	9.58E-02
Cer (d42:2)	0.98	4.13E-01
Cer (d42:3)	1.00	6.53E-01
Cer (d43:2)	0.92	9.92E-02
Cer (d44:2)	0.91	5.78E-02
Cer (t42:0)	0.87	2.89E-02
CerG (d40:1)	0.95	2.71E-01
CerG (d41:1)	0.92	2.65E-01
CerG (d42:1)	0.94	3.90E-01
CerG3 (d34:1)	1.00	9.13E-01
CerG3 (d40:1)	0.95	4.02E-01
CerG3 (d42:1)	0.94	3.90E-01
CerG3 (d42:2)	1.00	7.01E-01
LPC (12:0)	0.61	3.44E-05
LPC (14:0)	0.77	6.07E-04
LPC (14:1)	0.65	1.53E-05
LPC (15:1)	0.77	5.72E-04
LPC (16:0)	0.93	6.17E-02
LPC (16:1)	0.89	6.46E-02
LPC (17:0)	0.93	1.54E-01
LPC (18:0)	0.94	2.82E-01
LPC (18:1)	0.94	3.64E-01
LPC (18:2)	0.90	3.98E-02
LPC (19:0)	0.92	1.61E-01
LPC (19:1)	0.95	3.46E-01
LPC (20:0)	0.94	4.83E-01
LPC (20:1)	0.96	5.25E-01
LPC (20:4)	1.02	8.61E-01
LPC (22:0)	0.93	2.65E-01
LPC (22:1)	1.07	9.54E-01
LPC (24:0)	0.91	9.42E-02
LPC (24:1)	0.95	4.02E-01
LPC (26:1)	0.93	1.84E-01
LPE (16:0)	0.89	8.64E-03
LPE (18:1)	0.86	8.65E-03
LPE (18:2)	0.86	2.12E-02
LPE (20:4)	1.02	9.36E-01
LPE (22:5)	1.14	1.99E-01
LPE (22:6)	1.06	5.23E-01
OEA (C18:1)	1.19	9.05E-03
PC (28:0)	0.74	6.19E-03
PC (28:1)	0.61	1.53E-05
PC (29:0)	0.81	1.61E-02
PC (30:0)	0.83	9.05E-03
PC (30:1)	0.78	3.50E-03

Compound	Fold change	p-value
PC (31:0)	0.90	5.79E-02
PC (31:1)	0.88	6.46E-02
PC (31:2)	0.82	2.49E-03
PC (32:0)	0.95	9.95E-02
PC (32:1)	0.91	1.35E-01
PC (32:2)	0.84	1.16E-02
PC (33:0)	0.93	1.17E-01
PC (33:1)	0.92	6.40E-02
PC (33:2)	0.90	6.78E-02
PC (33:3)	0.83	2.04E-02
PC (34:0)	0.94	2.19E-01
PC (34:1)	0.97	4.02E-01
PC (34:2)	0.97	1.54E-01
PC (34:3)	0.89	5.82E-02
PC (34:4)	0.84	6.17E-02
PC (35:1)	0.95	1.12E-01
PC (35:2)	0.92	6.17E-02
PC (35:3)	0.93	1.06E-01
PC (36:1)	0.93	1.87E-01
PC (36:2)	0.93	2.83E-02
PC (36:3)	0.96	2.26E-01
PC (36:4)	0.96	2.74E-01
PC (36:5)	0.93	4.28E-01
PC (36:6)	0.83	4.44E-02
PC (37:1)	0.92	1.54E-01
PC (37:2)	0.86	1.01E-02
PC (37:3)	0.98	3.90E-01
PC (37:4)	1.00	6.74E-01
PC (37:6)	0.92	1.68E-01
PC (38:1)	0.93	1.69E-01
PC (38:2)	0.95	2.86E-01
PC (38:3)	0.99	8.18E-01
PC (38:4)	1.01	8.42E-01
PC (38:5)	0.98	6.76E-01
PC (38:6)	0.94	1.99E-01
PC (39:3)	0.96	4.45E-01
PC (39:6)	0.96	4.45E-01
PC (40:1)	0.97	3.90E-01
PC (40:2)	0.94	2.08E-01
PC (40:3)	0.96	3.50E-01
PC (40:4)	1.01	8.87E-01
PC (40:5)	0.98	7.15E-01
PC (40:6)	0.94	3.34E-01
PC (40:7)	0.96	2.50E-01
PC (40:8)	0.87	3.20E-02
PC (42:10)	0.87	5.36E-02
PC (42:2)	0.93	1.69E-01
PC (42:3)	0.95	2.08E-01
PC (42:5)	0.94	3.55E-01
PC (42:7)	0.94	1.98E-01
PC (42:9)	0.92	2.26E-01
PC (44:5)	0.98	5.62E-01
PE (34:1)	0.86	2.08E-02
PE (34:2)	0.82	1.39E-03
PE (36:1)	0.71	4.12E-05
PE (36:2)	0.74	3.44E-05
PE (36:4)	0.94	7.31E-02
PE (36:5)	0.82	1.69E-02
PE (38:4)	0.87	2.10E-02
PE (38:5)	0.92	5.73E-02
PE (38:6)	0.93	1.40E-01
PE (40:6)	0.90	8.89E-02
PEA (C16:0)	1.21	3.29E-03
PI (32:1)	0.95	7.20E-01
PI (36:3)	0.98	6.26E-01
SM (d30:1)	0.96	2.69E-01
SM (d32:0)	0.91	1.02E-01
SM (d32:1)	0.91	7.77E-02
SM (d32:2)	1.03	8.64E-01
SM (d34:0)	0.96	4.64E-01

Supplement

Continued from Table S6.6 All compounds analyzed in chapter 4.2.1. Corresponding fold change (stroke/control), FDR adjusted p-value.

Compound	Fold change	p-value
SM (d34:1)	0.98	5.23E-01
SM (d34:2)	0.98	6.76E-01
SM (d35:1)	0.98	3.06E-01
SM (d36:0)	0.96	3.95E-01
SM (d36:1)	0.99	2.28E-01
SM (d36:2)	1.00	4.02E-01
SM (d36:3)	1.03	9.83E-01
SM (d38:1)	0.92	4.44E-02
SM (d38:2)	0.96	1.42E-01
SM (d38:3)	1.03	7.84E-01
SM (d39:1)	0.91	1.11E-01
SM (d40:1)	0.94	2.11E-01
SM (d40:2)	0.95	2.08E-01
SM (d41:1)	0.94	3.34E-01
SM (d41:2)	0.96	3.89E-01
SM (d42:1)	0.95	3.97E-01
SM (d42:2)	0.98	4.64E-01
SM (d42:3)	0.99	6.53E-01
SM (d42:4)	1.03	9.63E-01
SM (d43:1)	0.91	1.68E-01
SM (d43:2)	0.94	1.85E-01
SM (d43:3)	0.95	2.65E-01
SM (d44:2)	0.95	2.10E-01
SM (d44:3)	0.97	3.90E-01
SM (t34:0)	0.92	9.95E-02
SM (t34:2)	0.99	6.92E-01
SM (t36:1)	1.01	7.40E-01
SM (t40:0)	0.92	2.08E-01
SM (t42:1)	0.96	2.35E-01
TG (48:2)	0.57	6.81E-06
TG (50:1)	0.75	1.58E-03
TG (50:2)	0.76	1.00E-03
TG (50:3)	0.72	2.57E-04
TG (52:2)	0.78	5.41E-03
TG (52:3)	0.79	6.19E-03
TG (52:4)	0.74	2.57E-03
TG (52:5)	0.68	2.65E-04
TG (53:2)	0.67	2.06E-04
TG (53:3)	0.71	4.43E-04
TG (53:4)	0.69	2.06E-04
TG (54:4)	0.76	3.40E-03
TG (54:5)	0.77	4.35E-03
TG (54:6)	0.73	1.41E-03
TG (56:5)	0.81	2.08E-02
TG (56:6)	0.81	1.92E-02
TG (56:7)	0.81	2.92E-02
TG (58:6)	0.76	3.40E-03
TG (58:7)	0.81	2.12E-02
PC (34:1)/PE (34:1)	1.15	6.43E-02
PC (34:2)/PE (34:2)	1.22	1.24E-03
PC (36:1)/PE (36:1)	1.29	4.79E-05
PC (36:2)/PE (36:2)	1.28	3.10E-05
PC (36:4)/PE (36:4)	1.08	1.98E-01
PC (36:5)/PE (36:5)	1.20	1.01E-02
PC (38:4)/PE (38:4)	1.14	7.16E-03
PC (38:5)/PE (38:5)	1.12	9.95E-02
PC (38:6)/PE (38:6)	1.10	2.95E-01
PC (40:6)/PE (40:6)	1.15	9.92E-02
SM (d40:1)/Cer (d40:1)	1.04	2.96E-01
SM (d40:2)/Cer (d40:2)	1.04	4.02E-01
SM (d42:1)/Cer (d42:1)	1.07	5.78E-02
SM (d42:2)/Cer (d42:2)	1.03	4.97E-01
SM (d42:3)/Cer (d42:3)	1.03	7.15E-01
SM (d43:2)/Cer (d43:2)	1.04	2.95E-01
SM (d44:2)/Cer (d44:2)	1.07	1.50E-01
1-Methylguanine	0.95	3.89E-01
2,3-Dihydroxybenzoate	0.78	6.57E-02
2,5-Dihydroxybenzoate	1.05	6.26E-01
2-Aminobutyric acid	1.08	2.50E-01
2'-Deoxyinosine	0.96	7.15E-01

Compound	Fold change	p-value
2-Hydroxybutyrate	1.37	6.75E-05
2-Hydroxy(iso)valeric acid	1.05	8.44E-01
2-Methylhippuric acid	0.91	2.96E-01
3-Phenyllactic acid	1.00	6.88E-01
4-Guanidinobutanoate	0.77	2.53E-04
4-Hydroxyproline	0.93	6.43E-02
5'-Methylthioadenosine	0.87	1.45E-03
5-Oxo-proline	0.98	6.11E-01
7-Methylguanine	0.99	7.58E-01
Acetylarginine	0.94	1.79E-01
Acetylcarnitine	1.03	6.88E-01
Adenosine	0.88	8.53E-01
Alanine	0.91	2.42E-02
Alpha-Aminoadipate	0.75	6.07E-04
Alpha-ketoglutaric acid	1.06	4.51E-01
Ascorbic acid-2-sulfate	0.89	6.95E-02
Asparagine	0.93	6.23E-02
Aspartate	0.95	1.99E-01
Aspartyl-Phenylalanine	1.05	5.19E-01
Carnitine	0.97	5.12E-01
Citrulline	0.97	5.95E-01
Creatine	0.95	4.02E-01
Creatinine	0.95	2.10E-01
Decanoylcarnitine	1.07	5.25E-01
Deoxycarnitine	0.88	5.78E-02
3-Aminoisobutyric acid	1.12	5.23E-01
Dipalmitoylphosphatidylcholine	1.02	8.60E-01
Glutamic Acid	0.92	4.02E-01
Glutamine	0.98	5.08E-01
Glycine	0.90	1.92E-02
Guanidinoacetate	1.01	8.18E-01
Guanidinoethyl sulfonate	0.90	1.72E-01
Guanosine	0.84	1.28E-01
Hypotaurine	0.85	1.57E-01
Imidazoleacetic Acid	0.90	4.01E-02
Kynurenic acid	0.80	3.60E-02
Kynurenine	0.81	6.61E-04
L-alpha-phosphatidylcholine	0.96	4.02E-01
L-Ergothioneine	1.01	6.94E-01
Methionine	0.96	3.90E-01
Methionine sulfoxide	0.78	3.76E-05
Methylimidazoleacetic acid	0.98	5.77E-01
Methylnicotinamide	0.95	7.65E-01
N4-Acetylcytidine	0.92	5.10E-01
N6-Acetyl-L-lysine	0.96	4.64E-01
N-Acetyl-DL-glutamic Acid	0.97	2.57E-01
N-Acetylhistamine	0.87	9.11E-02
N-Acetyl-L-carnosine	0.88	9.90E-02
N-Acetylneuraminic acid	0.94	4.87E-01
N-Amidino-L-aspartate	1.04	4.64E-01
N-Phenylacetylglutamine	0.87	4.94E-01
Palmitoylcarnitine	1.06	3.91E-01
Pantothenic acid	0.99	8.60E-01
Phenylacetic acid/Toluic acid	1.18	9.92E-02
Phenylalanine	0.92	3.90E-01
Proline	0.90	3.40E-03
Propionylcarnitine	0.73	6.81E-06
Pseudouridine	0.96	4.87E-01
S-Adenosylhomocysteine	1.06	8.77E-01
Serine	0.98	5.14E-01
Sphingomyelin	0.98	8.42E-01
Stachydrine	0.81	1.02E-01
Taurine	0.91	6.74E-02
Threonine	0.87	2.57E-03
Thymine	0.97	5.23E-01
Trigonelline	0.69	1.35E-03
Tryptophan	0.81	1.53E-05
Tyrosine	0.86	2.65E-04
Uridine	1.06	2.65E-01
Valine/Betaine	0.96	9.11E-02
Xanthine	0.94	3.18E-01

Table S6.7 All compounds analyzed in chapter 4.2.3. Corresponding fold change (bad outcome/good outcome), FDR adjusted p-value.

Compound	Fold change	p-value
AcCa (13:0)	1.46	6.70E-02
AcCa (14:1)	1.11	9.18E-01
AcCa (14:2)	1.05	9.34E-01
AcCa (16:0)	0.99	9.46E-01
AcCa (16:1)	1.09	5.55E-01
AcCa (18:0)	0.89	1.66E-01
AcCa (18:1)	1.05	5.05E-01
AcCa (24:0)	0.87	5.42E-02
AcCa (26:1)	1.00	9.06E-01
Cer (d40:0)	0.84	5.42E-02
Cer (d42:1)	0.92	4.49E-01
CerG (d40:1)	0.89	1.20E-01
CerG (d41:1)	0.92	2.91E-01
CerG3 (d42:2)	0.89	3.13E-01
LPC (14:0)	0.90	4.18E-01
LPC (16:0)	0.89	1.75E-02
LPC (16:1)	0.93	4.15E-01
LPC (17:0)	0.92	4.15E-01
LPC (18:0)	0.82	2.00E-03
LPC (18:1)	0.93	2.99E-01
LPC (18:2)	0.90	6.39E-02
LPC (19:0)	0.77	4.47E-03
LPC (19:1)	0.92	4.18E-01
LPC (20:1)	0.94	4.18E-01
LPC (20:4)	0.84	1.73E-02
LPC (22:0)	0.79	2.33E-03
LPC (22:1)	0.99	2.99E-01
LPC (24:0)	0.78	1.74E-03
LPC (24:1)	0.86	4.84E-02
LPE (16:0)	1.07	3.57E-01
LPE (20:4)	1.05	8.28E-01
LPE (22:5)	0.94	5.32E-01
LPE (22:6)	0.91	3.57E-01
PC (28:0)	0.92	5.45E-01
PC (29:0)	1.19	3.72E-01
PC (30:0)	1.05	8.86E-01
PC (30:1)	0.95	9.90E-01
PC (31:1)	1.18	2.72E-01
PC (32:0)	1.08	3.28E-01
PC (32:1)	1.03	6.51E-01
PC (32:2)	0.83	1.69E-01
PC (33:0)	1.20	6.18E-02
PC (33:1)	1.17	5.42E-02
PC (33:2)	1.07	5.45E-01
PC (33:3)	1.12	4.95E-01
PC (34:0)	0.91	1.23E-01
PC (34:1)	1.00	9.90E-01
PC (34:2)	0.98	8.08E-01
PC (34:4)	0.78	8.76E-02
PC (35:1)	1.12	1.26E-01
PC (35:2)	1.08	2.83E-01
PC (35:3)	1.01	7.88E-01
PC (36:1)	0.93	3.71E-01

Compound	Fold change	p-value
PC (36:2)	0.93	8.76E-02
PC (36:3)	0.92	1.23E-01
PC (36:4)	0.92	6.39E-02
PC (36:5)	0.98	8.93E-01
PC (37:1)	0.98	9.34E-01
PC (37:2)	0.94	3.48E-01
PC (37:3)	1.01	8.12E-01
PC (37:4)	1.02	7.27E-01
PC (37:6)	1.04	6.71E-01
PC (38:1)	0.93	2.58E-01
PC (38:2)	0.84	2.69E-02
PC (38:3)	0.80	5.71E-03
PC (38:5)	0.93	2.58E-01
PC (38:6)	0.93	2.26E-01
PC (39:6)	1.11	2.58E-01
PC (40:2)	0.93	2.83E-01
PC (40:6)	0.94	4.33E-01
PC (40:7)	0.91	2.90E-01
PC (40:8)	0.73	2.14E-03
PC (42:10)	0.68	4.31E-03
PC (42:3)	0.89	1.07E-01
PC (42:7)	0.97	8.86E-01
PC (42:9)	0.73	6.34E-03
PC (44:5)	0.95	6.90E-01
PE (36:4)	1.19	4.84E-02
PE (38:5)	1.19	2.73E-02
PE (38:6)	1.49	8.81E-04
SM (d32:0)	0.85	6.15E-02
SM (d32:1)	0.93	2.99E-01
SM (d34:0)	0.94	1.69E-01
SM (d34:1)	0.97	3.57E-01
SM (d34:2)	0.92	1.41E-01
SM (d35:1)	1.07	4.15E-01
SM (d36:0)	0.86	1.46E-01
SM (d36:1)	1.00	9.90E-01
SM (d36:2)	1.04	7.62E-01
SM (d36:3)	0.94	2.99E-01
SM (d38:1)	0.88	4.84E-02
SM (d38:2)	0.98	7.27E-01
SM (d38:3)	1.11	1.02E-01
SM (d40:1)	0.82	2.75E-03
SM (d42:2)	0.90	3.44E-02
SM (d42:3)	0.92	1.69E-01
SM (d43:2)	1.01	9.09E-01
SM (d43:3)	1.03	9.18E-01
SM (d44:2)	0.84	4.66E-02
SM (d44:3)	0.90	2.58E-01
SM (t36:1)	0.96	6.29E-01
PC (34:2)/PE (34:2)	0.70	1.93E-04
PC (36:4)/PE (36:4)	0.75	1.37E-03
PC (38:5)/PE (38:5)	0.72	2.33E-03
PC (38:6)/PE (38:6)	0.63	4.35E-05
PC (40:6)/PE (40:6)	0.66	1.80E-04

Table S6.8 All compounds analyzed in the brain 2 hours and 24 hours after MCAO. Corresponding fold change (FC, ipsilateral/contralateral). Paired Student's t-test, FDR adjusted p-value, n = 16 (2 hours)/15 (24 hours).

Compound	2 hours		24 hours	
	FC	p-value	FC	p-value
AcCa (14:0)	0.91	9.57E-01	1.21	1.38E-02
AcCa (16:0)	1.05	9.89E-01	1.60	2.87E-05
AcCa (16:1)	0.95	9.67E-01		
AcCa (18:0)			2.06	3.99E-06
Cer (d36:0)	1.08	3.09E-01		
Cer (d36:1)	1.06	2.15E-01	1.92	1.30E-05
Cer (d36:2)	0.98	9.89E-01		
Cer (d38:2)	1.01	9.89E-01	0.93	3.00E-01
Cer (d40:0)	0.96	9.89E-01	0.87	1.09E-01
Cer (d40:1)	1.00	9.89E-01	0.98	7.20E-01
Cer (d40:2)	0.97	9.67E-01	0.92	2.34E-01
Cer (d41:0)	0.94	9.74E-01	0.94	5.59E-01
Cer (d42:1)	1.00	9.89E-01	0.97	7.66E-01
Cer (d42:2)	0.97	9.76E-01	0.98	7.33E-01
Cer (d42:3)	0.95	9.67E-01	0.93	2.39E-01
Cer (d43:2)	0.93	9.67E-01	0.85	1.60E-01
Cer (d44:2)	0.92	8.16E-01	0.94	7.08E-01
CerG (d36:1-OH)	1.01	9.89E-01	0.98	6.64E-01
CerG (d36:1)	0.98	9.89E-01		
CerG (d36:2)	1.02	9.67E-01	0.98	7.44E-01
CerG (d38:0-OH)	0.98	9.96E-01		
CerG (d38:1)	0.96	9.57E-01		
CerG (d38:2)	1.00	9.94E-01		
CerG (d40:0-OH)	1.01	9.89E-01	0.96	5.59E-01
CerG (d40:1-OH)	0.96	9.57E-01	0.94	3.37E-01
CerG (d40:1)	0.97	9.89E-01	0.94	4.73E-01
CerG (d40:2)	0.97	9.67E-01	0.93	2.34E-01
CerG (d41:0-OH)			0.97	7.63E-01
CerG (d41:1-OH)	0.95	9.64E-01	0.93	1.88E-01
CerG (d41:1)	0.95	9.89E-01	0.91	3.25E-01
CerG (d41:2)	0.96	9.89E-01	0.91	7.66E-02
CerG (d42:0-OH)	0.95	9.67E-01	0.93	4.51E-01
CerG (d42:1-OH)	0.97	9.67E-01		
CerG (d42:2-OH)	0.94	8.85E-01	0.94	3.88E-01
CerG (d42:2)	0.97	9.74E-01	0.92	2.34E-01
CerG (d42:3)	0.95	9.64E-01	0.94	3.55E-01
CerG (d43:1)	0.93	9.64E-01	0.90	3.36E-01
CerG (d43:2-OH)	1.01	9.89E-01	0.92	1.41E-01
CerG (d43:2)	0.93	9.67E-01		
CerG (d44:2-OH)			0.94	5.04E-01
CerPE (d44:4)	0.96	9.89E-01	0.93	5.96E-01
Coenzyme Q9	0.96	7.46E-01	0.86	7.46E-03
Coenzyme Q10			0.83	1.74E-03
DAG (34:1)	0.99	9.93E-01	0.92	3.07E-01
DAG (36:1)	0.99	9.89E-01	0.99	9.08E-01
FA(20:4)	0.78	4.11E-01	0.87	2.41E-01
FA(22:6)			1.03	7.20E-01
GM1 (d36:1)	0.93	2.02E-01	0.91	7.67E-02
GM3 (d36:1)			0.85	8.14E-03
Gsulfo-Cer (d40:1-OH)	0.96	9.67E-01	0.96	6.39E-01
Gsulfo-Cer (d41:1)			0.92	2.28E-01
Gsulfo-Cer (d42:0)			0.98	7.80E-01
Gsulfo-Cer (d42:1-OH)	0.94	7.50E-01	0.91	9.09E-02
Gsulfo-Cer (d42:1)	0.97	9.64E-01	0.94	3.37E-01
Gsulfo-Cer (d42:2)	1.00	9.89E-01	0.98	6.88E-01
LPC (16:0)	1.08	9.89E-01	1.65	4.21E-05
LPC (16:1)	0.86	9.89E-01	0.88	9.41E-02
LPC (17:0)	1.16	9.89E-01	1.63	6.16E-05
LPC (18:0)	1.07	9.89E-01	1.45	8.72E-04
LPC (18:1)	1.06	9.97E-01	0.97	6.86E-01
LPC (20:0)	1.03	9.89E-01	1.21	4.10E-02
LPC (20:4)	0.93	9.89E-01	0.77	4.21E-04
LPC (22:0)	0.97	9.67E-01		
LPC (22:4)	0.93	9.89E-01		
LPC (22:6)	1.00	9.89E-01	0.71	6.69E-04
LPC (24:0)	0.91	9.57E-01	0.96	6.88E-01

Compound	2 hours		24 hours	
	FC	p-value	FC	p-value
LPE (22:6)	1.09	9.94E-01		
LPI (18:0)	1.24	9.89E-01	0.95	6.03E-01
LPI (20:4)	0.80	6.12E-01		
PC (28:0)	1.00	9.89E-01		
PC (29:0)	0.96	8.66E-01		
OEA (C18:1)			2.94	1.30E-05
PC (30:0)	0.99	9.89E-01	0.96	3.37E-01
PC (30:1)	0.99	9.94E-01		
PC (31:0)	0.96	2.97E-01	0.86	2.83E-04
PC (32:0)	1.02	9.57E-01	1.00	9.08E-01
PC (32:1)			0.97	3.36E-01
PC (32:2)	0.97	9.67E-01		
PC (33:0)	0.98	9.52E-01	0.90	9.42E-03
PC (33:1)	0.96	6.66E-01	0.91	4.46E-02
PC (34:0)	1.00	9.94E-01	0.96	3.37E-01
PC (34:1)	1.00	9.89E-01	1.01	7.21E-01
PC (34:2)	1.02	8.85E-01	1.06	1.60E-01
PC (34:3)	1.05	6.12E-01	1.15	1.01E-02
PC (35:1)	0.99	9.89E-01	0.94	2.60E-01
PC (36:1)	1.00	9.89E-01	0.99	7.66E-01
PC (36:2)			1.05	2.01E-01
PC (36:4)	0.99	9.89E-01	0.93	1.23E-02
PC (37:1)			0.94	3.37E-01
PC (37:4)	0.94	3.31E-01	0.87	7.20E-04
PC (37:5)	0.94	6.66E-01		
PC (38:1)	1.00	9.89E-01	0.98	7.44E-01
PC (38:2)	1.00	9.89E-01	1.01	9.14E-01
PC (38:3)	0.99	9.89E-01	0.97	6.88E-01
PC (38:4)	0.99	9.89E-01	0.95	1.37E-01
PC (38:5)	0.96	5.38E-01		
PC (38:6)	0.97	9.05E-01	0.93	1.51E-01
PC (39:1)	0.97	9.89E-01	0.95	5.43E-01
PC (39:5)	0.97	9.74E-01		
PC (39:6)	0.97	6.45E-01	0.86	5.57E-03
PC (39:7)	0.93	7.14E-01		
PC (40:1)	0.98	9.89E-01	0.99	9.02E-01
PC (40:2)	0.99	9.94E-01	0.99	9.08E-01
PC (40:4)	0.98	9.89E-01	0.96	5.04E-01
PC (40:6)	1.02	8.16E-01	1.03	4.51E-01
PC (40:7)	0.98	9.74E-01	1.01	7.44E-01
PC (40:8)	1.07	4.52E-01	1.13	2.94E-02
PC (41:1)	1.08	6.45E-01	1.01	8.84E-01
PC (41:2)	0.94	9.89E-01		
PC (42:1)	1.01	9.89E-01	1.01	8.78E-01
PC (42:2)	0.97	9.89E-01	0.98	8.12E-01
PC (42:3)	0.95	9.89E-01		
PC (42:4)	0.95	7.20E-01	0.91	6.03E-02
PC (42:6)			0.95	3.56E-01
PC (42:7)	1.01	9.89E-01	1.03	7.44E-01
PC (42:9)	0.94	7.56E-01		
PC (42:10)	1.01	9.89E-01	1.02	8.12E-01
PC (44:12)	0.96	9.57E-01	1.06	3.77E-01
PC (44:2)	0.98	9.89E-01	1.05	5.82E-01
PE (34:0)	0.99	9.89E-01		
PE (34:1)			1.00	9.82E-01
PE (34:2)	1.07	8.04E-02		
PE (36:1)	1.01	9.89E-01	0.98	8.12E-01
PE (36:2)	1.02	9.64E-01	1.01	7.80E-01
PE (36:3)			0.96	7.37E-01
PE (36:4)	1.00	9.89E-01	0.97	5.59E-01
PE (36:5)	0.99	9.89E-01	1.03	7.44E-01
PE (38:2)	1.01	9.89E-01	1.01	8.25E-01
PE (38:4)	0.99	9.74E-01	0.95	2.34E-01
PE (38:5)			0.99	9.55E-01
PE (38:6)	0.97	7.91E-01	0.95	1.34E-01
PE (40:4)	1.00	9.93E-01	0.98	7.44E-01

Supplement

Continued from Table S6.8 All compounds analyzed in the brain 2 hours and 24 hours after MCAO. Corresponding fold change (FC, ipsilateral/contralateral). Paired Student's t-test, FDR adjusted p-value, n = 16 (2 hours)/15 (24 hours).

Compound	2 hours		24 hours	
	FC	p-value	FC	p-value
PE (40:5)	0.95	3.31E-01		
PE (40:6)	1.00	9.94E-01	0.97	3.88E-01
PE (40:7)	1.03	7.68E-01	1.08	6.03E-02
PE (40:8)	1.00	9.94E-01	1.04	4.85E-01
PE (42:4)	0.97	8.25E-01		
PEA (C16:0)			10.19	3.12E-06
PG (44:12)	0.78	6.94E-02	0.71	2.28E-04
PI (36:1)	0.93	9.57E-01		
PI (36:2)	1.04	9.57E-01		
PI (36:4)	0.79	2.02E-01	0.87	1.23E-02
PI (37:4)	0.87	5.62E-01		
PI (38:3)			1.14	5.59E-02
PI (38:4)	0.86	4.95E-01	0.93	1.40E-01
PI (38:5)	0.80	1.02E-01	0.94	3.42E-01
PI (40:6)	0.98	9.89E-01	0.94	6.41E-01
PS (34:1)	0.96	7.76E-01	0.91	6.39E-02
PS (36:1)	1.01	9.89E-01	1.01	8.43E-01
PS (36:2)	1.00	9.93E-01		
PS (38:1)	1.00	9.89E-01	1.04	7.18E-01
PS (38:4)	0.99	9.89E-01	0.98	7.05E-01
PS (40:2)	0.99	9.93E-01	1.03	7.80E-01
PS (40:4)	1.00	9.89E-01	1.01	9.14E-01
PS (40:5)			1.00	9.72E-01
PS (40:6)	0.99	9.89E-01	0.94	1.10E-01
PS (42:2)	1.02	9.57E-01	0.99	8.12E-01
SM (d32:1)	0.92	3.31E-01		
SM (d34:0-OH)	0.84	2.89E-01		
SM (d34:1)	0.97	7.56E-01	0.92	6.41E-02
SM (d34:2)	0.96	9.67E-01	0.66	7.20E-04
SM (d35:1)	0.93	1.81E-01	0.69	1.86E-05
SM (d36:0)	0.96	7.14E-01		
SM (d36:1)	0.99	9.89E-01	0.89	1.91E-03
SM (d36:2)	0.94	1.86E-01	0.62	5.56E-07
SM (d37:1)	0.94	5.62E-01	0.81	6.69E-04
SM (d38:1)	0.98	9.57E-01	0.91	3.93E-02
SM (d40:0)	0.98	9.89E-01	1.00	8.64E-01
SM (d40:1)	0.98	9.89E-01	0.96	5.82E-01
SM (d41:2)	0.94	9.64E-01	0.88	1.17E-01
SM (d42:0)	0.99	9.89E-01		
SM (d42:1)	0.96	9.67E-01	0.96	6.39E-01
SM (d42:2)	0.97	9.89E-01	0.91	2.06E-01
SM (d42:3)			0.98	8.12E-01
SM (d44:2)	0.95	9.57E-01		
TG (50:1)			1.30	6.69E-04
TG (52:1)			1.26	1.15E-03
TG (54:1)			1.14	1.09E-01
PC (34:0)/PE (34:0)	1.00	9.89E-01		
PC (34:1)/PE (34:1)			1.02	6.71E-01
PC (34:2)/PE (34:2)	0.95	3.62E-01		
PC (36:1)/PE (36:1)	0.99	9.67E-01	1.01	8.60E-01
PC (36:2)/PE (36:2)			1.03	2.31E-01
PC (36:4)/PE (36:4)	0.99	8.10E-01	0.95	7.06E-02
PC (38:2)/PE (38:2)	0.99	9.67E-01	0.99	9.48E-01
PC (38:4)/PE (38:4)	1.00	9.89E-01	1.00	9.72E-01
PC (38:6)/PE (38:6)	1.00	9.97E-01	0.98	5.29E-01
PC (40:4)/PE (40:4)	0.99	9.89E-01	0.98	5.16E-01
PC (40:6)/PE (40:6)	1.02	5.59E-01	1.06	4.46E-02
PC (40:7)/PE (40:7)	0.95	4.95E-01	0.94	1.39E-01
PC (40:8)/PE (40:8)	1.07	4.77E-02	1.09	4.46E-02

Compound	2 hours		24 hours	
	FC	p-value	FC	p-value
PC (42:4)/PE (42:4)	0.98	7.76E-01		
1-Methyladenosine	1.00	9.89E-01		
3-(4-Hydroxyphenyl) lactate			1.75	6.57E-05
3-Hydroxy-3-methyl-glutarate			1.06	2.34E-01
3-Methylhistidine	1.53	9.58E-06	1.70	7.06E-02
4-Hydroxyproline	1.43	3.80E-04		
5'-Methylthioadenosine	0.88	4.21E-01	0.82	1.04E-02
5-Oxo-Proline	1.07	1.02E-01	1.01	7.66E-01
Acetylcarnitine	1.00	9.89E-01	2.25	2.92E-06
Adenine	0.67	2.62E-03	0.86	7.56E-02
Adenosine	0.91	6.45E-01	0.87	6.03E-02
Adenosine 5'-diphosphoribose	2.09	1.86E-01		
Adenosine 5'-monophosphate	1.13	5.75E-01		
Alanine	1.33	1.94E-02	1.15	5.59E-02
Alanylproline	1.43	1.19E-04		
Allantoin	1.98	5.72E-04	2.85	4.56E-03
Alpha-Aminoadipate	1.59	1.84E-05	2.76	4.66E-05
Amino(iso)butyric acid	1.02	9.74E-01	0.98	7.66E-01
Arginine			1.42	3.07E-06
Argininosuccinic acid	1.43	3.15E-04		
Ascorbic acid	1.10	9.89E-01		
Asparagine	1.19	3.11E-04	1.01	9.14E-01
Aspartate	1.00	9.89E-01	0.94	7.44E-01
Beta-Alanine	1.10	2.26E-02	1.02	8.03E-01
Betaine			3.05	1.18E-08
C12H22O11			1.43	8.98E-01
C6 sugar alcohol			1.40	1.30E-05
C6H12O6	2.02	2.52E-02	3.60	1.82E-06
C6H13O9P	0.80	8.26E-02	1.07	6.20E-01
Carnitine	1.08	2.18E-01	1.44	2.78E-05
Carnosine	0.94	5.68E-01	1.15	1.21E-02
Choline	1.04	7.55E-01	1.13	1.85E-02
Citrulline	2.46	5.58E-05	3.62	6.59E-06
Creatine	1.07	3.36E-01	1.01	8.49E-01
Creatinine	1.06	5.38E-01	1.23	8.14E-03
Cystathionine	1.36	4.82E-05	2.17	3.21E-07
Cytidine	0.98	9.89E-01	1.19	2.91E-02
Cytidine 5'-diphosphocholine	0.98	9.67E-01		
Deoxycarnitine	0.95	7.76E-01	1.17	2.69E-02
Deoxycytidine	1.07	2.89E-01		
Dimethylarginine	1.35	9.39E-03		
Fumaric acid	1.06	2.65E-01		
GABA	1.06	4.20E-01		
Glutamic Acid	1.03	8.60E-01	1.22	1.19E-01
Glutamine	1.07	1.20E-01	1.02	7.44E-01
Glutathione	0.86	1.02E-01	0.87	6.11E-02
Glycerolphosphate	1.10	2.49E-01		
Glycerophospho-choline	0.84	7.76E-02	0.87	6.14E-01
Glycine	1.19	8.94E-03	1.30	1.77E-03
Glycolate	0.99	9.89E-01	1.02	7.20E-01
Guanine	0.89	1.44E-01	0.91	1.49E-01
Guanosine	0.91	2.83E-01	0.88	4.68E-02
Guanosine 5'-monophosphate	1.16	7.46E-01		
Hexanoylcarnitine	1.05	8.16E-01		
Histidine	1.70	3.34E-06	1.62	1.24E-03
Hydroxyglutaric acid	0.96	8.85E-01	0.88	2.39E-01
Hypoxanthine	1.04	6.40E-01	1.02	7.20E-01
Inosine	0.89	2.85E-02	0.92	6.98E-02
Lactic acid	1.03	8.11E-01	1.09	5.59E-01

Supplement

Continued from Table S6.8 All compounds analyzed in the brain 2 hours and 24 hours after MCAO. Corresponding fold change (FC, ipsilateral/contralateral). Paired Student's *t*-test, FDR adjusted *p*-value, *n* = 16 (2 hours)/15 (24 hours).

Compound	2 hours		24 hours	
	FC	p-value	FC	p-value
Leucine	1.65	9.58E-06	1.58	2.33E-02
Malate			1.08	2.37E-01
Methionine	1.66	9.58E-06	1.47	5.31E-05
Methylnicotinamide			1.38	1.71E-04
Myo-Inositol	1.00	9.89E-01		
N(PAI)-methylhistidine			1.79	1.23E-04
N6,N6,N6-Trimethyl-lysine			0.88	4.56E-03
N-Acetylalanine	0.92	3.16E-01	0.86	1.29E-02
N-Acetylgarginine	1.09	2.89E-01		
N-Acetylaspartate	0.67	1.96E-01	0.84	5.25E-03
N-Acetylaspartyl glutamic acid			1.07	9.51E-02
N-Acetylglutamate			1.18	1.22E-02
N-Acetylglycine			1.72	4.56E-03
N-Acetylmethionine	1.09	1.56E-01		
N-Acetylneuraminate	0.96	8.85E-01		
N-Methylglutamate	1.00	8.66E-01		
Phosphoethanolamine	0.84	2.52E-02		
Orotic Acid			2.45	1.15E-06
Palmitoylcarnitine	1.03	7.20E-01	1.76	1.60E-06
Pantothenic Acid	1.04	7.76E-01		
Phenylalanine	1.59	1.84E-05	1.53	4.49E-03
Phospho(enol)pyruvic acid	1.47	3.18E-02		
Phosphocholine	0.97	9.67E-01		
Pipecolate	1.20	2.12E-02		
Proline	1.65	9.58E-06	1.35	1.24E-03
Propionylcarnitine	1.02	9.67E-01	3.08	1.03E-06
Pseudouridine	1.17	3.18E-02	1.27	5.57E-03
Pyridoxamine			1.44	1.86E-05
Riboflavin	1.51	4.03E-03		
Ribosephosphate	0.88	6.79E-02	1.07	4.68E-01
S-Adenosylhomo-cysteine	1.26	1.19E-04		
Saccharopine			1.50	9.42E-03
Serine	1.15	2.12E-02		
Sphingosine (d18:1)	1.02	9.57E-01		
Taurine	0.98	9.89E-01	1.02	7.33E-01
Thiamine	1.14	1.71E-02	1.27	3.87E-04
Trans-Aconitate			1.49	4.66E-05
Threonine	1.56	3.34E-06		
Trigonelline	1.26	5.13E-02	2.63	1.35E-05
Tryptophan	1.59	1.94E-05	1.69	7.20E-04
Tyrosine	1.63	1.84E-05	1.41	7.20E-04
Urate	2.53	4.51E-05	1.78	5.56E-07
Urea	1.14	6.77E-02	1.32	6.22E-03
Uridine	0.96	7.56E-01	1.14	1.23E-02
Uridine 5'-Diphospho-glucose	0.88	2.18E-01		
Uridine 5'-Diphospho-N-acetylgalactosamine	0.84	8.36E-03		
Valine	1.33	3.44E-04	1.94	9.90E-06
Xanthine	1.09	1.02E-01	1.11	3.16E-02

Table S6.9 All compounds analyzed in chapter 4.6.2. Fold change (FC, big infarct/small infarct group) with *p*-value (Student's *t*-test for plasma/liver, two-way repeated measures ANOVA with Sidak's post test for the brain). Pearson correlation coefficient (*r*) and corresponding *p*-value for the correlation with the infarct size. *N* = 9/7 (small infarct/big infarct) for 2 hours after MCAO, *n* = 8/7 (small infarct/big infarct) for 24 hours after MCAO.

[illegible]

Continued from Table S6.9 All compounds analyzed in chapter 4.3.2.

Compound	Brain (ipsilateral) - 2 hours				Brain (contralateral) - 2 hours				Plasma - 2 hours				Liver - 2 hours				Brain (ipsilateral) - 24 hours				Brain (contralateral) - 24 hours				Plasma - 24 hours				Liver - 24 hours				
	FC	p-value	r	p-value	FC	p-value	r	p-value	FC	p-value	r	p-value	FC	p-value	r	p-value	FC	p-value	r	p-value	FC	p-value	r	p-value	FC	p-value	r	p-value	FC	p-value	r	p-value	
0.95	3.9E-01	-0.05	8.4E-01	1.06	7.3E-02	0.39	1.4E-01																										
GMB (386.1)																																	
GSuHo_Cer (440:1-OH)	1.00	9.9E-01	0.25	3.5E-01	1.16	4.9E-02	0.27	3.1E-01																									
GSuHo_Cer (441:1)																																	
GSuHo_Cer (442:1-OH)	1.03	7.7E-01	0.24	3.8E-01	1.12	8.3E-02	0.17	5.3E-01																									
GSuHo_Cer (443:1)	1.04	7.0E-01	0.23	4.0E-01	1.22	7.5E-03	0.41	1.2E-01																									
GSuHo_Cer (442:2-OH)	1.04	6.1E-01	0.32	2.3E-01	1.14	5.4E-02	0.35	1.8E-01																									
GSuHo_Cer (442:2)	1.03	7.7E-01	0.16	5.4E-01	1.14	9.7E-03	0.43	9.8E-02																									
PC (16:0)	0.82	5.2E-01	-0.08	7.6E-01	0.97	9.2E-01	-0.09	7.3E-01			1.87	2.4E-01	0.10	7.3E-01	1.17	2.1E-01	0.34	2.1E-01	1.00	9.9E-01	-0.26	3.4E-01											
PC (16:1)	0.74	5.0E-01	-0.15	5.9E-01	0.79	5.2E-01	-0.24	3.7E-01																									
PC (17:0)	0.77	4.0E-01	-0.19	4.7E-01	0.83	6.4E-01	-0.22	4.1E-01			1.99	2.3E-01	0.10	7.1E-01	1.16	2.4E-01	0.26	3.5E-01	0.95	5.6E-01	-0.37	1.8E-01											
PC (18:0)	0.71	2.7E-01	-0.22	4.1E-01	0.83	6.9E-01	-0.19	4.8E-01			1.97	1.6E-01	0.16	5.6E-01	1.18	2.3E-01	0.39	1.5E-01	1.00	9.7E-01	-0.24	1.4E-01											
PC (18:1)	0.72	3.2E-01	-0.19	4.8E-01	0.88	7.4E-01	-0.16	5.5E-01			1.88	3.9E-01	0.04	8.7E-01	0.99	9.2E-01	0.05	8.6E-01	0.92	2.5E-01	-0.36	1.8E-01											
PC (18:2)											1.31	4.5E-01	0.09	7.4E-01																			
PC (19:0)											2.18	2.8E-01	0.08	7.6E-01																			
PC (19:1)																																	
PC (20:0)	0.73	3.2E-01	-0.21	4.3E-01	0.89	7.6E-01	-0.20	4.6E-01			1.97	4.1E-01	0.05	8.6E-01	0.89	2.0E-01	-0.27	3.3E-01	0.86	2.4E-01	-0.42	1.2E-01											
PC (20:1)																																	
PC (20:3)																																	
PC (20:4)																																	
PC (22:0)	0.71	3.7E-01	-0.18	5.1E-01	0.78	4.4E-01	-0.23	4.0E-01			1.76	4.1E-01	0.03	9.1E-01																			
PC (22:1)	0.77	4.1E-01	-0.16	5.6E-01	0.94	8.9E-01	-0.18	4.9E-01	0.91	3.1E-01	-0.52	4.1E-02																					
PC (22:4)																																	
PC (22:6)	0.69	2.7E-01	-0.23	4.0E-01	0.94	8.4E-01	-0.11	6.9E-01																									
PC (26:0)	0.64	2.4E-01	-0.23	3.9E-01	0.88	7.7E-01	-0.15	5.9E-01			1.63	3.5E-01	0.10	7.0E-01	0.81	1.2E-01	-0.60	1.8E-02	0.80	1.0E-01	-0.40	1.4E-01											
PC (26:1)	0.89	4.3E-01	-0.49	5.4E-02																													
PC (26:1)											0.92	2.2E-01	-0.55	2.9E-02																			
PC (26:1)																																	
PC (18:1)																																	
PC (18:2)																																	
PC (20:4)																																	
PC (22:6)																																	
PC (25:0)	0.78	4.3E-01	-0.12	6.7E-01	0.89	7.9E-01	-0.15	5.8E-01	0.90	5.2E-01	-0.17	5.3E-01																					
PC (25:0)	0.73	3.0E-01	-0.19	4.9E-01	0.88	7.9E-01	-0.15	5.8E-01																									
PC (18:2)																																	
PC (20:4)	0.59	1.3E-01	-0.34	2.0E-01	0.79	5.8E-01	-0.21	4.4E-01	0.68	5.8E-02	-0.59	1.6E-02																					
PC (28:1)																																	
PC (28:2)																																	
PC (28:3)																																	
PC (28:4)																																	
PC (29:0)	1.00	9.9E-01	0.17	5.3E-01	1.17	1.3E-02																											
PC (29:0)	0.96	6.1E-01	-0.04	8.9E-01	1.18	2.3E-02																											
PC (30:0)	0.95	3.2E-01	-0.21	4.3E-01	1.04	2.6E-01	0.42	1.0E-01	0.93	4.0E-01	-0.35	1.9E-01																					
PC (30:1)	0.92	3.3E-01	-0.06	8.4E-01	1.11	1.2E-01	0.36	1.6E-01	0.78	1.7E-01	-0.41	1.1E-01	0.99	9.6E-01	-0.07	8.1E-01																	
PC (30:2)																																	
PC (31:0)	0.95	4.1E-01	-0.23	3.8E-01	1.01	7.8E-01	-0.02	9.5E-01	0.95	6.4E-01	-0.33	2.2E-01	1.07	4.4E-01	0.06	8.1E-01	0.99	9.3E-01	-0.08	7.7E-01	1.05	6.1E-01	-0.16	5.6E-01	0.78	2.2E-01	-0.43	1.1E-01					
PC (32:0)	0.93	2.6E-01	-0.47	6.8E-02	0.94	5.0E-02	-0.26	3.4E-01	1.00	9.9E-01	-0.14	6.0E-01	1.06	4.4E-01	0.25	3.6E-01	0.98	7.7E-01	1.03	6.8E-01	-0.14	6.3E-01	1.04	5.7E-01	1.01	4.7E-01	-0.43	1.0E-01	1.02	9.9E-01	0.22	4.3E-01	
PC (32:1)																																	
PC (32:2)	0.93	3.9E-01	0.00	9.9E-01	1.11	9.7E-02	0.35	1.8E-01	0.82	2.8E-01	-0.33	2.2E-01	1.02	7.7E-01	0.00	9.9E-01	1.00	1.0E-00	-0.02	9.3E-01	1.05	5.4E-01	-0.17	5.4E-01	0.80	3.6E-01	-0.33	2.3E-01	0.92	9.8E-01	0.22	4.3E-01	
PC (33:0)	0.93	2.2E-01	-0.30	2.5E-01	1.01	5.7E-01	0.15	5.7E-01	0.98	8.9E-01	-0.27	3.1E-01	1.17	3.1E-01	0.22	4.7E-01																	
PC (33:1)	0.94	2.9E-01	-0.18	5.0E-01	1.02</																												

Compound	Brain (ipsilateral) -2 hours				Brain (contralateral) -2 hours				Plasma -2 hours				Liver -2 hours				Brain (ipsilateral) -24 hours				Brain (contralateral) -24 hours				Plasma -24 hours				Liver -24 hours										
	FC	p-value	r	infect	FC	p-value	r	infect	FC	p-value	r	infect	FC	p-value	r	infect	FC	p-value	r	infect	FC	p-value	r	infect	FC	p-value	r	infect	FC	p-value	r	infect							
0.92	3.9E-02	-0.47	0.92	0.32	0.97	0.32	0.18	0.12	1.8E-01	0.90	1.8E-01	-0.56	2.4E-02	1.00	9.7E-01	0.02	9.4E-01	0.97	6.8E-01	0.02	9.3E-01	1.02	7.9E-01	1.02	7.9E-01	-0.23	4.1E-01	1.16	2.2E-01	0.94	3.8E-02	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.96	4.2E-01	-0.22	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.91	3.4E-01	-0.30	0.91	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.93	5.4E-01	0.04	0.93	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01	0.35	2.0E-01	1.03	4.8E-01	0.12	6.6E-01
0.94	4.7E-01	-0.08	0.94	0.38	1.1E-01	0.13	0.25	0.15	7.1E-02	0.90	7.1E-02	-0.50	1.5E-02	0.89	1.7E-01	-0.38	1.5E-01	0.96	1.5E-01	0.87	1.5E-01	0.96	6.5E-01	0.96	6.5E-01	-0.40	1.4E-01	1.03	4.8E-01	0.12	6.6E-01	1.09	2.9E-01						

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[illegible]

Compound	Brain (ipsilateral) - 2 hours				Brain (contralateral) - 2 hours				Plasma - 2 hours				Liver - 2 hours				Brain (ipsilateral) - 24 hours				Brain (contralateral) - 24 hours				Plasma - 24 hours				Liver - 24 hours			
	FC	p-value	r	FC	p-value	r	FC	p-value	r	FC	p-value	r	FC	p-value	r	FC	p-value	r	FC	p-value	r	FC	p-value	r	FC	p-value	r	FC	p-value	r		
	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect	big/small	corel.	infect		
Alanylproline	0.99	9.0E-01	0.11	6.9E-01	1.10	4.2E-01	0.14	6.0E-01	0.95	5.6E-01	-0.27	3.0E-01	0.78	8.8E-02	-0.38	1.9E-01	0.93	5.4E-01	-0.06	8.3E-01	1.03	7.9E-01	-0.08	7.9E-01	1.04	8.5E-01	-0.09	7.4E-01	1.20	1.2E-01	0.08	7.8E-01
Alanine	1.26	5.5E-02	0.38	1.4E-01	1.04	6.8E-01	0.15	5.8E-01	0.95	5.6E-01	-0.27	3.0E-01	0.78	8.8E-02	-0.38	1.9E-01	0.93	5.4E-01	-0.06	8.3E-01	1.03	7.9E-01	-0.08	7.9E-01	1.04	8.5E-01	-0.09	7.4E-01	1.20	1.2E-01	0.08	7.8E-01
Alanylglycine	1.58	2.1E-02	0.53	3.6E-02	1.15	7.2E-02	0.32	2.3E-01	0.88	6.5E-01	-0.39	1.4E-01	0.88	2.7E-01	-0.39	1.4E-01	0.88	3.7E-01	-0.14	6.1E-01	0.95	3.9E-01	-0.18	5.2E-01	0.86	4.5E-01	-0.26	3.4E-01	0.86	2.5E-01	-0.33	2.4E-01
Alloisoleucine	1.25	2.1E-02	0.53	3.5E-02	1.32	1.3E-03	0.68	3.3E-03	0.99	9.6E-01	-0.03	9.0E-01	0.89	6.7E-01	-0.10	7.1E-01	1.03	8.9E-01	0.12	6.8E-01	0.96	8.9E-01	-0.16	5.6E-01	0.86	3.1E-01	-0.50	5.6E-02	0.89	8.0E-01	-0.16	5.7E-01
Alloisoleucine	0.92	2.7E-01	-0.39	1.4E-01	0.87	3.5E-02	-0.46	7.3E-02	0.76	3.2E-02	-0.59	1.7E-02	0.90	2.9E-01	-0.10	7.0E-01	0.95	6.0E-01	-0.25	3.7E-01	1.08	4.2E-01	0.01	9.8E-01	0.86	3.4E-01	-0.51	7.1E-02	0.92	3.8E-01	-0.16	6.0E-01
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
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Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
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Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
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Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
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Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																								
Alloisoleucine	0.94	6.2E-01	-0.17	5.3E-01	0.79	5.7E-02	-0.57	2.0E-02																</								

[illegible]

Continued from Table S6.9 All compounds analyzed in chapter 4.3.2.

Compound	Brain (ipsilateral) - 2 hours				Brain (contralateral) - 2 hours				Plasma - 2 hours				Liver - 2 hours				Brain (ipsilateral) - 24 hours				Brain (contralateral) - 24 hours				Plasma - 24 hours				Liver - 24 hours			
	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r	FC big/small	p-value	r		
Pantothenic acid	0.93	3.4E-01	-0.30	2.6E-01	0.93	3.4E-01	-0.31	2.4E-01	0.48	2.1E-01	-0.30	2.6E-01	0.87	1.8E-01	-0.32	2.3E-01	0.81	2.9E-01	-0.32	2.5E-01	0.92	4.2E-01	-0.36	1.8E-01	0.76	1.9E-01	-0.39	1.5E-01	0.85	3.6E-01	-0.44	1.0E-01
Phenylalanine	1.05	6.1E-01	0.08	7.8E-01	0.93	3.0E-01	-0.32	2.3E-01	1.11	6.3E-01	0.16	5.5E-01	0.67	1.1E-01	-0.39	1.3E-01																
Phosphatidylcholine (34:3)																																
Phosphoenolpyruvic acid	1.16	4.4E-01	0.16	5.6E-01	0.77	6.8E-02	-0.28	2.9E-01																								
Phosphocholine	1.01	9.5E-01	0.11	7.0E-01	1.08	4.3E-01	0.34	1.9E-01																								
Phosphocholine-amine	0.96	6.1E-01	-0.38	4.5E-01	1.06	4.0E-01	0.25	3.5E-01																								
Pyrodoate	1.36	3.3E-01	0.14	6.0E-01	1.04	7.6E-01	0.04	8.8E-01																								
Proline	1.12	2.1E-01	0.29	2.8E-01	1.03	6.1E-01	0.18	5.1E-01	0.81	4.3E-02	-0.55	2.8E-02	0.85	1.1E-01	-0.37	1.0E-01	1.00	9.9E-01	0.15	6.0E-01	1.12	1.9E-01	0.02	9.5E-01								
Propionylcarnitine	0.98	7.9E-01	0.02	9.5E-01	0.97	6.8E-01	-0.09	7.4E-01	0.35	5.4E-02	-0.62	1.1E-02	1.34	3.9E-01	0.30	2.5E-01	0.97	8.3E-01	-0.25	3.7E-01	0.95	7.7E-01	-0.13	6.4E-01	0.78	4.5E-01	-0.31	2.6E-01	0.72	6.0E-01		
Pseudouridine	1.26	4.8E-02	0.43	1.0E-01	1.09	2.0E-01	0.09	7.3E-01	1.01	6.2E-01	0.05	8.5E-01					0.88	6.1E-01	-0.15	6.0E-01	0.88	5.2E-01	-0.28	3.0E-01								
Pyridoxal																		0.93	5.7E-01	-0.16	5.8E-01	0.93	4.1E-01	-0.39	1.5E-01							
Pyridoxamine																																
Pyruvate																																
Riboflavin	1.51	3.6E-03	0.75	8.6E-04	1.16	2.6E-01	0.15	5.8E-01																								
Ribosephosphate	0.99	9.6E-01	-0.11	6.9E-01	0.97	6.4E-01	-0.10	7.0E-01																								
Saccharopane																																
S-Adenosyl-homocysteine	0.99	8.8E-01	-0.18	4.9E-01	0.99	9.3E-01	0.03	9.2E-01																								
Sedohexulose-phosphate																																
Serine	1.01	9.1E-01	-0.06	8.1E-01	0.99	7.6E-01	0.09	7.3E-01	0.93	4.4E-01	-0.18	5.1E-01	0.96	7.0E-01	-0.21	4.3E-01																
Sphingomyelin (d18:1)																																
Sphingosine (d18:1)	1.03	7.3E-01	-0.05	8.5E-01	0.96	4.5E-01	-0.36	1.7E-01																								
Taurine	0.85	4.5E-02	-0.46	8.3E-02	0.95	3.4E-01	-0.21	4.3E-01	1.14	3.7E-01	0.23	4.0E-01	0.91	3.8E-01	0.02	9.5E-01	0.81	7.2E-02	-0.43	1.1E-01	0.97	7.9E-01	-0.14	6.2E-01	0.85	2.4E-01	-0.37	1.7E-01	1.13	2.3E-01		
Thiamine	1.13	2.8E-01	0.22	4.1E-01	1.27	2.1E-02	0.50	4.7E-02	1.15	6.3E-01	0.03	9.0E-01					0.99	8.8E-01	-0.11	7.1E-01	0.95	6.3E-01	-0.34	2.1E-01	1.46	2.8E-01	0.31	2.6E-01	1.11	5.4E-01		
Threonine	1.05	4.7E-01	0.08	7.8E-01	0.98	6.6E-01	-0.17	5.3E-01	0.97	6.8E-01	-0.26	3.3E-01	0.89	2.6E-01	-0.25	3.5E-01																
Thymidine																																
Thymine																																
Transaconitate																																
Transferrine	1.08	8.7E-01	0.03	9.2E-01	0.91	1.7E-01	-0.25	3.5E-01	0.75	9.3E-02	-0.54	3.2E-02	0.91	7.7E-01	0.02	9.4E-01	0.95	7.0E-01	0.03	9.2E-01	0.86	2.1E-01	-0.35	2.1E-01	1.04	8.5E-01	0.11	7.0E-01				
Trigonelline																																
Tryptophan	1.04	6.9E-01	0.16	5.6E-01	0.94	2.6E-01	-0.18	5.0E-01	0.99	9.4E-01	-0.06	8.2E-01	0.85	3.0E-02	-0.63	9.7E-03	1.10	6.4E-01	0.20	4.8E-01	1.04	7.6E-01	-0.02	9.4E-01	1.48	3.0E-02	0.62	1.4E-02	0.98	9.0E-01		
Tyrosine	1.15	1.8E-01	0.39	1.4E-01	0.97	5.4E-01	-0.03	9.3E-01	0.93	3.8E-01	-0.28	2.9E-01	0.99	9.5E-01	-0.07	8.0E-01	0.88	9.3E-01	0.12	6.6E-01	1.03	8.5E-01	0.05	8.6E-01	1.51	3.1E-02	0.44	1.0E-01	1.01	9.6E-01		
Uracil																																
Urate	1.63	4.4E-04	0.71	2.2E-03	1.08	3.0E-01	0.21	4.4E-01									1.31	2.4E-01	0.36	2.3E-01	1.08	7.9E-01	-0.18	5.3E-01	1.52	3.1E-02	0.55	3.4E-02				
Urea	1.30	1.2E-01	0.25	3.5E-01	1.31	2.3E-02	0.47	6.5E-02	1.06	5.5E-01	0.09	7.4E-01	0.63	1.6E-02	-0.57	2.2E-02	0.71	3.1E-01	-0.21	4.5E-01	0.73	3.0E-01	-0.31	2.7E-01	1.02	9.0E-01	-0.22	4.2E-01	0.90	7.0E-01		
Uridine 5'-acetylglucosamine	1.06	4.4E-01	0.27	3.1E-01	1.02	5.2E-01	0.11	6.7E-01	0.68	1.4E-01	-0.54	3.1E-02	0.76	6.6E-02	-0.37	1.5E-01	0.91	3.8E-01	-0.34	2.1E-01	0.99	9.4E-01	-0.18	5.3E-01								
Uridine 5'-phosphoglucose	0.87	1.5E-01	-0.17	5.2E-01	1.08	3.0E-01	0.26	3.4E-01																								
Uridine 5'-Diphospho-N-acetylglucosamine	0.97	7.0E-01	0.15	5.9E-01	1.06	3.2E-01	0.18	5.1E-01																								
Uridine 5'-di-phospho-N-acetylglucosamine																																
Uridine-5-Monophosphate																																
Valine	1.06	5.8E-01	0.04	8.7E-01	0.90	2.0E-01	-0.17	5.3E-01	0.87	5.6E-02	-0.46	7.6E-02	1.04	6.5E-01	0.04	8.9E-01	0.95	7.5E-01	-0.15	5.9E-01	0.89	3.5E-01	-0.45	9.4E-02	1.15	3.7E-01	-0.05	8.6E-01	0.90	5.5E-01		
Valine/Betaine																																
Xanthine	1.10	1.8E-01	0.46	7.2E-02	1.08	3.0E-01	0.30	2.6E-01																								
Xanthosine																																

6.3 MS² and LC library

The MS² and LC library are available online: <https://data.mendeley.com/datasets/9m89d3wjj5/1>

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Literature

- [1] Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials; Board on Health Care Services; Board on Health Sciences Policy; Institute of Medicine. C.M. Micheel, S.J. Nass, G.S. Omenn (Eds.) *Evolution of Translational Omics: Lessons Learned and the Path Forward*, Washington (DC), 2012,10.17226/13297.
- [2] F. Matsuda, Technical Challenges in Mass Spectrometry-Based Metabolomics, *Mass Spectrom (Tokyo)*, 5 (2016) S0052,10.5702/massspectrometry.S0052.
- [3] E. Riekeberg, R. Powers, New frontiers in metabolomics: from measurement to insight, *F1000Research*, 6 (2017) 1148,10.12688/f1000research.11495.1.
- [4] M.A. Alves, S. Lamichhane, A. Dickens, A. McGlinchey, H.C. Ribeiro, P. Sen, F. Wei, T. Hyotylainen, M. Oresic, Systems biology approaches to study lipidomes in health and disease, *Biochimica et biophysica acta. Molecular and cell biology of lipids*, 1866 (2021) 158857,10.1016/j.bbalip.2020.158857.
- [5] M. Kanehisa, S. Goto, M. Furumichi, M. Tanabe, M. Hirakawa, KEGG for representation and analysis of molecular networks involving diseases and drugs, *Nucleic acids research*, 38 (2010) D355-360,10.1093/nar/gkp896.
- [6] E. Fahy, S. Subramaniam, R.C. Murphy, M. Nishijima, C.R. Raetz, T. Shimizu, F. Spener, G. van Meer, M.J. Wakelam, E.A. Dennis, Update of the LIPID MAPS comprehensive classification system for lipids, *Journal of lipid research*, 50 Suppl (2009) S9-14,10.1194/jlr.R800095-JLR200.
- [7] A.C. Schrimpe-Rutledge, S.G. Codreanu, S.D. Sherrod, J.A. McLean, Untargeted Metabolomics Strategies-Challenges and Emerging Directions, *Journal of the American Society for Mass Spectrometry*, 27 (2016) 1897-1905,https://doi.org/10.1007/s13361-016-1469-y.
- [8] D.S. Wishart, Y.D. Feunang, A. Marcu, A.C. Guo, K. Liang, R. Vazquez-Fresno, T. Sajed, D. Johnson, C. Li, N. Karu, Z. Sayeeda, E. Lo, N. Assempour, M. Berjanskii, S. Singhal, D. Arndt, Y. Liang, H. Badran, J. Grant, A. Serra-Cayuela, Y. Liu, R. Mandal, V. Neveu, A. Pon, C. Knox, M. Wilson, C. Manach, A. Scalbert, HMDB 4.0: the human metabolome database for 2018, *Nucleic acids research*, 46 (2018) D608-D617,10.1093/nar/gkx1089.
- [9] T. Cajka, O. Fiehn, Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics, *Anal Chem*, 88 (2016) 524-545,https://doi.org/10.1021/acs.analchem.5b04491.
- [10] A. Ribbenstedt, H. Ziarrusta, J.P. Benskin, Development, characterization and comparisons of targeted and non-targeted metabolomics methods, *PloS one*, 13 (2018) e0207082,https://doi.org/10.1371/journal.pone.0207082.
- [11] O. Yanes, R. Tautenhahn, G.J. Patti, G. Siuzdak, Expanding coverage of the metabolome for global metabolite profiling, *Anal Chem*, 83 (2011) 2152-2161,https://doi.org/10.1021/ac102981k.

- [12] J. Draper, A.J. Lloyd, R. Goodacre, M. Beckmann, Flow infusion electrospray ionisation mass spectrometry for high throughput, non-targeted metabolite fingerprinting: a review, *Metabolomics : Official journal of the Metabolomic Society*, 9 (2013) S4-S29,10.1007/s11306-012-0449-x.
- [13] G.A. Gowda, D. Djukovic, Overview of mass spectrometry-based metabolomics: opportunities and challenges, *Methods Mol Biol*, 1198 (2014) 3-12,10.1007/978-1-4939-1258-2_1.
- [14] L. Konermann, E. Ahadi, A.D. Rodriguez, S. Vahidi, Unraveling the mechanism of electrospray ionization, *Anal Chem*, 85 (2013) 2-9,10.1021/ac302789c.
- [15] S. Banerjee, S. Mazumdar, Electrospray ionization mass spectrometry: a technique to access the information beyond the molecular weight of the analyte, *International journal of analytical chemistry*, 2012 (2012) 282574,10.1155/2012/282574.
- [16] M.R. Morad, A. Rajabi, M. Razavi, S.R. Sereshkeh, A Very Stable High Throughput Taylor Cone-jet in Electrohydrodynamics, *Scientific reports*, 6 (2016) 38509,10.1038/srep38509.
- [17] M. Stuber, T. Reemtsma, Evaluation of three calibration methods to compensate matrix effects in environmental analysis with LC-ESI-MS, *Analytical and bioanalytical chemistry*, 378 (2004) 910-916,10.1007/s00216-003-2442-8.
- [18] K.K. Murray, R.K. Boyd, M.N. Eberlin, G.J. Langley, L. Li, Y. Naito, Definitions of terms relating to mass spectrometry (IUPAC Recommendations 2013), *Pure Appl Chem*, 85 (2013) 1515-1609,10.1351/Pac-Rec-06-04-06.
- [19] A. Michalski, E. Damoc, J.P. Hauschild, O. Lange, A. Wiegand, A. Makarov, N. Nagaraj, J. Cox, M. Mann, S. Horning, Mass spectrometry-based proteomics using Q Exactive, a high-performance benchtop quadrupole Orbitrap mass spectrometer, *Molecular & cellular proteomics : MCP*, 10 (2011) M111 011015,10.1074/mcp.M111.011015.
- [20] A. Makarov, E. Denisov, A. Kholomeev, W. Balschun, O. Lange, K. Strupat, S. Horning, Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer, *Anal Chem*, 78 (2006) 2113-2120,10.1021/ac0518811.
- [21] M. Hardman, A.A. Makarov, Interfacing the orbitrap mass analyzer to an electrospray ion source, *Anal Chem*, 75 (2003) 1699-1705,10.1021/ac0258047.
- [22] A.R. Johnson, E.E. Carlson, Collision-Induced Dissociation Mass Spectrometry: A Powerful Tool for Natural Product Structure Elucidation, *Anal Chem*, 87 (2015) 10668-10678,10.1021/acs.analchem.5b01543.
- [23] J.C. Heaton, X. Wang, W.E. Barber, S.M. Buckenmaier, D.V. McCalley, Practical observations on the performance of bare silica in hydrophilic interaction compared with C18 reversed-phase liquid chromatography, *Journal of chromatography. A*, 1328 (2014) 7-15,https://doi.org/10.1016/j.chroma.2013.12.058.

- [24] T. Cajka, O. Fiehn, Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry, *Trends in analytical chemistry : TRAC*, 61 (2014) 192-206,10.1016/j.trac.2014.04.017.
- [25] K. Contrepois, L. Jiang, M. Snyder, Optimized Analytical Procedures for the Untargeted Metabolomic Profiling of Human Urine and Plasma by Combining Hydrophilic Interaction (HILIC) and Reverse-Phase Liquid Chromatography (RPLC)-Mass Spectrometry, *Molecular & cellular proteomics : MCP*, 14 (2015) 1684-1695,https://doi.org/10.1074/mcp.M114.046508.
- [26] H.G. Gika, G.A. Theodoridis, R.S. Plumb, I.D. Wilson, Current practice of liquid chromatography-mass spectrometry in metabolomics and metabonomics, *Journal of pharmaceutical and biomedical analysis*, 87 (2014) 12-25,https://doi.org/10.1016/j.jpba.2013.06.032.
- [27] T. Zullig, H.C. Kofeler, High Resolution Mass Spectrometry in Lipidomics, *Mass spectrometry reviews*, 40 (2021) 162-176,10.1002/mas.21627.
- [28] J.R. Bonney, B.M. Prentice, Perspective on Emerging Mass Spectrometry Technologies for Comprehensive Lipid Structural Elucidation, *Anal Chem*, 93 (2021) 6311-6322,10.1021/acs.analchem.1c00061.
- [29] E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H.P. Singer, J. Hollender, Identifying small molecules via high resolution mass spectrometry: communicating confidence, *Environmental science & technology*, 48 (2014) 2097-2098,10.1021/es5002105.
- [30] X. Domingo-Almenara, J.R. Montenegro-Burke, H.P. Benton, G. Siuzdak, Annotation: A Computational Solution for Streamlining Metabolomics Analysis, *Anal Chem*, 90 (2018) 480-489,10.1021/acs.analchem.7b03929.
- [31] C. Guijas, J.R. Montenegro-Burke, X. Domingo-Almenara, A. Palermo, B. Warth, G. Hermann, G. Koellensperger, T. Huan, W. Uritboonthai, A.E. Aisporna, D.W. Wolan, M.E. Spilker, H.P. Benton, G. Siuzdak, METLIN: A Technology Platform for Identifying Knowns and Unknowns, *Anal Chem*, 90 (2018) 3156-3164,10.1021/acs.analchem.7b04424.
- [32] B. Zhou, J.F. Xiao, L. Tuli, H.W. Ransom, LC-MS-based metabolomics, *Molecular bioSystems*, 8 (2012) 470-481,https://doi.org/10.1039/c1mb05350g.
- [33] B.L. Milman, Towards a full reference library of MSⁿ spectra. Testing of a library containing 3126 MS² spectra of 1743 compounds, *Rapid Commun Mass Sp*, 19 (2005) 2833-2839,https://doi.org/10.1002/rcm.2131.
- [34] F.W. McLafferty, D.A. Stauffer, S.Y. Loh, C. Wesdemiotis, Unknown identification using reference mass spectra. Quality evaluation of databases, *Journal of the American Society for Mass Spectrometry*, 10 (1999) 1229-1240,https://doi.org/10.1016/S1044-0305(99)00104-X.
- [35] A.W. Bristow, K.S. Webb, A.T. Lubben, J. Halket, Reproducible product-ion tandem mass spectra on various liquid chromatography/mass spectrometry instruments for the development of

spectral libraries, *Rapid communications in mass spectrometry* : RCM, 18 (2004) 1447-1454,10.1002/rcm.1492.

[36] R. Jansen, G. Lachatre, P. Marquet, LC-MS/MS systematic toxicological analysis: comparison of MS/MS spectra obtained with different instruments and settings, *Clinical biochemistry*, 38 (2005) 362-372,10.1016/j.clinbiochem.2004.11.003.

[37] S.S. Virani, A. Alonso, E.J. Benjamin, M.S. Bittencourt, C.W. Callaway, A.P. Carson, A.M. Chamberlain, A.R. Chang, S. Cheng, F.N. Delling, L. Djousse, M.S.V. Elkind, J.F. Ferguson, M. Fornage, S.S. Khan, B.M. Kissela, K.L. Knutson, T.W. Kwan, D.T. Lackland, T.T. Lewis, J.H. Lichtman, C.T. Longenecker, M.S. Loop, P.L. Lutsey, S.S. Martin, K. Matsushita, A.E. Moran, M.E. Mussolino, A.M. Perak, W.D. Rosamond, G.A. Roth, U.K.A. Sampson, G.M. Satou, E.B. Schroeder, S.H. Shah, C.M. Shay, N.L. Spartano, A. Stokes, D.L. Tirschwell, L.B. VanWagner, C.W. Tsao, Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association, *Circulation*, 141 (2020) e139-e596,10.1161/CIR.0000000000000757.

[38] R.L. Sacco, S.E. Kasner, J.P. Broderick, L.R. Caplan, J.J. Connors, A. Culebras, M.S. Elkind, M.G. George, A.D. Hamdan, R.T. Higashida, B.L. Hoh, L.S. Janis, C.S. Kase, D.O. Kleindorfer, J.M. Lee, M.E. Moseley, E.D. Peterson, T.N. Turan, A.L. Valderrama, H.V. Vinters, An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association, *Stroke*, 44 (2013) 2064-2089,10.1161/STR.0b013e318296aeca.

[39] R.A. Grysiewicz, K. Thomas, D.K. Pandey, Epidemiology of ischemic and hemorrhagic stroke: incidence, prevalence, mortality, and risk factors, *Neurologic clinics*, 26 (2008) 871-895, vii,10.1016/j.ncl.2008.07.003.

[40] N.P. Torres-Aguila, C. Carrera, E. Muino, N. Cullell, J. Carcel-Marquez, C. Gallego-Fabrega, J. Gonzalez-Sanchez, A. Bustamante, P. Delgado, L. Ibanez, L. Heitsch, J. Krupinski, J. Montaner, J. Marti-Fabregas, C. Cruchaga, J.M. Lee, I. Fernandez-Cadenas, Clinical Variables and Genetic Risk Factors Associated with the Acute Outcome of Ischemic Stroke: A Systematic Review, *Journal of stroke*, 21 (2019) 276-289,10.5853/jos.2019.01522.

[41] H.P. Adams, Jr., P.H. Davis, E.C. Leira, K.C. Chang, B.H. Bendixen, W.R. Clarke, R.F. Woolson, M.D. Hansen, Baseline NIH Stroke Scale score strongly predicts outcome after stroke: A report of the Trial of Org 10172 in Acute Stroke Treatment (TOAST), *Neurology*, 53 (1999) 126-131,10.1212/wnl.53.1.126.

[42] G. Saposnik, M.D. Hill, M. O'Donnell, J. Fang, V. Hachinski, M.K. Kapral, Variables associated with 7-day, 30-day, and 1-year fatality after ischemic stroke, *Stroke*, 39 (2008) 2318-2324,10.1161/STROKEAHA.107.510362.

[43] T. Brott, H.P. Adams, Jr., C.P. Olinger, J.R. Marler, W.G. Barsan, J. Biller, J. Spilker, R. Holleran, R. Eberle, V. Hertzberg, et al., Measurements of acute cerebral infarction: a clinical examination scale, *Stroke*, 20 (1989) 864-870,10.1161/01.str.20.7.864.

- [44] J. Rankin, Cerebral vascular accidents in patients over the age of 60. II. Prognosis, *Scottish medical journal*, 2 (1957) 200-215,10.1177/003693305700200504.
- [45] B. Farrell, J. Godwin, S. Richards, C. Warlow, The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results, *Journal of neurology, neurosurgery, and psychiatry*, 54 (1991) 1044-1054,10.1136/jnnp.54.12.1044.
- [46] J.P. Broderick, O. Adeoye, J. Elm, Evolution of the Modified Rankin Scale and Its Use in Future Stroke Trials, *Stroke*, 48 (2017) 2007-2012,10.1161/STROKEAHA.117.017866.
- [47] J.L. Saver, CLINICAL PRACTICE. Cryptogenic Stroke, *The New England journal of medicine*, 374 (2016) 2065-2074,10.1056/NEJMcp1503946.
- [48] T. Peisker, B. Koznar, I. Stetkarova, P. Widimsky, Acute stroke therapy: A review, *Trends in cardiovascular medicine*, 27 (2017) 59-66,10.1016/j.tcm.2016.06.009.
- [49] S. Bansal, K.S. Sangha, P. Khatri, Drug treatment of acute ischemic stroke, *American journal of cardiovascular drugs : drugs, devices, and other interventions*, 13 (2013) 57-69,10.1007/s40256-013-0007-6.
- [50] W. Hacke, M. Kaste, E. Bluhmki, M. Brozman, A. Davalos, D. Guidetti, V. Larrue, K.R. Lees, Z. Medeghri, T. Machnig, D. Schneider, R. von Kummer, N. Wahlgren, D. Toni, Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke, *The New England journal of medicine*, 359 (2008) 1317-1329,10.1056/NEJMoa0804656.
- [51] R.G. Nogueira, A.P. Jadhav, D.C. Haussen, A. Bonafe, R.F. Budzik, P. Bhuva, D.R. Yavagal, M. Ribo, C. Cognard, R.A. Hanel, C.A. Sila, A.E. Hassan, M. Millan, E.I. Levy, P. Mitchell, M. Chen, J.D. English, Q.A. Shah, F.L. Silver, V.M. Pereira, B.P. Mehta, B.W. Baxter, M.G. Abraham, P. Cardona, E. Veznedaroglu, F.R. Hellinger, L. Feng, J.F. Kirmani, D.K. Lopes, B.T. Jankowitz, M.R. Frankel, V. Costalat, N.A. Vora, A.J. Yoo, A.M. Malik, A.J. Furlan, M. Rubiera, A. Aghaebrahim, J.M. Olivot, W.G. Tekle, R. Shields, T. Graves, R.J. Lewis, W.S. Smith, D.S. Liebeskind, J.L. Saver, T.G. Jovin, Thrombectomy 6 to 24 Hours after Stroke with a Mismatch between Deficit and Infarct, *The New England journal of medicine*, 378 (2018) 11-21,10.1056/NEJMoa1706442.
- [52] G.W. Albers, M.P. Marks, S. Kemp, S. Christensen, J.P. Tsai, S. Ortega-Gutierrez, R.A. McTaggart, M.T. Torbey, M. Kim-Tenser, T. Leslie-Mazwi, A. Sarraj, S.E. Kasner, S.A. Ansari, S.D. Yeatts, S. Hamilton, M. Mlynash, J.J. Heit, G. Zaharchuk, S. Kim, J. Carrozzella, Y.Y. Palesch, A.M. Demchuk, R. Bammer, P.W. Lavori, J.P. Broderick, M.G. Lansberg, Thrombectomy for Stroke at 6 to 16 Hours with Selection by Perfusion Imaging, *The New England journal of medicine*, 378 (2018) 708-718,10.1056/NEJMoa1713973.
- [53] T. Brott, J. Bogousslavsky, Treatment of acute ischemic stroke, *The New England journal of medicine*, 343 (2000) 710-722,10.1056/NEJM200009073431007.
- [54] D. Belov Kirdajova, J. Kriska, J. Tureckova, M. Anderova, Ischemia-Triggered Glutamate Excitotoxicity From the Perspective of Glial Cells, *Frontiers in cellular neuroscience*, 14 (2020) 51,10.3389/fncel.2020.00051.

- [55] N.J. Abbott, L. Ronnback, E. Hansson, Astrocyte-endothelial interactions at the blood-brain barrier, *Nature reviews. Neuroscience*, 7 (2006) 41-53,10.1038/nrn1824.
- [56] Y. Yang, G.A. Rosenberg, Blood-brain barrier breakdown in acute and chronic cerebrovascular disease, *Stroke*, 42 (2011) 3323-3328,10.1161/STROKEAHA.110.608257.
- [57] R.L. Jayaraj, S. Azimullah, R. Beiram, F.Y. Jalal, G.A. Rosenberg, Neuroinflammation: friend and foe for ischemic stroke, *Journal of neuroinflammation*, 16 (2019) 142,10.1186/s12974-019-1516-2.
- [58] M. Gelderblom, F. Leypoldt, K. Steinbach, D. Behrens, C.U. Choe, D.A. Siler, T.V. Arumugam, E. Orthey, C. Gerloff, E. Tolosa, T. Magnus, Temporal and spatial dynamics of cerebral immune cell accumulation in stroke, *Stroke*, 40 (2009) 1849-1857,10.1161/STROKEAHA.108.534503.
- [59] C. Iadecola, J. Anrather, The immunology of stroke: from mechanisms to translation, *Nature medicine*, 17 (2011) 796-808,10.1038/nm.2399.
- [60] F. Liu, L.D. McCullough, Middle cerebral artery occlusion model in rodents: methods and potential pitfalls, *Journal of biomedicine & biotechnology*, 2011 (2011) 464701,10.1155/2011/464701.
- [61] A. Durukan, T. Tatlisumak, Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia, *Pharmacology, biochemistry, and behavior*, 87 (2007) 179-197,10.1016/j.pbb.2007.04.015.
- [62] A.Y. Abramov, A. Scorziello, M.R. Duchen, Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27 (2007) 1129-1138,10.1523/JNEUROSCI.4468-06.2007.
- [63] H. Chen, Y.S. Song, P.H. Chan, Inhibition of NADPH oxidase is neuroprotective after ischemia-reperfusion, *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 29 (2009) 1262-1272,10.1038/jcbfm.2009.47.
- [64] C.M. Maier, L. Hsieh, T. Crandall, P. Narasimhan, P.H. Chan, Evaluating therapeutic targets for reperfusion-related brain hemorrhage, *Annals of neurology*, 59 (2006) 929-938,10.1002/ana.20850.
- [65] G.W. Kim, T. Kondo, N. Noshita, P.H. Chan, Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals, *Stroke*, 33 (2002) 809-815,10.1161/hs0302.103745.
- [66] Y.S. Song, Y.S. Lee, P. Narasimhan, P.H. Chan, Reduced oxidative stress promotes NF-kappaB-mediated neuroprotective gene expression after transient focal cerebral ischemia: lymphocytotropic cytokines and antiapoptotic factors, *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 27 (2007) 764-775,10.1038/sj.jcbfm.9600379.

- [67] A. Saito, T. Hayashi, S. Okuno, T. Nishi, P.H. Chan, Modulation of proline-rich akt substrate survival signaling pathways by oxidative stress in mouse brains after transient focal cerebral ischemia, *Stroke*, 37 (2006) 513-517,10.1161/01.STR.0000198826.56611.a2.
- [68] C.H. Wong, S. Bozinovski, P.J. Hertzog, M.J. Hickey, P.J. Crack, Absence of glutathione peroxidase-1 exacerbates cerebral ischemia-reperfusion injury by reducing post-ischemic microvascular perfusion, *Journal of neurochemistry*, 107 (2008) 241-252,10.1111/j.1471-4159.2008.05605.x.
- [69] B. Hoehn, M.A. Yenari, R.M. Sapolsky, G.K. Steinberg, Glutathione peroxidase overexpression inhibits cytochrome C release and proapoptotic mediators to protect neurons from experimental stroke, *Stroke*, 34 (2003) 2489-2494,10.1161/01.STR.0000091268.25816.19.
- [70] A.M. Leone, R.M. Palmer, R.G. Knowles, P.L. Francis, D.S. Ashton, S. Moncada, Constitutive and inducible nitric oxide synthases incorporate molecular oxygen into both nitric oxide and citrulline, *The Journal of biological chemistry*, 266 (1991) 23790-23795
- [71] C. Iadecola, F. Zhang, R. Casey, M. Nagayama, M.E. Ross, Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 17 (1997) 9157-9164
- [72] Z. Huang, P.L. Huang, N. Panahian, T. Dalkara, M.C. Fishman, M.A. Moskowitz, Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase, *Science*, 265 (1994) 1883-1885,10.1126/science.7522345.
- [73] Z. Huang, P.L. Huang, J. Ma, W. Meng, C. Ayata, M.C. Fishman, M.A. Moskowitz, Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine, *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 16 (1996) 981-987,10.1097/00004647-199609000-00023.
- [74] D.N. Atochin, A. Wang, V.W. Liu, J.D. Critchlow, A.P. Dantas, R. Looft-Wilson, T. Murata, S. Salomone, H.K. Shin, C. Ayata, M.A. Moskowitz, T. Michel, W.C. Sessa, P.L. Huang, The phosphorylation state of eNOS modulates vascular reactivity and outcome of cerebral ischemia in vivo, *The Journal of clinical investigation*, 117 (2007) 1961-1967,10.1172/JCI29877.
- [75] J. Castillo, R. Rama, A. Davalos, Nitric oxide-related brain damage in acute ischemic stroke, *Stroke*, 31 (2000) 852-857,10.1161/01.str.31.4.852.
- [76] Z.Q. Chen, R.T. Mou, D.X. Feng, Z. Wang, G. Chen, The role of nitric oxide in stroke, *Medical gas research*, 7 (2017) 194-203,10.4103/2045-9912.215750.
- [77] C. Dominguez, P. Delgado, A. Vilches, P. Martin-Gallan, M. Ribo, E. Santamarina, C. Molina, N. Corbeto, V. Rodriguez-Sureda, A. Rosell, J. Alvarez-Sabin, J. Montaner, Oxidative stress after thrombolysis-induced reperfusion in human stroke, *Stroke*, 41 (2010) 653-660,10.1161/STROKEAHA.109.571935.

- [78] S. Lorenzano, N.S. Rost, M. Khan, H. Li, F.O. Lima, M.B. Maas, R.E. Green, T.K. Thankachan, A.J. Dipietro, K. Arai, A.T. Som, L.D. Pham, O. Wu, G.J. Harris, E.H. Lo, J.B. Blumberg, P.E. Milbury, S.K. Feske, K.L. Furie, Oxidative Stress Biomarkers of Brain Damage: Hyperacute Plasma F2-Isoprostane Predicts Infarct Growth in Stroke, *Stroke*, 49 (2018) 630-637,10.1161/STROKEAHA.117.018440.
- [79] I. Zitnanova, P. Siarnik, B. Kollar, M. Chomova, P. Pazderova, L. Andrezalova, M. Jezovicova, K. Konarikova, L. Laubertova, Z. Krivosikova, L. Slezakova, P. Turcani, Oxidative Stress Markers and Their Dynamic Changes in Patients after Acute Ischemic Stroke, *Oxidative medicine and cellular longevity*, 2016 (2016) 9761697,10.1155/2016/9761697.
- [80] J.S. Leinonen, J.P. Ahonen, K. Lonnrot, M. Jehkonen, P. Dastidar, G. Molnar, H. Alho, Low plasma antioxidant activity is associated with high lesion volume and neurological impairment in stroke, *Stroke*, 31 (2000) 33-39,10.1161/01.str.31.1.33.
- [81] J.H. Liu, T.W. Wang, Y.Y. Lin, W.C. Ho, H.C. Tsai, S.P. Chen, A.M. Lin, T.Y. Liu, H.T. Wang, Acrolein is involved in ischemic stroke-induced neurotoxicity through spermidine/spermine-N1-acetyltransferase activation, *Experimental neurology*, 323 (2020) 113066,10.1016/j.expneurol.2019.113066.
- [82] J. Song, J. Park, Y. Oh, J.E. Lee, Glutathione suppresses cerebral infarct volume and cell death after ischemic injury: involvement of FOXO3 inactivation and Bcl2 expression, *Oxidative medicine and cellular longevity*, 2015 (2015) 426069,10.1155/2015/426069.
- [83] R. Shirley, E.N. Ord, L.M. Work, Oxidative Stress and the Use of Antioxidants in Stroke, *Antioxidants (Basel)*, 3 (2014) 472-501,10.3390/antiox3030472.
- [84] G.C. Jickling, H. Xu, B. Stamova, B.P. Ander, X. Zhan, Y. Tian, D. Liu, R.J. Turner, M. Mesias, P. Verro, J. Khoury, E.C. Jauch, A. Pancioli, J.P. Broderick, F.R. Sharp, Signatures of cardioembolic and large-vessel ischemic stroke, *Annals of neurology*, 68 (2010) 681-692,10.1002/ana.22187.
- [85] K. Fassbender, S. Rossol, T. Kammer, M. Daffertshofer, S. Wirth, M. Dollman, M. Hennerici, Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease, *Journal of the neurological sciences*, 122 (1994) 135-139,10.1016/0022-510x(94)90289-5.
- [86] C.J. Smith, H.C. Emsley, C.M. Gavin, R.F. Georgiou, A. Vail, E.M. Barberan, G.J. del Zoppo, J.M. Hallenbeck, N.J. Rothwell, S.J. Hopkins, P.J. Tyrrell, Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome, *BMC neurology*, 4 (2004) 2,10.1186/1471-2377-4-2.
- [87] B.H. Clausen, M. Wirenfeldt, S.S. Hogedal, L.H. Frich, H.H. Nielsen, H.D. Schroder, K. Ostergaard, B. Finsen, B.W. Kristensen, K.L. Lambertsen, Characterization of the TNF and IL-1 systems in human brain and blood after ischemic stroke, *Acta neuropathologica communications*, 8 (2020) 81,10.1186/s40478-020-00957-y.
- [88] H. Offner, S. Subramanian, S.M. Parker, M.E. Afentoulis, A.A. Vandenbark, P.D. Hurn, Experimental stroke induces massive, rapid activation of the peripheral immune system, *Journal of*

cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 26 (2006) 654-665,10.1038/sj.jcbfm.9600217.

[89] C.H. Wong, C.N. Jenne, W.Y. Lee, C. Leger, P. Kubes, Functional innervation of hepatic iNKT cells is immunosuppressive following stroke, *Science*, 334 (2011) 101-105,10.1126/science.1210301.

[90] Y.Y. Wang, S.Y. Lin, Y.H. Chuang, W.H. Sheu, K.C. Tung, C.J. Chen, Activation of hepatic inflammatory pathways by catecholamines is associated with hepatic insulin resistance in male ischemic stroke rats, *Endocrinology*, 155 (2014) 1235-1246,10.1210/en.2013-1593.

[91] A. Ottani, D. Giuliani, C. Mioni, M. Galantucci, L. Minutoli, A. Bitto, D. Altavilla, D. Zaffe, A.R. Botticelli, F. Squadrito, S. Guarini, Vagus nerve mediates the protective effects of melanocortins against cerebral and systemic damage after ischemic stroke, *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 29 (2009) 512-523,10.1038/jcbfm.2008.140.

[92] J. Ivanisevic, G. Siuzdak, The Role of Metabolomics in Brain Metabolism Research, *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology*, 10 (2015) 391-395,10.1007/s11481-015-9621-1.

[93] P. Liu, R. Li, A.A. Antonov, L. Wang, W. Li, Y. Hua, H. Guo, L. Chen, Y. Tian, F. Xu, Z. Zhang, Y. Zhu, Y. Huang, Discovery of Metabolite Biomarkers for Acute Ischemic Stroke Progression, *Journal of proteome research*, 16 (2017) 773-779,10.1021/acs.jproteome.6b00779.

[94] Y. Lee, A. Khan, S. Hong, S.H. Jee, Y.H. Park, A metabolomic study on high-risk stroke patients determines low levels of serum lysine metabolites: a retrospective cohort study, *Molecular bioSystems*, 13 (2017) 1109-1120,10.1039/c6mb00732e.

[95] W.T. Kimberly, Y. Wang, L. Pham, K.L. Furie, R.E. Gerszten, Metabolite profiling identifies a branched chain amino acid signature in acute cardioembolic stroke, *Stroke*, 44 (2013) 1389-1395,10.1161/STROKEAHA.111.000397.

[96] D. Wang, J. Kong, J. Wu, X. Wang, M. Lai, GC-MS-based metabolomics identifies an amino acid signature of acute ischemic stroke, *Neuroscience letters*, 642 (2017) 7-13,10.1016/j.neulet.2017.01.039.

[97] M. Liu, K. Zhou, H. Li, X. Dong, G. Tan, Y. Chai, W. Wang, X. Bi, Potential of serum metabolites for diagnosing post-stroke cognitive impairment, *Molecular bioSystems*, 11 (2015) 3287-3296,10.1039/c5mb00470e.

[98] E. Tanaka, Y. Ogawa, R. Fujii, T. Shimonaka, Y. Sato, T. Hamazaki, T. Nagamura-Inoue, H. Shintaku, M. Tsuji, Metabolomic analysis and mass spectrometry imaging after neonatal stroke and cell therapies in mouse brains, *Scientific reports*, 10 (2020) 21881,10.1038/s41598-020-78930-x.

[99] T.H. Shin, D.Y. Lee, S. Basith, B. Manavalan, M.J. Paik, I. Rybinnik, M.M. Mouradian, J.H. Ahn, G. Lee, Metabolome Changes in Cerebral Ischemia, *Cells*, 9 (2020),10.3390/cells9071630.

- [100] A. Au, Metabolomics and Lipidomics of Ischemic Stroke, *Advances in clinical chemistry*, 85 (2018) 31-69,10.1016/bs.acc.2018.02.002.
- [101] J.N. van der Veen, J.P. Kennelly, S. Wan, J.E. Vance, D.E. Vance, R.L. Jacobs, The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease, *Biochimica et biophysica acta. Biomembranes*, 1859 (2017) 1558-1572,10.1016/j.bbamem.2017.04.006.
- [102] C.J. DeLong, Y.J. Shen, M.J. Thomas, Z. Cui, Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway, *The Journal of biological chemistry*, 274 (1999) 29683-29688,10.1074/jbc.274.42.29683.
- [103] C.J. Pynn, N.G. Henderson, H. Clark, G. Koster, W. Bernhard, A.D. Postle, Specificity and rate of human and mouse liver and plasma phosphatidylcholine synthesis analyzed in vivo, *Journal of lipid research*, 52 (2011) 399-407,10.1194/jlr.D011916.
- [104] M. Uhlen, L. Fagerberg, B.M. Hallstrom, C. Lindskog, P. Oksvold, A. Mardinoglu, A. Sivertsson, C. Kampf, E. Sjostedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C.A. Szgyarto, J. Odeberg, D. Djureinovic, J.O. Takanen, S. Hober, T. Alm, P.H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J.M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, F. Ponten, Proteomics. Tissue-based map of the human proteome, *Science*, 347 (2015) 1260419,10.1126/science.1260419.
- [105] M. Uhlen, M.J. Karlsson, W. Zhong, A. Tebani, C. Pou, J. Mikes, T. Lakshmikanth, B. Forsstrom, F. Edfors, J. Odeberg, A. Mardinoglu, C. Zhang, K. von Feilitzen, J. Mulder, E. Sjostedt, A. Hober, P. Oksvold, M. Zwahlen, F. Ponten, C. Lindskog, A. Sivertsson, L. Fagerberg, P. Brodin, A genome-wide transcriptomic analysis of protein-coding genes in human blood cells, *Science*, 366 (2019),10.1126/science.aax9198.
- [106] J.J. Secades, Citicoline: pharmacological and clinical review, 2016 update, *Revista de neurologia*, 63 (2016) S1-S73
- [107] M.Y. Martynov, E.I. Gusev, Current knowledge on the neuroprotective and neuroregenerative properties of citicoline in acute ischemic stroke, *Journal of experimental pharmacology*, 7 (2015) 17-28,10.2147/JEP.S63544.
- [108] R.M. Adibhatla, J.F. Hatcher, Cytidine 5'-diphosphocholine (CDP-choline) in stroke and other CNS disorders, *Neurochemical research*, 30 (2005) 15-23,10.1007/s11064-004-9681-8.
- [109] A.J. Marti-Carvajal, C. Valli, C.E. Marti-Amarista, I. Sola, J. Marti-Fabregas, X. Bonfill Cosp, Citicoline for treating people with acute ischemic stroke, *The Cochrane database of systematic reviews*, 8 (2020) CD013066,10.1002/14651858.CD013066.pub2.
- [110] T. Hulsen, J. de Vlieg, W. Alkema, BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams, *BMC genomics*, 9 (2008) 488,https://doi.org/10.1186/1471-2164-9-488.

- [111] R. Chen, G.I. Mias, J. Li-Pook-Than, L. Jiang, H.Y. Lam, E. Miriami, K.J. Karczewski, M. Hariharan, F.E. Dewey, Y. Cheng, M.J. Clark, H. Im, L. Habegger, S. Balasubramanian, M. O'Huallachain, J.T. Dudley, S. Hillenmeyer, R. Haraksingh, D. Sharon, G. Euskirchen, P. Lacroute, K. Bettinger, A.P. Boyle, M. Kasowski, F. Grubert, S. Seki, M. Garcia, M. Whirl-Carrillo, M. Gallardo, M.A. Blasco, P.L. Greenberg, P. Snyder, T.E. Klein, R.B. Altman, A.J. Butte, E.A. Ashley, M. Gerstein, K.C. Nadeau, H. Tang, M. Snyder, Personal omics profiling reveals dynamic molecular and medical phenotypes, *Cell*, 148 (2012) 1293-1307, <https://doi.org/10.1016/j.cell.2012.02.009>.
- [112] M.A. Kamleh, T.M.D. Ebbels, K. Spagou, P. Masson, E.J. Want, Optimizing the Use of Quality Control Samples for Signal Drift Correction in Large-Scale Urine Metabolic Profiling Studies, *Anal Chem*, 84 (2012) 2670-2677, <https://doi.org/10.1021/ac202733q>.
- [113] J. Vogel, C. Mobius, W. Kuschinsky, Early delineation of ischemic tissue in rat brain cryosections by high-contrast staining, *Stroke*, 30 (1999) 1134-1141, [10.1161/01.str.30.5.1134](https://doi.org/10.1161/01.str.30.5.1134).
- [114] T.N. Lin, Y.Y. He, G. Wu, M. Khan, C.Y. Hsu, Effect of brain edema on infarct volume in a focal cerebral ischemia model in rats, *Stroke*, 24 (1993) 117-121, [10.1161/01.str.24.1.117](https://doi.org/10.1161/01.str.24.1.117).
- [115] J. Chong, O. Soufan, C. Li, I. Caraus, S. Li, G. Bourque, D.S. Wishart, J. Xia, MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis, *Nucleic acids research*, 46 (2018) W486-W494, <https://doi.org/10.1093/nar/gky310>.
- [116] Z. Pang, J. Chong, G. Zhou, D.A. de Lima Morais, L. Chang, M. Barrette, C. Gauthier, P.E. Jacques, S. Li, J. Xia, MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights, *Nucleic acids research*, 49 (2021) W388-W396, [10.1093/nar/gkab382](https://doi.org/10.1093/nar/gkab382).
- [117] A. Frokjaer, C. Knox, E. Lim, T. Jewison, V. Law, D.D. Hau, P. Liu, B. Gautam, S. Ly, A.C. Guo, J. Xia, Y. Liang, S. Shrivastava, D.S. Wishart, SMPDB: The Small Molecule Pathway Database, *Nucleic acids research*, 38 (2010) D480-487, [10.1093/nar/gkp1002](https://doi.org/10.1093/nar/gkp1002).
- [118] D. Yekutieli, Y. Benjamini, Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics, *J Stat Plan Infer*, 82 (1999) 171-196, [Doi 10.1016/S0378-3758\(99\)00041-5](https://doi.org/10.1016/S0378-3758(99)00041-5).
- [119] J. Folberth, K. Begemann, O. Jöhren, M. Schwaninger, A. Othman, MS(2) and LC libraries for untargeted metabolomics: Enhancing method development and identification confidence, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 1145 (2020) 122105, [10.1016/j.jchromb.2020.122105](https://doi.org/10.1016/j.jchromb.2020.122105).
- [120] S. Wernisch, S. Pennathur, Evaluation of coverage, retention patterns, and selectivity of seven liquid chromatographic methods for metabolomics, *Analytical and bioanalytical chemistry*, 408 (2016) 6079-6091, [10.1007/s00216-016-9716-4](https://doi.org/10.1007/s00216-016-9716-4).
- [121] M. Comisso, A. Anesi, S. Dal Santo, F. Guzzo, Performance comparison of electrospray ionization and atmospheric pressure chemical ionization in untargeted and targeted liquid chromatography/mass spectrometry based metabolomics analysis of grapeberry metabolites, *Rapid Commun Mass Sp*, 31 (2017) 292-300, <https://doi.org/10.1002/rcm.7789>.

- [122] T. Kind, M. Scholz, O. Fiehn, How large is the metabolome? A critical analysis of data exchange practices in chemistry, *PloS one*, 4 (2009) e5440, <https://doi.org/10.1371/journal.pone.0005440>.
- [123] A. Cappiello, G. Famiglioni, P. Palma, E. Pierini, V. Termopoli, H. Trufelli, Overcoming matrix effects in liquid chromatography-mass spectrometry, *Anal Chem*, 80 (2008) 9343-9348, <https://doi.org/10.1021/ac8018312>.
- [124] D.P. Kloos, H. Lingeman, W.M. Niessen, A.M. Deelder, M. Giera, O.A. Mayboroda, Evaluation of different column chemistries for fast urinary metabolic profiling, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 927 (2013) 90-96, [10.1016/j.jchromb.2013.02.017](https://doi.org/10.1016/j.jchromb.2013.02.017).
- [125] N. Drouin, S. Rudaz, J. Schappler, Sample preparation for polar metabolites in bioanalysis, *The Analyst*, 143 (2017) 16-20, <https://doi.org/10.1039/c7an01333g>.
- [126] H. Gika, G. Theodoridis, Sample preparation prior to the LC-MS-based metabolomics/metabonomics of blood-derived samples, *Bioanalysis*, 3 (2011) 1647-1661, <https://doi.org/10.4155/bio.11.122>.
- [127] R. Wawrzyniak, A. Kosnowska, S. Macioszek, R. Bartoszewski, M. Jan Markuszewski, New plasma preparation approach to enrich metabolome coverage in untargeted metabolomics: plasma protein bound hydrophobic metabolite release with proteinase K, *Scientific reports*, 8 (2018) 9541, <https://doi.org/10.1038/s41598-018-27983-0>.
- [128] E.J. Want, G. O'Maille, C.A. Smith, T.R. Brandon, W. Uritboonthai, C. Qin, S.A. Trauger, G. Siuzdak, Solvent-dependent metabolite distribution, clustering, and protein extraction for serum profiling with mass spectrometry, *Anal Chem*, 78 (2006) 743-752, <https://doi.org/10.1021/ac051312t>.
- [129] S.J. Bruce, I. Tavazzi, V. Parisod, S. Rezzi, S. Kochhar, P.A. Guy, Investigation of human blood plasma sample preparation for performing metabolomics using ultrahigh performance liquid chromatography/mass spectrometry, *Anal Chem*, 81 (2009) 3285-3296, <https://doi.org/10.1021/ac8024569>.
- [130] A.M. De Livera, M. Sysi-Aho, L. Jacob, J.A. Gagnon-Bartsch, S. Castillo, J.A. Simpson, T.P. Speed, Statistical methods for handling unwanted variation in metabolomics data, *Anal Chem*, 87 (2015) 3606-3615, [10.1021/ac502439y](https://doi.org/10.1021/ac502439y).
- [131] R. Wei, G. Li, A.B. Seymour, High-throughput and multiplexed LC/MS/MS method for targeted metabolomics, *Anal Chem*, 82 (2010) 5527-5533, <https://doi.org/10.1021/ac100331b>.
- [132] S. Naz, H. Gallart-Ayala, S.N. Reinke, C. Mathon, R. Blankley, R. Chaleckis, C.E. Wheelock, Development of a Liquid Chromatography-High Resolution Mass Spectrometry Metabolomics Method with High Specificity for Metabolite Identification Using All Ion Fragmentation Acquisition, *Anal Chem*, 89 (2017) 7933-7942, <https://doi.org/10.1021/acs.analchem.7b00925>.

- [133] H. Oberacher, V. Reinstadler, M. Kreidl, M.A. Stravs, J. Hollender, E.L. Schymanski, Annotating Nontargeted LC-HRMS/MS Data with Two Complementary Tandem Mass Spectral Libraries, *Metabolites*, 9 (2018), <https://doi.org/10.3390/metabo9010003>.
- [134] G. Fave, M. Beckmann, A.J. Lloyd, S. Zhou, G. Harold, W. Lin, K. Taillart, L. Xie, J. Draper, J.C. Mathers, Development and validation of a standardized protocol to monitor human dietary exposure by metabolite fingerprinting of urine samples, *Metabolomics : Official journal of the Metabolomic Society*, 7 (2011) 469-484, [10.1007/s11306-011-0289-0](https://doi.org/10.1007/s11306-011-0289-0).
- [135] E. Werner, J.F. Heilier, C. Ducruix, E. Ezan, C. Junot, J.C. Tabet, Mass spectrometry for the identification of the discriminating signals from metabolomics: current status and future trends, *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 871 (2008) 143-163, [10.1016/j.jchromb.2008.07.004](https://doi.org/10.1016/j.jchromb.2008.07.004).
- [136] J. Montaner, L. Ramiro, A. Simats, S. Tiedt, K. Makris, G.C. Jickling, S. DeBette, J.C. Sanchez, A. Bustamante, Multilevel omics for the discovery of biomarkers and therapeutic targets for stroke, *Nature reviews. Neurology*, 16 (2020) 247-264, [10.1038/s41582-020-0350-6](https://doi.org/10.1038/s41582-020-0350-6).
- [137] T.R. Koves, J.R. Ussher, R.C. Noland, D. Slentz, M. Mosedale, O. Ilkayeva, J. Bain, R. Stevens, J.R. Dyck, C.B. Newgard, G.D. Lopaschuk, D.M. Muoio, Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance, *Cell metabolism*, 7 (2008) 45-56, [10.1016/j.cmet.2007.10.013](https://doi.org/10.1016/j.cmet.2007.10.013).
- [138] J.S. Bhatti, G.K. Bhatti, P.H. Reddy, Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies, *Biochimica et biophysica acta. Molecular basis of disease*, 1863 (2017) 1066-1077, [10.1016/j.bbadis.2016.11.010](https://doi.org/10.1016/j.bbadis.2016.11.010).
- [139] E.H. Lo, T. Dalkara, M.A. Moskowitz, Mechanisms, challenges and opportunities in stroke, *Nature reviews. Neuroscience*, 4 (2003) 399-415, [10.1038/nrn1106](https://doi.org/10.1038/nrn1106).
- [140] J.M. Rutkowski, T.A. Knotts, K.D. Ono-Moore, C.S. McCain, S. Huang, D. Schneider, S. Singh, S.H. Adams, D.H. Hwang, Acylcarnitines activate proinflammatory signaling pathways, *American journal of physiology. Endocrinology and metabolism*, 306 (2014) E1378-1387, [10.1152/ajpendo.00656.2013](https://doi.org/10.1152/ajpendo.00656.2013).
- [141] M. Guasch-Ferre, Y. Zheng, M. Ruiz-Canela, A. Hruby, M.A. Martinez-Gonzalez, C.B. Clish, D. Corella, R. Estruch, E. Ros, M. Fito, C. Dennis, I.M. Morales-Gil, F. Aros, M. Fiol, J. Lapetra, L. Serra-Majem, F.B. Hu, J. Salas-Salvado, Plasma acylcarnitines and risk of cardiovascular disease: effect of Mediterranean diet interventions, *The American journal of clinical nutrition*, 103 (2016) 1408-1416, [10.3945/ajcn.116.130492](https://doi.org/10.3945/ajcn.116.130492).
- [142] A. Brevik, M.B. Veierod, C.A. Drevon, L.F. Andersen, Evaluation of the odd fatty acids 15:0 and 17:0 in serum and adipose tissue as markers of intake of milk and dairy fat, *European journal of clinical nutrition*, 59 (2005) 1417-1422, [10.1038/sj.ejcn.1602256](https://doi.org/10.1038/sj.ejcn.1602256).
- [143] V. Foulon, M. Sniekers, E. Huysmans, S. Asselberghs, V. Mahieu, G.P. Mannaerts, P.P. Van Veldhoven, M. Casteels, Breakdown of 2-hydroxylated straight chain fatty acids via peroxisomal 2-

hydroxyphytanoyl-CoA lyase: a revised pathway for the alpha-oxidation of straight chain fatty acids, *The Journal of biological chemistry*, 280 (2005) 9802-9812,10.1074/jbc.M413362200.

[144] M. Schrader, H.D. Fahimi, Peroxisomes and oxidative stress, *Biochimica et biophysica acta*, 1763 (2006) 1755-1766,10.1016/j.bbamcr.2006.09.006.

[145] A. Vanella, A. Russo, R. Acquaviva, A. Campisi, C. Di Giacomo, V. Sorrenti, M.L. Barcellona, L - propionyl-carnitine as superoxide scavenger, antioxidant, and DNA cleavage protector, *Cell biology and toxicology*, 16 (2000) 99-104,10.1023/a:1007638025856.

[146] N. Cichon, M. Bijak, E. Miller, M. Niwald, J. Saluk, Poststroke depression as a factor adversely affecting the level of oxidative damage to plasma proteins during a brain stroke, *Oxidative medicine and cellular longevity*, 2015 (2015) 408745,10.1155/2015/408745.

[147] Z. Cai, L.J. Yan, Protein Oxidative Modifications: Beneficial Roles in Disease and Health, *Journal of biochemical and pharmacological research*, 1 (2013) 15-26

[148] R. Brouns, R. Verkerk, T. Aerts, D. De Surgeloose, A. Wauters, S. Scharpe, P.P. De Deyn, The role of tryptophan catabolism along the kynurenine pathway in acute ischemic stroke, *Neurochemical research*, 35 (2010) 1315-1322,10.1007/s11064-010-0187-2.

[149] Q. Wang, D. Liu, P. Song, M.H. Zou, Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation, *Front Biosci (Landmark Ed)*, 20 (2015) 1116-1143,10.2741/4363.

[150] L.F. Mohammad-Zadeh, L. Moses, S.M. Gwaltney-Brant, Serotonin: a review, *Journal of veterinary pharmacology and therapeutics*, 31 (2008) 187-199,10.1111/j.1365-2885.2008.00944.x.

[151] O. Vasicek, A. Lojek, M. Ciz, Serotonin and its metabolites reduce oxidative stress in murine RAW264.7 macrophages and prevent inflammation, *Journal of physiology and biochemistry*, 76 (2020) 49-60,10.1007/s13105-019-00714-3.

[152] H. Wu, T.H. Denna, J.N. Storkersen, V.A. Gerriets, Beyond a neurotransmitter: The role of serotonin in inflammation and immunity, *Pharmacological research*, 140 (2019) 100-114,10.1016/j.phrs.2018.06.015.

[153] T. Siepmann, A.I. Penzlin, J. Kepplinger, B.M. Illigens, K. Weidner, H. Reichmann, K. Barlinn, Selective serotonin reuptake inhibitors to improve outcome in acute ischemic stroke: possible mechanisms and clinical evidence, *Brain and behavior*, 5 (2015) e00373,10.1002/brb3.373.

[154] D.R. Sell, C.M. Strauch, W. Shen, V.M. Monnier, 2-aminoadipic acid is a marker of protein carbonyl oxidation in the aging human skin: effects of diabetes, renal failure and sepsis, *The Biochemical journal*, 404 (2007) 269-277,10.1042/BJ20061645.

[155] S. Zeitoun-Ghandour, O.I. Leszczyszyn, C.A. Blindauer, F.M. Geier, J.G. Bundy, S.R. Sturzenbaum, C. elegans metallothioneins: response to and defence against ROS toxicity, *Molecular bioSystems*, 7 (2011) 2397-2406,10.1039/c1mb05114h.

- [156] D.L. Goh, A. Patel, G.H. Thomas, G.S. Salomons, D.S. Schor, C. Jakobs, M.T. Geraghty, Characterization of the human gene encoding alpha-aminoacidase (AADAT), *Molecular genetics and metabolism*, 76 (2002) 172-180,10.1016/s1096-7192(02)00037-9.
- [157] J.A. Rothwell, F. Madrid-Gambin, M. Garcia-Aloy, C. Andres-Lacueva, C. Logue, A.M. Gallagher, C. Mack, S.E. Kulling, Q. Gao, G. Pratico, L.O. Dragsted, A. Scalbert, Biomarkers of intake for coffee, tea, and sweetened beverages, *Genes & nutrition*, 13 (2018) 15,10.1186/s12263-018-0607-5.
- [158] J. Satriano, Arginine pathways and the inflammatory response: interregulation of nitric oxide and polyamines: review article, *Amino acids*, 26 (2004) 321-329,10.1007/s00726-004-0078-4.
- [159] M.A. Avila, E.R. Garcia-Trevijano, S.C. Lu, F.J. Corrales, J.M. Mato, Methylthioadenosine, *The international journal of biochemistry & cell biology*, 36 (2004) 2125-2130,10.1016/j.biocel.2003.11.016.
- [160] R.M. Adibhatla, J.F. Hatcher, K. Sailor, R.J. Dempsey, Polyamines and central nervous system injury: spermine and spermidine decrease following transient focal cerebral ischemia in spontaneously hypertensive rats, *Brain research*, 938 (2002) 81-86,10.1016/s0006-8993(02)02447-2.
- [161] C.M. Henley, K. Wey, A. Takashima, C. Mills, E. Granmayeh, I. Krishnappa, C.S. Robertson, S-adenosylmethionine decarboxylase activity is decreased in the rat cortex after traumatic brain injury, *Journal of neurochemistry*, 69 (1997) 259-265,10.1046/j.1471-4159.1997.69010259.x.
- [162] B. Moreno, I. Lopez, B. Fernandez-Diez, M. Gottlieb, C. Matute, M.V. Sanchez-Gomez, M. Domercq, A. Giralt, J. Alberch, K.W. Collon, H. Zhang, J.M. Parent, M. Teixido, E. Giralt, V. Cena, I. Posadas, E. Martinez-Pinilla, P. Villoslada, R. Franco, Differential neuroprotective effects of 5'-deoxy-5'-methylthioadenosine, *PloS one*, 9 (2014) e90671,10.1371/journal.pone.0090671.
- [163] P.G. Sreekumar, D.R. Hinton, R. Kannan, Methionine sulfoxide reductase A: Structure, function and role in ocular pathology, *World journal of biological chemistry*, 2 (2011) 184-192,10.4331/wjbc.v2.i8.184.
- [164] N. Antonios, D.J. Angiolillo, S. Silliman, Hypertriglyceridemia and ischemic stroke, *European neurology*, 60 (2008) 269-278,10.1159/000157880.
- [165] L. Yang, P. Lv, W. Ai, L. Li, S. Shen, H. Nie, Y. Shan, Y. Bai, Y. Huang, H. Liu, Lipidomic analysis of plasma in patients with lacunar infarction using normal-phase/reversed-phase two-dimensional liquid chromatography-quadrupole time-of-flight mass spectrometry, *Analytical and bioanalytical chemistry*, 409 (2017) 3211-3222,10.1007/s00216-017-0261-6.
- [166] T. Dziedzic, A. Slowik, E.A. Gryz, A. Szczudlik, Lower serum triglyceride level is associated with increased stroke severity, *Stroke*, 35 (2004) e151-152,10.1161/01.STR.0000128705.63891.67.
- [167] M. Jain, A. Jain, N. Yerragondur, R.D. Brown, A. Rabinstein, B.S. Jahromi, L. Vaidyanathan, B. Blyth, L.G. Stead, The Triglyceride Paradox in Stroke Survivors: A Prospective Study, *Neuroscience journal*, 2013 (2013) 870608,10.1155/2013/870608.

- [168] E. Sidorov, D.K. Sanghera, J.K.P. Vanamala, Biomarker for Ischemic Stroke Using Metabolome: A Clinician Perspective, *Journal of stroke*, 21 (2019) 31-41,10.5853/jos.2018.03454.
- [169] H. Sun, J. Zhao, D. Zhong, G. Li, Potential serum biomarkers and metabonomic profiling of serum in ischemic stroke patients using UPLC/Q-TOF MS/MS, *PloS one*, 12 (2017) e0189009,10.1371/journal.pone.0189009.
- [170] R. Muralikrishna Adibhatla, J.F. Hatcher, Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia, *Free radical biology & medicine*, 40 (2006) 376-387,10.1016/j.freeradbiomed.2005.08.044.
- [171] R.M. Adibhatla, J.F. Hatcher, Role of Lipids in Brain Injury and Diseases, *Future lipidology*, 2 (2007) 403-422,10.2217/17460875.2.4.403.
- [172] G.Y. Sun, P.B. Shelat, M.B. Jensen, Y. He, A.Y. Sun, A. Simonyi, Phospholipases A2 and inflammatory responses in the central nervous system, *Neuromolecular medicine*, 12 (2010) 133-148,10.1007/s12017-009-8092-z.
- [173] M.N. Hoda, I. Singh, A.K. Singh, M. Khan, Reduction of lipoxidative load by secretory phospholipase A2 inhibition protects against neurovascular injury following experimental stroke in rat, *Journal of neuroinflammation*, 6 (2009) 21,10.1186/1742-2094-6-21.
- [174] S.H. Law, M.L. Chan, G.K. Marathe, F. Parveen, C.H. Chen, L.Y. Ke, An Updated Review of Lysophosphatidylcholine Metabolism in Human Diseases, *International journal of molecular sciences*, 20 (2019),10.3390/ijms20051149.
- [175] A. Perrakis, W.H. Moolenaar, Autotaxin: structure-function and signaling, *Journal of lipid research*, 55 (2014) 1010-1018,10.1194/jlr.R046391.
- [176] S. Bhattarai, S. Sharma, H. Ara, U. Subedi, G. Sun, C. Li, M.S. Bhuiyan, C. Kevil, W.P. Armstrong, M.T. Minvielle, S. Miriyala, M. Panchatcharam, Disrupted Blood-Brain Barrier and Mitochondrial Impairment by Autotaxin-Lysophosphatidic Acid Axis in Postischemic Stroke, *Journal of the American Heart Association*, 10 (2021) e021511,10.1161/JAHA.121.021511.
- [177] I.A. Rahman, K. Tsuboi, T. Uyama, N. Ueda, New players in the fatty acyl ethanolamide metabolism, *Pharmacological research*, 86 (2014) 1-10,10.1016/j.phrs.2014.04.001.
- [178] E. Esposito, M. Cordaro, S. Cuzzocrea, Roles of fatty acid ethanolamides (FAE) in traumatic and ischemic brain injury, *Pharmacological research*, 86 (2014) 26-31,10.1016/j.phrs.2014.05.009.
- [179] P. Garg, R.S. Duncan, S. Kaja, P. Koulen, Intracellular mechanisms of N-acyl ethanolamine-mediated neuroprotection in a rat model of stroke, *Neuroscience*, 166 (2010) 252-262,10.1016/j.neuroscience.2009.11.069.
- [180] M.R. Laursen, J. Hansen, C. Elkjaer, N. Stavnager, C.B. Nielsen, K. Pryds, J. Johnsen, J.M. Nielsen, H.E. Botker, M. Johannsen, Untargeted metabolomics reveals a mild impact of remote ischemic conditioning on the plasma metabolome and alpha-hydroxybutyrate as a possible

cardioprotective factor and biomarker of tissue ischemia, *Metabolomics : Official journal of the Metabolomic Society*, 13 (2017) 67,10.1007/s11306-017-1202-2.

[181] E. Mosharov, M.R. Cranford, R. Banerjee, The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes, *Biochemistry*, 39 (2000) 13005-13011,10.1021/bi001088w.

[182] C. Ye, B.M. Sutter, Y. Wang, Z. Kuang, B.P. Tu, A Metabolic Function for Phospholipid and Histone Methylation, *Molecular cell*, 66 (2017) 180-193 e188,10.1016/j.molcel.2017.02.026.

[183] W.E. Gall, K. Beebe, K.A. Lawton, K.P. Adam, M.W. Mitchell, P.J. Nakhle, J.A. Ryals, M.V. Milburn, M. Nannipieri, S. Camastra, A. Natali, E. Ferrannini, alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population, *PloS one*, 5 (2010) e10883,10.1371/journal.pone.0010883.

[184] B. Senior, L. Loridan, Direct regulatory effect of ketones on lipolysis and on glucose concentrations in man, *Nature*, 219 (1968) 83-84,10.1038/219083a0.

[185] P.F. Devaux, Static and dynamic lipid asymmetry in cell membranes, *Biochemistry*, 30 (1991) 1163-1173,10.1021/bi00219a001.

[186] M. Dashti, W. Kulik, F. Hoek, E.C. Veerman, M.P. Peppelenbosch, F. Rezaee, A phospholipidomic analysis of all defined human plasma lipoproteins, *Scientific reports*, 1 (2011) 139,10.1038/srep00139.

[187] Z. Li, D.E. Vance, Phosphatidylcholine and choline homeostasis, *Journal of lipid research*, 49 (2008) 1187-1194,10.1194/jlr.R700019-JLR200.

[188] C.J. Walkey, L. Yu, L.B. Agellon, D.E. Vance, Biochemical and evolutionary significance of phospholipid methylation, *The Journal of biological chemistry*, 273 (1998) 27043-27046,10.1074/jbc.273.42.27043.

[189] X. Jin, R.H. Wang, H. Wang, C.L. Long, Brain protection against ischemic stroke using choline as a new molecular bypass treatment, *Acta pharmacologica Sinica*, 36 (2015) 1416-1425,10.1038/aps.2015.104.

[190] R.M. Adibhatla, J.F. Hatcher, R.J. Dempsey, Effects of citicoline on phospholipid and glutathione levels in transient cerebral ischemia, *Stroke*, 32 (2001) 2376-2381,10.1161/hs1001.096010.

[191] O. Hurtado, A. Cardenas, J.M. Pradillo, J.R. Morales, F. Ortego, T. Sobrino, J. Castillo, M.A. Moro, I. Lizasoain, A chronic treatment with CDP-choline improves functional recovery and increases neuronal plasticity after experimental stroke, *Neurobiology of disease*, 26 (2007) 105-111,10.1016/j.nbd.2006.12.005.

[192] A. Gomez-Munoz, N. Presa, A. Gomez-Larrauri, I.G. Rivera, M. Trueba, M. Ordonez, Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate, *Progress in lipid research*, 61 (2016) 51-62,10.1016/j.plipres.2015.09.002.

- [193] I. Azizkhanian, S.A. Sheth, A.T. Iavarone, S. Lee, V. Kakarla, J.D. Hinman, Plasma Lipid Profiling Identifies Biomarkers of Cerebral Microvascular Disease, *Frontiers in neurology*, 10 (2019) 950,10.3389/fneur.2019.00950.
- [194] C. Zhong, Z. Lu, B. Che, S. Qian, X. Zheng, A. Wang, X. Bu, J. Zhang, Z. Ju, T. Xu, Y. Zhang, Choline Pathway Nutrients and Metabolites and Cognitive Impairment After Acute Ischemic Stroke, *Stroke*, 52 (2021) 887-895,10.1161/STROKEAHA.120.031903.
- [195] M. Beridze, T. Sanikidze, R. Shakarishvili, N. Intskirveli, N.M. Bornstein, Selected acute phase CSF factors in ischemic stroke: findings and prognostic value, *BMC neurology*, 11 (2011) 41,10.1186/1471-2377-11-41.
- [196] E. Cuadrado, A. Rosell, N. Colome, M. Hernandez-Guillamon, T. Garcia-Berrocso, M. Ribo, A. Alcazar, A. Ortega-Aznar, M. Salinas, F. Canals, J. Montaner, The proteome of human brain after ischemic stroke, *Journal of neuropathology and experimental neurology*, 69 (2010) 1105-1115,10.1097/NEN.0b013e3181f8c539.
- [197] F. Fluri, M.K. Schuhmann, C. Kleinschnitz, Animal models of ischemic stroke and their application in clinical research, *Drug design, development and therapy*, 9 (2015) 3445-3454,10.2147/DDDT.S56071.
- [198] B. Seminotti, J.C. da Silva, R.T. Ribeiro, G. Leipnitz, M. Wajner, Free Radical Scavengers Prevent Argininosuccinic Acid-Induced Oxidative Stress in the Brain of Developing Rats: a New Adjuvant Therapy for Argininosuccinate Lyase Deficiency?, *Molecular neurobiology*, 57 (2020) 1233-1244,10.1007/s12035-019-01825-0.
- [199] Y. Yabuki, N. Shioda, Y. Yamamoto, M. Shigano, K. Kumagai, M. Morita, K. Fukunaga, Oral L-citrulline administration improves memory deficits following transient brain ischemia through cerebrovascular protection, *Brain research*, 1520 (2013) 157-167,10.1016/j.brainres.2013.05.011.
- [200] R.H. Boger, K. Sydow, J. Borlak, T. Thum, H. Lenzen, B. Schubert, D. Tsikas, S.M. Bode-Boger, LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases, *Circulation research*, 87 (2000) 99-105,10.1161/01.res.87.2.99.
- [201] P. Vallance, A. Leone, A. Calver, J. Collier, S. Moncada, Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis, *Journal of cardiovascular pharmacology*, 20 Suppl 12 (1992) S60-62,10.1097/00005344-199204002-00018.
- [202] X. Hu, D. Atzler, X. Xu, P. Zhang, H. Guo, Z. Lu, J. Fassett, E. Schwedhelm, R.H. Boger, R.J. Bache, Y. Chen, Dimethylarginine dimethylaminohydrolase-1 is the critical enzyme for degrading the cardiovascular risk factor asymmetrical dimethylarginine, *Arteriosclerosis, thrombosis, and vascular biology*, 31 (2011) 1540-1546,10.1161/ATVBAHA.110.222638.
- [203] K. Sydow, T. Munzel, ADMA and oxidative stress, *Atherosclerosis. Supplements*, 4 (2003) 41-51,10.1016/s1567-5688(03)00033-3.

- [204] R. Brouns, B. Marescau, I. Possemiers, R. Sheorajpanday, P.P. De Deyn, Dimethylarginine levels in cerebrospinal fluid of hyperacute ischemic stroke patients are associated with stroke severity, *Neurochemical research*, 34 (2009) 1642-1649,10.1007/s11064-009-9954-3.
- [205] S. Chen, N. Li, M. Deb-Chatterji, Q. Dong, J.T. Kielstein, K. Weissenborn, H. Worthmann, Asymmetric dimethylarginine as marker and mediator in ischemic stroke, *International journal of molecular sciences*, 13 (2012) 15983-16004,10.3390/ijms131215983.
- [206] V. Vitvitsky, M. Thomas, A. Ghorpade, H.E. Gendelman, R. Banerjee, A functional transsulfuration pathway in the brain links to glutathione homeostasis, *The Journal of biological chemistry*, 281 (2006) 35785-35793,10.1074/jbc.M602799200.
- [207] S.J. Chan, C. Chai, T.W. Lim, M. Yamamoto, E.H. Lo, M.K. Lai, P.T. Wong, Cystathionine beta-synthase inhibition is a potential therapeutic approach to treatment of ischemic injury, *ASN neuro*, 7 (2015),10.1177/1759091415578711.
- [208] T. Morikawa, M. Kajimura, T. Nakamura, T. Hishiki, T. Nakanishi, Y. Yukutake, Y. Nagahata, M. Ishikawa, K. Hattori, T. Takenouchi, T. Takahashi, I. Ishii, K. Matsubara, Y. Kabe, S. Uchiyama, E. Nagata, M.M. Gadalla, S.H. Snyder, M. Suematsu, Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway, *Proceedings of the National Academy of Sciences of the United States of America*, 109 (2012) 1293-1298,10.1073/pnas.1119658109.
- [209] F. Tian, R. Liu, C. Fan, Y. Sun, X. Huang, Z. Nie, X. Zhao, X. Pu, Effects of Thymoquinone on Small-Molecule Metabolites in a Rat Model of Cerebral Ischemia Reperfusion Injury Assessed using MALDI-MSI, *Metabolites*, 10 (2020),10.3390/metabo10010027.
- [210] M.M. Nielsen, K.L. Lambertsen, B.H. Clausen, M. Meyer, D.R. Bhandari, S.T. Larsen, S.S. Poulsen, B. Spengler, C. Janfelt, H.S. Hansen, Mass spectrometry imaging of biomarker lipids for phagocytosis and signalling during focal cerebral ischaemia, *Scientific reports*, 6 (2016) 39571,10.1038/srep39571.
- [211] Y. Inose, Y. Kato, K. Kitagawa, S. Uchiyama, N. Shibata, Activated microglia in ischemic stroke penumbra upregulate MCP-1 and CCR2 expression in response to lysophosphatidylcholine derived from adjacent neurons and astrocytes, *Neuropathology : official journal of the Japanese Society of Neuropathology*, 35 (2015) 209-223,10.1111/neup.12182.
- [212] M. Bieber, J. Gronewold, A.C. Scharf, M.K. Schuhmann, F. Langhauser, S. Hopp, S. Mencl, E. Geuss, J. Leinweber, J. Guthmann, T.R. Doeppner, C. Kleinschnitz, G. Stoll, P. Kraft, D.M. Hermann, Validity and Reliability of Neurological Scores in Mice Exposed to Middle Cerebral Artery Occlusion, *Stroke*, 50 (2019) 2875-2882,10.1161/STROKEAHA.119.026652.
- [213] D.C. Tong, M.A. Yenari, G.W. Albers, M. O'Brien, M.P. Marks, M.E. Moseley, Correlation of perfusion- and diffusion-weighted MRI with NIHSS score in acute (<6.5 hour) ischemic stroke, *Neurology*, 50 (1998) 864-870,10.1212/wnl.50.4.864.

- [214] U.V. Wesley, V.J. Bhute, J.F. Hatcher, S.P. Palecek, R.J. Dempsey, Local and systemic metabolic alterations in brain, plasma, and liver of rats in response to aging and ischemic stroke, as detected by nuclear magnetic resonance (NMR) spectroscopy, *Neurochemistry international*, 127 (2019) 113-124,10.1016/j.neuint.2019.01.025.
- [215] B.M. Arendt, D.W. Ma, B. Simons, S.A. Noureldin, G. Therapondos, M. Guindi, M. Sherman, J.P. Allard, Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine to phosphatidylethanolamine, *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*, 38 (2013) 334-340,10.1139/apnm-2012-0261.
- [216] Z. Li, L.B. Agellon, T.M. Allen, M. Umeda, L. Jewell, A. Mason, D.E. Vance, The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis, *Cell metabolism*, 3 (2006) 321-331,10.1016/j.cmet.2006.03.007.
- [217] S.M. Abdeldyem, T. Goda, S.A. Khodeir, S. Abou Saif, S. Abd-Elsalam, Nonalcoholic fatty liver disease in patients with acute ischemic stroke is associated with more severe stroke and worse outcome, *Journal of clinical lipidology*, 11 (2017) 915-919,10.1016/j.jacl.2017.04.115.
- [218] N.J. Alkayed, I. Harukuni, A.S. Kimes, E.D. London, R.J. Traystman, P.D. Hurn, Gender-linked brain injury in experimental stroke, *Stroke*, 29 (1998) 159-165; discussion 166,10.1161/01.str.29.1.159.
- [219] B. Manwani, F. Liu, V. Scranton, M.D. Hammond, L.H. Sansing, L.D. McCullough, Differential effects of aging and sex on stroke induced inflammation across the lifespan, *Experimental neurology*, 249 (2013) 120-131,10.1016/j.expneurol.2013.08.011.
- [220] M. Resseguie, J. Song, M.D. Niculescu, K.A. da Costa, T.A. Randall, S.H. Zeisel, Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 21 (2007) 2622-2632,10.1096/fj.07-8227com.
- [221] A.K. Bonkhoff, A. Karch, R. Weber, J. Wellmann, K. Berger, Female Stroke: Sex Differences in Acute Treatment and Early Outcomes of Acute Ischemic Stroke, *Stroke*, 52 (2021) 406-415,10.1161/STROKEAHA.120.032850.
- [222] M. Dichgans, R. Malik, I.R. Konig, J. Rosand, R. Clarke, S. Gretarsdottir, G. Thorleifsson, B.D. Mitchell, T.L. Assimes, C. Levi, C.J. O'Donnell, M. Fornage, U. Thorsteinsdottir, B.M. Psaty, C. Hengstenberg, S. Seshadri, J. Erdmann, J.C. Bis, A. Peters, G.B. Boncoraglio, W. Marz, J.F. Meschia, S. Kathiresan, M.A. Ikram, R. McPherson, K. Stefansson, C. Sudlow, M.P. Reilly, J.R. Thompson, P. Sharma, J.C. Hopewell, J.C. Chambers, H. Watkins, P.M. Rothwell, R. Roberts, H.S. Markus, N.J. Samani, M. Farrall, H. Schunkert, Shared genetic susceptibility to ischemic stroke and coronary artery disease: a genome-wide analysis of common variants, *Stroke*, 45 (2014) 24-36,10.1161/STROKEAHA.113.002707.
- [223] L. Han, Q. Wu, C. Wang, Y. Hao, J. Zhao, L. Zhang, R. Fan, Y. Liu, R. Li, Z. Chen, T. Zhang, S. Chen, J. Ma, S. Liu, X. Peng, S. Duan, Homocysteine, Ischemic Stroke, and Coronary Heart Disease

in Hypertensive Patients: A Population-Based, Prospective Cohort Study, *Stroke*, 46 (2015) 1777-1786,10.1161/STROKEAHA.115.009111.

[224] D.E. Vance, Phospholipid methylation in mammals: from biochemistry to physiological function, *Biochimica et biophysica acta*, 1838 (2014) 1477-1487,10.1016/j.bbamem.2013.10.018.

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Curriculum vitae

Personal details

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Education

Since 06/2016	PhD student Institute for Experimental and Clinical Pharmacology and Toxikology, University of Lübeck Project: <i>A metabolomics and lipidomics profile of ischemic stroke</i> Supervisor: Prof. Dr. Markus Schwaninger
2011 – 2016	State examination (Staatsexamen) - Food chemistry Department of Chemistry and Pharmacy Friedrich-Alexander University Erlangen/Nürnberg Final grade: 1,7 Thesis project: <i>Cyanotoxine in Nahrungsergänzungsmitteln</i> German Federal Institute for Risk Assessment (BfR), Berlin Supervisor: Dr. Cindy Schöne
2003 – 2011	Allgemeine Hochschulreife Albert-Schweitzer-Gymnasium, Erlangen Final grade: 1,0

Working experience

Since 01/2021	Research associate - Scientific head of the Bioanalytic Core Facility (Mass spectrometry laboratory) University Medical Center Schleswig-Holstein, Lübeck Center of Brain, Behavior and Metabolism (CBBM), University of Lübeck
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- Providing liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis
- Organisation of the mass spectrometry laboratory

06/2016 – 12/2020

Research associate

University Medical Center Schleswig-Holstein, Lübeck
Institute for Experimental and Clinical Pharmacology and
Toxicology, University of Lübeck

- Method development and optimization in the field of liquid chromatography coupled to mass spectrometry (LC-MS/MS)
- Main research: Lipidomics and Metabolomics analysis

10/2015 – 04/2016

Guest scientist

German Federal Institute for Risk Assessment (BfR),
Berlin

- Method development and validation in the field of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

Software and language skills

Software

Microsoft Office

Data acquisition and data processing software:

- Xcalibur
- TraceFinder
- Compound Discoverer

Statistics:

- SPSS
- Prism

Languages

German: native speaker
English: fluent

Lübeck, 18.03.2022

