Lipid profiling of brain tissue and blood after traumatic brain injury

A review of human and experimental studies

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Declaration of interest:

None

Abstract

Traumatic brain injury (TBI) is a neurological condition which affects a large number of individuals worldwide, across all ages. It can lead to major physical, cognitive and psychological impairment, and represents a considerable health cost burden. TBI is a heterogeneous condition and there has been intense effort over the last decade to identify better biomarkers, which would enable an optimum and personalized treatment. The brain is highly enriched in a variety of lipids, including fatty acids, glycerophospholipids, glycerolipids, sterols and sphingolipids. There is accumulating evidence in clinical studies in TBI patients and also in experimental models of TBI, that injury triggers a complex pattern of changes in various lipid classes. Such changes can be detected in blood (plasma/serum), cerebrospinal fluid and also in brain tissue. They provide new insights into the pathophysiology of TBI, and have biomarker potential. Here, we review the various changes reported and discuss the scope and value of these lipid focused studies within the TBI field.

Keywords

Traumatic brain injury, phospholipids, cardiolipin, biomarker, plasma, free fatty acids

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[1] **Abbreviations:**

ApoE: Apolipoprotein E
ARA: Arachidonic acid (20:4n-6)
BBB: Blood-brain barrier
CCI: Controlled cortical impact
CNS: Central nervous system
CSF: Cerebrospinal fluid
CT: Computer tomography
DHA: Docosahexaenoic acid (22:6n-3)
GCS: Glasgow Coma Scale
LysoPL: Lysophospholipid(s)
MRI: Magnetic resonance imaging
PC: Phosphatidylcholine
PE: Phosphatidylethanolamine
PGE_2: Prostaglandin E_2
PI: Phosphatidylinositol
PL: Phospholipid(s)
PLA_2: Phospholipase A_2
PND: Postnatal day
PS: Phosphatidylserine
SM: Sphingomyelin
TBI: Traumatic brain injury

1. Introduction

Traumatic brain injury (TBI) is a major health problem worldwide and is associated with a significant socioeconomic burden [1, 2]. It is a neurological condition which can lead to life-changing physical, psychological and cognitive changes [3, 4]. There is also accumulating evidence that TBI significantly increases the risk of developing neurodegenerative disease, such as dementia [5-8]. TBI is a major cause of death and disability below 45 years of age in Western countries [9], and the growing number of TBI cases, including an increased prevalence in the elderly, highlights the need to develop sensitive TBI diagnostic and prognostic tools, and improved treatment. TBI can occur in a variety of contexts (e.g. traffic accidents, falls, assaults and military combat). It is a very heterogeneous condition, and the considerable variation between patients implies a significant need for personalized management. Collaborative efforts worldwide, such as the CENTER-TBI and Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) initiatives, have been addressing the need for the acquisition of comprehensive datasets, and the development of complex outcome assessment batteries, which could ultimately lead to improved effectiveness in neurotrauma [10, 11].

The severity of TBI is graded neurologically using the Glasgow Coma Scale (GCS), based on motor, eye and verbal responses which evaluate the patient's level of consciousness. However, the GCS has limited clinical value and is unsatisfactory [12]. Additional information can be obtained through imaging methods such as magnetic resonance imaging (MRI) and computer tomography (CT), which provide more objective information on the magnitude and localization of the injury [13]. However, CT scans lack sensitivity in mild to moderate diffuse brain injury, and the availability and feasibility of MRI limits its broad clinical use. It is worth underlining that, in this type of injury, advanced neuroimaging techniques, such as diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS) and functional MRI (fMRI) provide useful objective information of diagnostic and prognostic value [14-17]. Therefore, there is a need for additional biomarkers for TBI, with high specificity and sensitivity [18], which would enable a refinement of diagnosis and prognosis of outcome. This need has led to a sustained effort over the last decade to identify various types of biomarkers of TBI in blood [19-21]. Among these biomarkers there is a very limited representation of lipids, although the brain is the organ which is most enriched in lipids, that represent more than 60% of its dry weight.

Lipids are a major class of cellular components, which show a wide diversity. They have a variety of structural and signalling roles, and a key role in energy storage and supply. The developments in biochemical analysis, instrumentation and bioinformatics have led to the development of lipidomics, i.e. the study of lipid classes, lipid networks and pathways, and have allowed insights into the complexity of lipid profiles and their dynamics during development, in aging and in disease states. The

efforts of the LIPID MAPS consortium have led to a classification of lipids, which is regularly updated, and also a standardization of protocols and an improved availability of high-quality standards (http://www.lipidmaps.org). Lipids are grouped under the following major categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides [22]. Methods based on mass spectrometry have enabled the qualitative and quantitative analysis of lipids in complex tissue matrices, either in a targeted or untargeted mode [23]. Reference material for specific tissues has become available, e.g. Quehenberger and collaborators [24] have published an analysis of plasma lipids in a human plasma standard reference material. This material was prepared by obtaining plasma samples from 100 individuals between 40 and 50 years of age, ethnically representative of the US population, including an equal number of men and women.

The central nervous system (CNS) contains thousands of different lipid species and most brain lipids are synthesized locally and are separated from the peripheral compartment by the blood-brain barrier (BBB) [25]. Using an untargeted lipidomic approach, Bozek *et al.* [26] carried out a comprehensive brain lipidome analysis in several species: human, chimpanzee, macaque and mouse. Their analysis showed that in humans, in evolutionary terms, there is an increased brain lipid specificity as shown by comparison to the kidney or muscle lipidome, with striking differences in this specificity seen even between chimpanzees and humans, who have a high degree of genetic relatedness. Between brain regions (considering the prefrontal cortex, the primary visual area and the cerebellum), there were also lipid differences. Interestingly, there was a 3-fold acceleration of lipid specialization and divergence in the neocortex vs. the cerebellum. Therefore, there was greater lipidome divergence between the brain and non-neural tissues in species that show greater cognitive complexity, and a faster divergence of lipids enriched in brain compared with lipids enriched in non-neural tissues. These observations suggest a link between the evolution of the brain lipidome and the evolution of brain functionality and its increasing complexity on the phylogenetic scale. This supports the idea that lipids fulfil unique roles in the CNS.

TBI disrupts tissue architecture and leads to complex alterations in all tissue components, including lipids. Measurable and minimally invasive biomarkers which are reliable indicators of the CNS injury and predict the evolution and outcome, are pivotal tools for optimized therapeutic management. Changes in lipids which can be detected peripherally (blood, plasma or serum) have been increasingly reported after TBI. The aim of this review is to summarize findings of alterations in lipids following TBI in humans and also in experimental models of TBI, in both the peripheral compartment (blood, plasma, serum) and central compartment (brain tissue, cerebral microdialysate and cerebrospinal fluid (CSF)), in order to identify a potential lipid signature of TBI which may have unique value in the growing armamentarium of biomarkers for this condition. The CNS is enriched in specific lipids, i.e. fatty acids,

phospholipids (PL), sphingolipids, glycerolipids and sterols, with complex structural and signalling roles [25, 27]. These species are the major lipids in the studies discussed here, and they are briefly reviewed below. Their general structures are shown in Figure 1 [28].

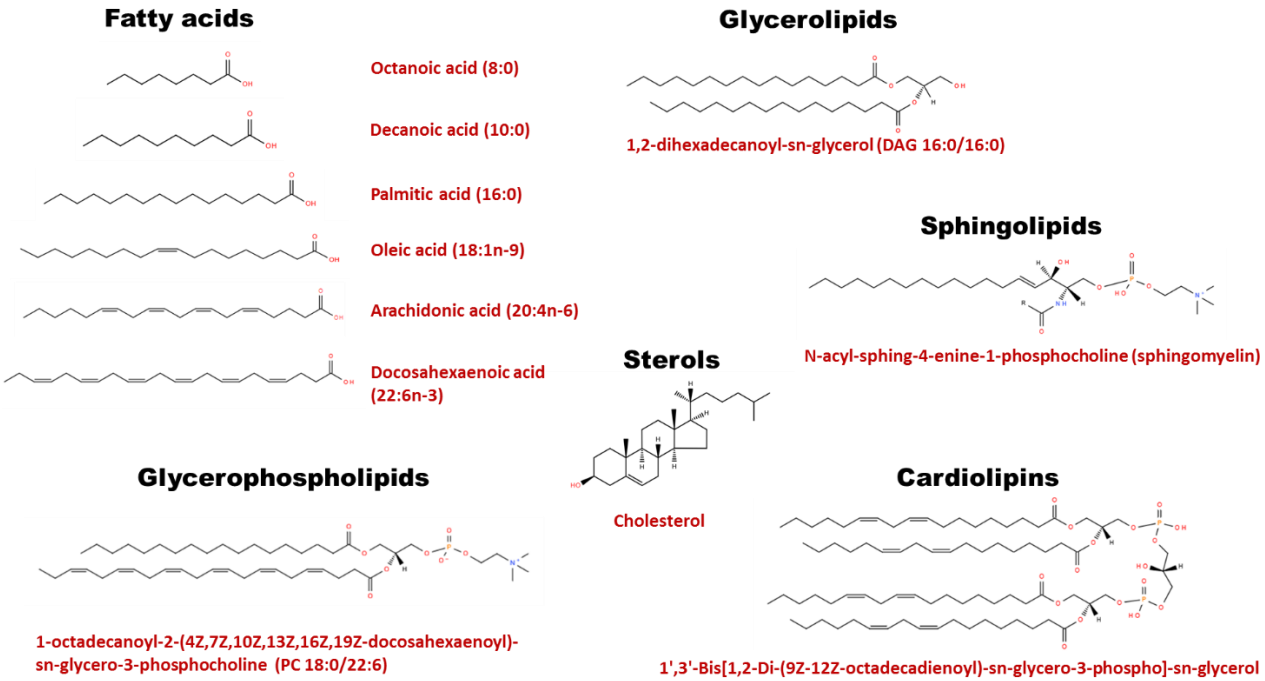

Figure 1: *Examples of lipid species structures from the main classes of lipids (LIPID MAPS; https://www.lipidmaps.org/)*

Fatty acids are essential building blocks of more complex lipids. They have varying chain lengths and can be saturated (e.g. palmitic acid, 16:0), monounsaturated (e.g. oleic acid 18:1n-9) or polyunsaturated (e.g. α-linolenic acid, 18:3n-3). Polyunsaturated fatty acids such as arachidonic acid (ARA, 20:4n-6) or docosahexaenoic acid (DHA, 22:6n-3), have structural and signalling roles, can activate a variety of receptors and can also give rise to a large array of lipid mediators, such as the ARA-derived eicosanoids and the DHA-related docosanoids [29, 30]. *Phospholipids* (or glycerophospholipids) are integral components of cell membranes and are by far the most abundant lipid species in the brain (45% of the dry weight). They have a glycerol backbone whose hydroxyl groups in the *sn-1* and *sn-2* positions are linked to fatty acids, whereas the polar phosphodiester group is linked to the *sn-3* carbon. The esterified acyl residues can be saturated (e.g. palmitic acid or stearic acid) or unsaturated, the latter ranging from monounsaturated fatty acids (e.g. oleic acid) to polyunsaturated fatty acids (e.g. ARA or DHA). In general, PL have in *sn-1* a saturated fatty acyl residue, and in *sn-2* an unsaturated fatty acyl residue. Major PL species in cell membranes, based on the different polar group at *sn-3*, include phosphatidylcholine (PC), phosphatidylethanolamine (PE),

phosphatidylserine (PS) and phosphatidylinositol (PI). In mammalian cells, the distribution of PL differs between the outer vs. the inner leaflet of the membrane lipid bilayer: the outer leaflet contains mainly PC, whereas PS and PE are found in the inner leaflet [31]. There is also a difference in the lipid composition between grey and white matter, as reported in humans [32]. PE is the most abundant in grey matter (30.7%), followed by PC (25.1%), cholesterol (19.6%), PS (7.2%), PI (3.9%), and sphingomyelin (SM) (3.2%). In white matter, cholesterol dominates (26.9%), followed by PE (19.6%), PC (11.8%), PS (9%), SM (4.4%), and PI (4%). Modulation of PL composition is essential for maintaining cellular and subcellular structures and for creating distinct lipid microdomains within membranes. The hydrolysis of PL by phospholipases, which cleave acyl residues in *sn-1* or *sn-2*, produces lysophospholipids (lysoPL), which can act as intrinsic regulators of various biological processes [33]; for example, specific lysoPL species such as lysoPC, can have pro-inflammatory effects in nervous tissue [34, 35]. *Sphingolipids* are derived from the amino-alcohol sphingosine and include ceramides, phosphosphingolipids, such as SM, and glycosphingolipids. SM is distinct from glycerol-containing PL, as it is composed of mostly long saturated fatty acid chains. *Glycerolipids* are mono-, di- or tri-substituted glycerols, i.e. they have a glycerol backbone linked to a variable number of fatty acyl residues. Diacylglycerols can be a source of neuroactive endocannabinoids, such as 2-arachidonoylglycerol, and the disruption of this biosynthetic pathway affects neurogenesis and synaptic plasticity [36]. *Sterols* are also important components of membrane lipids; cholesterol, a major sterol in mammals, is highly enriched in the brain, which contains about 20% of the body cholesterol [37]. It is essential for normal neural development, a precursor of neurosteroids such as dehydroepiandrosterone and allopregnanolone, and like SM, it is a major constituent of myelin. *Cardiolipins* are glycerophospholipids which are essential for the structure and function of mitochondria, where they accumulate in the inner membrane [38]. Cardiolipins contain a glycerol backbone and two phosphatidylglycerols, with four fatty acid chains [39]. Compared to other PL, cardiolipins are present in cells in low abundance [39]. There is a large number of cardiolipins in the brain, with longer chain fatty acids which are more unsaturated [38]. Using gas cluster ion beam secondary ion mass spectrometry, regional specific variations have been reported in the brain, with more unsaturated species with longer carbon chains localised in the cortical regions and cardiolipins with shorter chains and fewer double bonds in the hippocampus [39]. Comparison of human brain and heart cardiolipins revealed 26 brain specific cardiolipin species [40]. The distinct brain cardiolipin profile has also been confirmed in rats [40].

2. Pathophysiology of TBI

TBI may be penetrating or non-penetrating, focal or diffuse, and can be accompanied by concomitant traumatic injury to other parts of the body. It is an event resulting from the absorption by the brain of

part of the energy associated with an external mechanical force (linear, rotational, and translational), not necessarily acting directly on the head, and causing acceleration/deceleration of the cerebral tissue. The energy associated with blast waves is also capable of producing head injuries similar to those occurring when a mechanical force is acting. These different types of forces can be mimicked experimentally in animals (mostly rodents), in models such as: the controlled cortical impact (CCI), the fluid percussion, the weight-drop or the blast wave model [41]. The impact of the injury in these models can be assessed both in terms of tissue damage and in terms of neurological impairment, using various behavioural tests.

The primary injury in TBI is a direct consequence of the impact of such forces on the brain. The damage created during the primary phase leads to a cascade of processes which amplify the injury - this constitutes the secondary injury phase. This phase is characterised by mitochondrial dysfunction and energy deficit, oxidative stress, cell death, neuroinflammation and may or may not be accompanied by hypoxia or ischaemia [42-44]. The depletion in energy stores and the collapse in ionic gradients lead to an overflow of the excitatory amino acids glutamate and aspartate and a massive influx of calcium. One of the consequences of the increased intracellular calcium levels is the activation of phospholipases. This is a superfamily of hydrolase enzymes, divided into groups and subgroups based on their specific patterns of cleavage; these enzymes can modify the composition of cellular membranes, by cleaving PL and releasing free fatty acids and diacylglycerols [45, 46]. One of the most well studied enzymes in this family is phospholipase A_2 (PLA_2), which is specific for the *sn-2* position of the glycerol phosphate backbone of PL. The released free fatty acids, such as ARA (an omega-6 fatty acid) and DHA (an omega-3 fatty acid) can be further metabolised into eicosanoids and docosanoids, which have powerful modulatory effects on the inflammatory processes triggered by the injury.

Shohami *et al.* [47] were the first to report changes in PLA_2 activity in a TBI model, i.e. closed head injury in rats. At 15 minutes after trauma, a 75% increase in PLA_2 activity was found at the injury epicenter and at 4 hours, the whole contused brain hemisphere showed elevated PLA_2 activity. At the injury site, PLA_2 activity was still 245% of control values at 24 hours after injury. A significant correlation was found between the elevation in PLA_2 activity and the levels of the proinflammatory eicosanoid prostaglandin E_2 (PGE_2) in the injured hemisphere. The elevation in PGE_2 was abolished when animals were pre-treated with a PLA_2 inhibitor. These results showed that increase in brain phospholipase activity is a very early event following neurotrauma, and that this mechanism is involved in the degradation of PL, and the subsequent increased production of fatty acid-derived lipid mediators, which have intrinsic biological effects and contribute to the pathophysiology of TBI. The three decades since these first observations were reported have seen a steady accumulation of studies on lipid changes after TBI, in patients and animal models, which will be discussed below.

3. Lipid changes in human traumatic brain injury

3.1. Serum and plasma

3.1.1. Phospholipids

More than four decades ago, Heller and colleagues reported changes in serum PL in 4 patients with brain injury and subsequent hypoxia [48] . Patients with acute hypoxia had an increase in PL, whereas a decrease was seen in patients with chronic hypoxia. The study did not analyse specific PL classes. In 2016, a study in adolescent ice hockey players showed that 55 hours post-concussion, 82% of the plasma metabolite variance between players with or without concussion was explained by 10 components, each including 9 metabolites [49]. The first component, accounting for 28.21% of the variance, consisted solely of PC species. PC, lysoPC, as well as SM, were also part of the other components. In the same year, Emmerich *et al.* reported a detailed lipid analysis of plasma from soldiers with mild TBI (no further description), incurred several years before the study, i.e. during military service in 2008-2010 [50]. Significant decreases in plasma levels of PC (-19%), lysoPC (-24%), PE (-26%), lysoPE (-24%), PI (-30%) and SM (-17%) were detected, compared to the levels in control soldiers. Changes were more marked in soldiers who had also developed post-traumatic stress disorder. Further analysis of the various lipid species within these classes, showed that the decreases were seen across saturated-, monounsaturated-, and polyunsaturated fatty acid-containing species. The study also assessed the impact of isoforms of the apolipoprotein E (ApoE) on these changes. ApoE is a protein involved in lipid metabolism and transport, and the ApoE gene has three alleles: ApoE2, ApoE3 and ApoE4. Some differences were noted as a function of the ApoE4 genotype. Thus, ApoE4-negative individuals with mild TBI showed significant decreases in monounsaturated fatty acid-containing lysoPC, whereas ApoE4 positive individuals showed no difference compared to controls. Similar results were seen for saturated-, monounsaturated-, and polyunsaturated fatty acid-containing PE species. In individuals with mild TBI, the ratio of ARA to DHA was also significantly decreased in PE. When data was stratified for the ApoE4 genotype, the non-carriers had significantly lower PI than the controls, and ApoE4 carriers had significantly higher lysoPC levels than the controls, whereas no difference was detected for non-carriers. Ether PL (in which the glycerol backbone has an ether or vinyl-ether bond at the *sn-1* position) levels showed similar patterns of change. Ether PE was reduced by 25% in mild TBI, whereas no difference could be seen in ether PC. The ApoE4 genotype also influenced the ether PE, with significantly lower ether PE in ApoE4 non-carriers compared to carriers, which was also seen for etherPC. This trend was also seen for etherPC species. In a subsequent analysis, the same group also found no effect of mild TBI on PS plasma levels in soldiers [51]. The degree of saturation of PS was not changed by mild TBI and no overall effect of the ApoE4 gene was detected. However, PS 38:4 was increased in the ApoE4 non-carrier mild TBI group

compared to ApoE4 non-carrier controls. Using plasma metabolomics, a 6-metabolite panel was characterized in a cohort of collegiate athletes, discriminating concussed athletes from age-, sex- and sports-matched controls within 6 hours, as well as at 2, 3, and 7 days post-injury [52]. The panel consisted of five lipids, including increased PE P-16:0/20:4, and lysoPC 20:4/0:0, and decreased PE 16:0/22:6, and was validated in an independent, clinical TBI cohort.

3.1.2. Cholesterol

In active duty soldiers, there was no effect of mild TBI on total cholesterol levels or its degree of unsaturation in plasma [51]. However, the ApoE4 status had an effect. Cholesterol esters of chain length 20 were significantly higher in plasma in non-carriers after mild TBI compared with control non-carriers.

3.1.3. Free fatty acids

The early report from Heller and colleagues indicated an initial loss of triglycerides and esterified fatty acids, which corresponded with an increase in free fatty acids [48]. As part of the TBICARE project, Orešič and colleagues identified the fatty acids 8:0 and 10:0 as upregulated metabolites in the serum of patients with moderate and severe TBI, within 12 hours of the injury [53]. Both remained elevated during the first week after TBI. Metabolites in patients with mild TBI followed the same pattern, however the upregulation was not as pronounced. Furthermore, elevated levels were associated with poor outcome in these patients, based on the Glasgow Outcome Scale Extended. The 6-metabolite mild TBI (concussion) panel described by Fiandaca and colleagues also included two fatty acids, increased 18:0 and decreased oxidised 16:0 [52]. Thomas and colleagues found a positive correlation between serum metabolite cluster 4 (fatty acids, e.g. ARA) and positive MRI findings in the right caudate, left lateral ventricle, right lateral orbital gyrus, right middle frontal gyrus and left middle occipital gyrus in TBI patients [54]. Furthermore, amongst others, serum linoleic acid levels were indicative of a positive MRI finding.

3.1.4. Cardiolipin

To our knowledge, cardiolipin has not been measured in human plasma after TBI. However, Anthonymuthu and colleagues have measured nine brain specific cardiolipins in the plasma of patients with cardiac arrest, within 6 hours of resuscitation [40]. A combination of three of these cardiolipin species (70:3, 72:5, and 78:11) correlated well with three clinical measures of neurological injury (Full Outline of UnResponsiveness score, GCS, Pittsburgh Cardiac Arrest Category), with higher values in patients with poor neurological or functional outcome, and predicted poor discharge status. Cardiolipin (70:5) on its own, with a cut off of 0.93 pmol/mL, was 83% sensitive and 90% specific in

predicting neurological outcome, and thus might be a suitable biomarker for brain injury in this context.

3.2. Cerebrospinal fluid

3.2.1. Phospholipids

In 2003, Kay and colleagues measured PL in the lipoprotein fraction of ventricular CSF pooled from 27 patients with severe TBI (GCS<8 within 24 hours of admission) [55]. Comparison with 6 pooled lumbar CSF samples from 150 controls (patients with suspected neurological disease), showed a significant increase in PL (0.29 ± 0.09 μM vs. 0.44 μM) after TBI. Pasvogel and colleagues measured PL changes over 6 days in the CSF of 10 patients with TBI, with a range of GCS from 3 to 11 at admission [56]. Levels of PE and PC were above normal ranges on day 1-5, and PS and lysoPC were elevated on day 1-6, indicating a disruption of cell membranes in the CNS after TBI. The group further analysed the data stratified for patients who survived (6/10) and who died [57]. All PL levels were elevated above normal CSF levels, and all levels apart from lysoPC on day 3 and SM on day 1, were higher in patients who died, than in those who survived. PE, PC and SM levels were only slightly higher in patients who survived than in control patients, whereas levels of PS and lysoPC were also elevated in surviving patients. A second increase in PE, PC, PS, and SM was seen on day 4 (last measurement before death), potentially indicating secondary injury.

3.2.2. Cholesterol

In a study assessing cholesterol in the CSF of patients with TBI in the acute phase (exact time unspecified), a pooled sample of 27 severe TBI patients showed a significant 5-fold increase in non-esterified cholesterol in the lipoprotein fraction of ventricular CSF compared to controls [55].

3.2.3. Free fatty acids

A study measuring free fatty acid levels in CSF after TBI, showed significantly higher levels of myristic acid, palmitic acid, oleic acid, linoleic acid, ARA and DHA in 15 patients within 48 hours of the insult, compared to control patients without TBI [58]. In general, free fatty acids were significantly elevated at 24 and 48 hours after injury and returned towards control levels at 96 hours post-injury. Furthermore, higher CSF levels of total polyunsaturated fatty acids (linoleic acid, ARA and DHA combined) as well as myristic acid, palmitic acid and ARA individually, one week after TBI, were associated with a worse outcome at discharge, defined as a Glasgow Outcome Score of less than 4, which indicates severe disability or death. Similarly, the same group found significantly higher levels of the same fatty acids in CSF within 24 hours in patients with subarachnoid haemorrhage (mainly from ruptured aneurysms) [59], indicating that this pattern is not specific for TBI. Medium-chain fatty

acids, including 8:0 and 10:0, which were upregulated in the serum of TBI patients, were also detectable in high concentrations in the brain microdialysates from the patients [53].

Free fatty acids increase when PL are degraded. Another end-product of PL degradation is glycerol, and several studies have measured glycerol in microdialysis samples after TBI [60-62]. Significantly higher glycerol levels were seen in TBI patients with unfavourable prognosis [61], and in the first 72 hours in TBI patients who died [62]. Furthermore, a peak interstitial glycerol level above 150 µmol/L had a 100% positive predictive value for an unfavourable outcome, therefore indicating that glycerol levels correlate with the severity of brain damage [60].

3.3. Brain tissue

3.3.1. Cardiolipin

Two-dimensional liquid chromatography mass spectrometry analysis of brain tissue from the right temporal lobe from a patient with severe TBI in the acute stage revealed numerous oxidised cardiolipins in the penumbra of the contused brain tissue, but no oxidation products of PC or PE [63]. In contusional brain tissue from severe TBI patients, increased mitophagy has been reported, along with a decreased mitochondrial to genomic DNA ratio [64]. Animal experiments confirmed increased mitochondrial autophagy early after CCI [64], and studies in primary cortical neurons indicate that mitophagy is mediated via the translocation of cardiolipin to the outer mitochondrial membrane [65]. Therefore, it could be speculated that cardiolipin also mediates mitophagy in human TBI.

4. Lipid changes in experimental traumatic brain injury

4.1. Serum and plasma

4.1.1. Phospholipids

Several experimental TBI studies have reported alterations in plasma PL. Three months after CCI, 6 months old male C57BL/6 mice had overall lower PC, PE and PI levels in plasma [66]. The decrease in PI was significant and equally distributed between saturated-, monounsaturated-, and polyunsaturated species. PC and PE only showed significant decreases in monounsaturated species. Additionally, the DHA to ARA ratio was significantly lower within the PE species. Similar results were reported by the same group using a closed head injury mouse model, to simulate mild TBI [67], and over a longer timeline, i.e. at 3, 12 and 24 months post-injury. Levels of PC, lysoPC, PE, lysoPE, and PI were significantly decreased compared to their own 24 hours post-injury values. This decrease was injury specific and was greater than the effect of normal ageing. No major changes were seen in plasma PL after mild TBI at the acute time point (24 hours) vs. controls, whereas significant changes occurred in the chronic phase. Relative to control mice, plasma levels of PC, lysoPC,, PE, lysoPE and PI were lower in TBI mice at all chronic time points, with significantly lower levels of PC, PE and PI at 3,

12 and 24 months, lower lysoPC and lysoPE at 3 and 24 months, and lower SM at 24 months only. This is consistent with reported lower plasma PL in soldiers at a chronic time point after mild TBI [50]. Overall, results indicated a decrease in PL in the injury phase from 24 hours to 3 months, followed by a recovery phase to 6 months, and PLs remained overall lower in TBI mice than control mice up to 24 months [67]. The analysis showed that the decreases in PL were evenly distributed between saturated-, monounsaturated-, and polyunsaturated fatty acids at 3 and 24 months for PC and lysoPC. In contrast, at 3 months only saturated and polyunsaturated fatty acid-containing PE decreased, and only saturated and monounsaturated lysoPE species decreased. At 12 months post-injury, levels of saturated and polyunsaturated PC species were decreased, and in PE and PI only polyunsaturated species decreased, whereas only monounsaturated species were affected in lysoPE. Twenty-four months after injury, polyunsaturated PE, lysoPE and PI were decreased, and additionally monounsaturated lysoPE species decreased. Apart from a significant increase in saturated PE species, no other PL was affected in the acute phase (24 hours). EtherPC, ether lysoPC, and ether lysoPE were not different in plasma from TBI animals compared to control animals. However, ether PE was significantly higher at 24 hours, but significantly lower at 3, 12 and 24 months post-injury in TBI animals. In general, DHA-containing PL species were decreased after injury, and only etherPC-containing DHA was significantly increased at 24 hours post-injury. At 12 months post-injury, the majority of DHA-containing PL species were significantly decreased. A similar pattern was seen for ARA-containing PL species. Overall, this experimental closed head TBI model led to changes similar to those seen in soldiers with chronic mild TBI.

Hogan and colleagues used untargeted lipidomics and identified a panel of 26 serum lipids that discriminated between rats with TBI (CCI of the lateral frontoparietal cortex) and control rats with 85% accuracy [68] at a subacute time, i.e. day 3 and 7 post-injury. Of these lipids, 7 PL (PE 20:4/16:0, 18:2/18:0, 18:0/22:4; PC 20:2/18:0, 16:0/16:0, 18:2/16:0, 18:2/22:1; SM d18:1/22:1; lysoPC 20:2) were decreased relatively to controls, whereas PS 16:0/20:4 and oxidised lysoPC 18:2 were increased. All PL, apart from SM, were not significantly different between day 3 and day 7 post-injury. Using a metabolomics approach, Bahado-Singh and colleagues were able to clearly discriminate closed head TBI mouse serum samples from control serum samples, based on decreased levels of PC 34:4 and methionine sulfoxide in the acute phase (4 and 24 hours post-injury) [69]. A 6-component serum metabolomic panel including SM 18:0 and 18:1 could be used to differentiate early (4 hours) from late (24 hours post-injury) serum samples, with higher levels in the latter. Plasma PC was not altered 1 hour after lateral fluid percussion in rats, although changes in brain PC were identified [70]. In a pig TBI model, serum PL metabolism was altered 24 hours post-CCI, but no longer at 7 days [71].

Sheth and colleagues used a targeted lipid profiling approach to detect changes in rat plasma sphingolipids [72]. Sphingolipids, mostly SM, increased after CCI. The highest increase was seen in two SM species; SM 37:1 was significantly increased at 4, 24 and 48 hours, and SM 38:8 was significantly increased at 24 and 48 hours after TBI. Both returned to normal by 7 days after injury. The increase in SM was correlated with the lesion volume. Another group investigating SM changes used a blast injury model of TBI. Four days after mild to moderate blast trauma in mice, SM 22:0, 24:0 and 22:1 were significantly reduced in plasma compared to their level in control mice [73]. This was accompanied by a decrease in the precursor dihydro SM 22:0 and 24:0. In contrast, dihydro SM 16:0 was increased significantly, and a non-significant increase in SM 16:0 was also recorded.

4.1.2. Cholesterol

The 26 lipid panel reported by Hogan and collaborators (see above), differentiating control animals from rats with TBI, also included cholesterol sulphate, which was significantly reduced in serum after TBI compared to control animals in the first week after injury [68].

4.1.3. Free fatty acids

Hogan *et al*. showed that the free fatty acid 18:0 was significantly more abundant in rat TBI samples [68]. The release of free fatty acids after focal cold injury was also studied in cats [74]. When no subsequent cerebral ischaemia developed, plasma levels at 5-7 hours post-injury were similar to levels before the injury. In animals with a malignant increase in intracranial pressure and subsequent cerebral ischaemia, plasma levels of 16:0, 18:0, 18:1, 18:2 were significantly increased. In CSF, most free fatty acids were too low to measure prior to injury. In contrast, almost all fatty acids were higher in oedema fluid 5-7 hours after injury than in the control CSF. However, they were below the levels in plasma, with the exception of the omega-6 fatty acid ARA, which was higher in the interstitially drained oedema fluid.

4.1.4. Cardiolipin

In naïve rat plasma, cardiolipins make up less than 0.001% of PL [38]. After CCI injury in postnatal day (PND) 17 rats, the phospholipidome was significantly different between naïve and TBI groups, at 4 and 24 hours. Cardiolipins were mainly responsible for the differences, and a time-dependent enrichment in brain specific cardiolipins was noted. The increase in these brain specific cardiolipins at 24 hours post-injury correlated significantly with the decrease in these species in the injured cortex. Increased cardiolipins in plasma included cardiolipin 70:3 and cardiolipin 72:5 [38]. Both these cardiolipins were also increased in the plasma of cardiac arrest patients with neurological injuries [40], as described above.

4.2. Brain tissue

4.2.1. Phospholipids

4.2.1.1. Single TBI

Four and 24 hours after fluid percussion injury, rats had significantly lower PL levels in the perilesional area [75]. A slight decrease was already seen 10 minutes after injury. After a single CCI in juvenile (PND17) rats, a significant increase in lysoPC was found 3 hours post-CCI in the lesioned cortex [76]. In a subsequent study, a 2- and 5- fold increase in lysoPC, and a 1.7- and 5-fold increase in lysoPE were shown in the pericontusional cortex at 4 and 24 hours post-injury, respectively [38]. In both studies, PL did not change significantly at these time points. Similarly, concussion induced by an impact acceleration weight drop model, produced no significant changes in brain PC content in the acute and subacute period (2, 6, 24, 48 and 120 hours post-injury) [77]. However, 1 hour after lateral fluid percussion injury, ^{1}H NMR metabolomics identified a significant decrease in PC and glyceroPC of 23% and 19% in the cortex and hippocampus of adult rats, respectively [70]. Twenty-four hours after cryogenic injury, PL containing ARA and DHA were significantly reduced in the injured hemisphere of rats [78]. Chitturi and colleagues noted a significant increase in PC and PL biosynthesis in the injured hemisphere of rats (PND31) at 72 hours post-lateral fluid percussion [79]. Bayir and colleagues found no changes in SM in the acute phase (3 hours post-injury) of TBI in rats [76]. In contrast to this, significantly elevated levels of SM were shown in the lesioned hemisphere of mice in the subacute phase, 2 and 7 days after CCI [80]. In particular, SM species containing 14:0, 16:0, 24:0 and 24:1 were increased, with increases in SM 14:0 and 16:0 already seen at 24 hours. The precursor dihydrosphingosine was also significantly increased at 1, 2 and 7 days post-injury. One and 3 days after CCI, the metabolic profile of TBI rats and control rats in the hippocampus was significantly different [81]. Although the identified biomarker panel did not include PL, the mechanistic pathway analysis identified proteins involved in PL metabolism as well as fatty acid degradation. Abdullah and colleagues investigated brain PL changes at a chronic time point (3 months after injury) [66]. Additionally to the changes in plasma PL levels described in the previous section, PC, SM, and PE were significantly increased (+28%, +37%, +32%, respectively) in the hippocampus, but decreased (-34%, -25%, -31%, respectively) in the cerebellum. In the cortex, PC (-7%) and PE (-18%) were significantly decreased. In contrast to PI plasma levels, no changes were seen in tissue PI levels. Increases in the hippocampus were mediated by significant increases in PC saturated and monounsaturated species, monounsaturated species in PE, and saturated species in PI. In the cortex, significant decreases in polyunsaturated PC species, and across all PE species, were seen. Cerebellum analysis showed significantly decreased PC (all species) and monounsaturated PE species. The DHA to ARA ratio was also altered by TBI. Decreases in this ratio were seen in PC and PE in the hippocampus and the cortex, and in PI in the cortex. An increase in the DHA to ARA ratio was seen in the cerebellum in PE and in

the hippocampus in PI. EtherPC increased significantly in the hippocampus and decreased significantly in the cerebellum, whereas the only change in ether PE was a decrease in the cortex.

Bayir and colleagues investigated oxidative modifications in PL after CCI in the juvenile PND17 rat model [76]. Three hours post-injury, no oxidation of PL was detected, but at 24 hours after injury, PS was markedly oxidised in the ipsilateral cortex, especially PS species containing DHA. PE, PC, and PI were slightly oxidised at this time point. This indicates a specific PL species oxidation after TBI, since oxidation did not follow the relative abundance of PL species. These results were confirmed in a later study with a similar setup. No change in oxidised PC and PE occurred 3 hours after CCI in juvenile rats [63].

4.2.1.2. Repetitive TBI

Ojo and colleagues used a repetitive injury model (5 hits over 9 days) in adult C57BL/6 mice [82]. This resulted in an increase in total cortical PC at 24 hours and 12 months. Total PI significantly decreased at 3 and increased at 12 months. LysoPE was significantly increased at 24 hours, 3 and 12 months post-injury. The increases in PC at 24 hours and 12 months were seen amongst all levels of unsaturation. Changes in PI were mediated through significant decreases in polyunsaturated fatty acid-containing species at 3 months and increases in polyunsaturated fatty acid-containing species at 12 months. ARA and DHA-containing PC were increased at 24 hours and 12 months, whereas ARA-containing PI were decreased at 3 months post-injury. Additionally, an increase in total ether PC was shown at 24 hours and 12 months. In the hippocampus, total PE, PC, SM significantly increased at 24 hours, 6, and 12 months compared to sham mice. PI increased at 24 hours and 12 months. LysoPE increased at 3, 6, 12 months, however lysoPC only increased at 24 hours post-injury. The increases at 24 hours were distributed equally amongst saturated-, monounsaturated-, and polyunsaturated fatty acid-containing species. Six months after injury, the increase was evenly distributed amongst PE, whereas only polyunsaturated PC and monounsaturated SM increased. At 12 months post-injury, saturated-, monounsaturated-, and polyunsaturated PE species increased, whereas within PC, only monounsaturated and polyunsaturated species increased, and only saturated and monounsaturated SM species increased. Significant increases in hippocampal ARA-containing PC, PE, and PI were shown at 24 hours and 12 months. ARA-containing lysoPC increased 24 hours after the last injury. DHA-containing species increased in PC at 24 hours and 12 months, in PE at 24 hours, 6 and 12 months, in PI at 12 months and in lysoPC 24 hours after the last injury. Ether PE increased at 24 hours, 6, and 12 months, while ether PC only increased 6 months after the last injury. Muza and colleagues also used a repetitive mild TBI model with 3 CCI per week, over one month [83]. They demonstrated an ApoE genotype specific effect on PL. Overall, ApoE4-positive mice had significantly higher levels of SM and PC compared to ApoE3-positive mice; and TBI showed an injury effect in ApoE4-positive mice, with

462 significantly higher levels of SM (10%) and PC compared to sham operated ApoE4-positive mice. The
463 increase was seen across saturated-, monounsaturated-, and polyunsaturated PC species.

464 **4.2.1.3. Imaging Studies**

465 Mass spectrometry imaging offers the advantage of displaying regional differences in PL with high
466 spatial resolution, compared to tissue analysis on brain homogenates. In a rat CCI model, Roux and
467 colleagues showed that low mass SM, which is normally detected in the ventricular region, was
468 detected in the injury area 3 days after injury [84]. Intermediate mass SM decreased in the region of
469 injury at day 1 and 3, but this change was not significant. Overall SM decreased in grey matter at 1 day
470 post-injury, but increased at day 3 and 7. Ceramide (several species), a breakdown product of SM,
471 showed higher levels at the injured site between day 1 and 3, followed by a drop on day 7. PE 16:0/22:6
472 and PE 18:0/22:6 decreased significantly in the injured area at day 3 post-injury. Similarly, PC showed
473 decreases at 24 hours post-injury, and PC and PI showed decreases at day 3, followed by increases at
474 day 7. DAG, a product of hydrolysis of PL by phospholipase C, was increased at 3 days, but the
475 increased levels were attenuated by day 7. Han and colleagues showed that the CCI lesion and
476 perilesional area 3 days after injury could be distinguished from an uninjured area in the contralateral
477 cortex using principal component analysis [85]. Part of the discriminating lipid profile was an
478 upregulation in PC 16:1/16:0 and a downregulation of PE 28:5, PS 18:0/22:6 and PI 38:4. Li and
479 colleagues found a downregulation of PI in the peripheral region of a TBI injury in rats 3 days post-
480 injury [86]. In juvenile PND17 rats, PI *m/z* 883.5 and 885.5 were both significantly decreased at the
481 point of impact 3 hours post-injury and additionally, *m/z* 885.5 was significantly decreased in the
482 hippocampus and *m/z* 883.5 in the thalamus [87]. No changes were noted in the adjacent cortical
483 region.

484 Mallah and colleagues used a three dimensional matrix-assisted laser desorption/ionization-mass
485 spectrometry imaging (MALDI-MSI) approach to study the dynamics of lipid changes after CCI in the
486 rat cortex, and found specific lipid profiles in the uninjured cortex, the lesion interior and the lesion
487 exterior, 3 days post-injury [88]. They showed that PE 38:1/PC35:1 was highly expressed in the
488 uninjured cortex but was absent in the lesion, whereas PC 38:7 and PC 38:4 were both highly
489 expressed in the lesion but not in the contralateral cortex. Furthermore, PC 42:9 and *m/z* 723.508
490 were elevated at the injured site.

491 Gou and colleagues specifically monitored changes in DHA-containing lipids after TBI in adult rats, in
492 the fluid percussion injury model [89]. Three days after injury, DHA and lysoPE 22:6 were significantly
493 increased in the injury area, but PE P-18:0/22:6 (the plasmalogen form), PE 18:0/22:6, and PS
494 18:0/22:6 were significantly decreased. DHA was also significantly increased at 1 day post-injury at the

injury site and then decreased gradually, but stayed significantly higher than in controls. LysoPE 22:6 was increased at day 1 and further increased until day 3, and returned to normal afterwards. Lysophosphatidylglycerol 22:6 levels were similar to controls over the first 3 days and increased significantly on day 5 and day 7 post-injury in the lesion area. PE P-18:0/22:6 was significantly decreased in the injured area from day 1 and stayed significantly lower for the 7 days post-injury. PE 18:0/22:6 and PS 18:0/22:6 were significantly decreased from day 1, and increased afterwards until day 7 post-injury, when they were still significantly lower than in controls.

4.2.2. Cholesterol

Adult rats receiving a cryogenic brain injury had an 18% decrease in cholesterol in the lesioned hemisphere 24 hours post-injury [78]. Similarly, a significant decrease in cholesterol was seen in rats at 10 minutes, 4 hours and 24 hours after fluid percussion injury in the perilesional area [75]. The study by Roux and collaborators showed a strong signal for cholesterol throughout the rat adult brain, and it was decreased at 3 and 7 days post-CCI in the injured area [84]. Interestingly, this was followed by an increase in cholesterol in the corpus callosum, dentate gyrus and the internal capsule after 3 days. Cholesterol esters were not detected in the brain in control animals, but increased significantly in the injured area at 3 and 7 days post-injury, the time points at which the decline in the cholesterol signal was seen.

4.2.3. Free fatty acids

Rats subjected to fluid percussion injury had significantly higher free fatty acid levels (+119%) in the hippocampus 5 minutes after injury [90]. At 20 minutes post-injury, levels were still elevated, but this was no longer statistically significant. A similar pattern was seen for 18:0 and ARA, which both increased significantly at 5 minutes (+180%) and stayed elevated at 20 minutes. No change in 16:0 and 18:1 was seen. In another fluid percussion study, all free fatty acids (16:0, 18:0, 18:1, 18:3, ARA and DHA) were significantly higher in the fluid percussion-injured area in rats at 4 and 24 hours post-injury [75], and 18:0 and ARA were already significantly increased 10 minutes after the injury.

Dhillon and colleagues subjected rats to CCI and assessed free fatty acids at 30 minutes, 2.5 hours and 24 hours after the injury [91]. All free fatty acids (16:0, 18:0, 18:1, ARA) were significantly higher in the injury site compared to control animals at all time points. In the region adjacent to the injury site, only 18:0 and ARA were significantly elevated at all time points. In the ipsilateral hippocampus, ARA was significantly increased at 30 minutes and 2.5 hours after the injury and 18:0 was significantly increased 2.5 hours after the injury. In the contralateral cortex, only ARA at 2.5 hours post-injury was elevated. Similarly, total free fatty acids, as well as 16:0, 18:0, 18:1, and ARA increased significantly in the injury area in the cortex of male rats 30 minutes after the injury [92]. At 6 hours, total free fatty

acids, as well as 16:0, 18:0, and ARA were still significantly elevated. In the region adjacent to the injury, total free fatty acids as well as 16:0 and 18:0 were also significantly elevated 30 minutes after the injury, and remained so at 6 hours after the injury, including ARA levels. In the ipsilateral hippocampus, total free fatty acids as well as 16:0, 18:0,18:1 and ARA were significantly elevated 30 minutes post-injury, and at 6 hours all fatty acids returned to control levels. In the contralateral cortex, no change in free fatty acids was seen at any time point. Homayoun and colleagues investigated changes in free fatty acids in rats subjected to CCI, at a subacute (4 days) and chronic (35 days) time point [93]. At both time points, significantly higher free fatty acids were detected in the frontal right cortex (injury site), compared to sham operated animals, with much higher levels at 4 days post-injury. Free fatty acids were also significantly elevated in the contralateral frontal left cortex; however, these increases were less than in the injured cortex. In other regions (occipital cortex, hippocampus, cerebellum and brainstem), total free fatty acids did not change after TBI. Individual free fatty acids (16:0, 18:0, 18:1, ARA, and DHA) were all significantly increased at 4 days post-injury in the injured cortex, whereas on the contralateral side all free fatty acids were increased, but only the increase in DHA was significant. Additionally, increases in ARA and DHA were significant in the occipital right cortex. These changes remained stable at 35 days post-injury for 18:0, 18:1 and ARA in the frontal right cortex, and for ARA in the occipital right cortex. Furthermore, at 35 days post-injury, 18:0, 18:1 and ARA were significantly increased in the frontal left cortex. Lipid metabolism was significantly increased in the injured hemisphere of rats (PND31) compared to sham TBI and TBI uninjured hemispheres at 72 hours post-fluid percussion [79]. Furthermore, the uninjured hemisphere also showed a significant increase in lipid metabolism, compared to sham TBI. In a pig TBI model, ARA metabolism was altered 7 days after CCI in the grey and white matter of the injured hemisphere [71]. Glycerolipid metabolism was also affected.

Anthonymuthu and colleagues investigated the time course of fatty acid oxidation after CCI in juvenile rats (PND17) [94]. The amount of non-enzymatic oxidised lipids was similar in TBI and in naïve animals, therefore, the majority of oxidised lipids were generated by enzymatic oxidation. One hour after TBI, 16 octadecanoids, 89 eicosanoids, 96 docosanoids, and 20 oxidised ultra-long chain polyunsaturated fatty acids were significantly increased. Of these, 3 octadecanoids, 47 eicosanoids, 55 docosanoids, and 10 ultra-long chain polyunsaturated fatty acids remained elevated at 4 hours, and 14 eicosanoids, 4 docosanoids, and 1 ultra-long chain polyunsaturated fatty acid remained elevated at 24 hours post injury. Of the elevated docosanoids, 8 were specialised pro-resolving mediators. Overall, both pro- and anti-inflammatory oxidised free fatty acids were produced after TBI and were elevated at 1 hour after injury, and at 4 hours both decreased, but were still above naïve levels. Pro-inflammatory

mediators returned to normal at 24 hours post-injury, while anti-inflammatory mediators remained elevated.

4.2.4. Cardiolipin

TBI leads to an increased energy demand in the brain, which results in increased mitochondrial respiration and subsequent reactive oxygen species production. This oxidative stress can damage mitochondria, which in turn will translocate cardiolipin to the outer mitochondria membrane resulting in autophagy, as early as one hour after CCI, in juvenile (PND17) rats [64]. This elimination of damaged mitochondria precedes cardiolipin-induced apoptosis and is neuroprotective after TBI.

Using MALDI-MSI, Li and colleagues reported a down-regulation of cardiolipins in the perilesional area of a CCI injury in adult rats [86]. Another imaging study confirmed these results in PND17 rats [87]. Three hours after injury, cardiolipin levels were significantly decreased in the injured region, in the hippocampus and in the thalamus. However, no changes were noted in the adjacent cortical region. Similarly, in PND17 rats, cardiolipin level decreased significantly in the pericontusional cortex at 4 hours (-96%) and 24 hours (-89%) after CCI injury, with a uniform decrease across all cardiolipin species [38]. Chao and colleagues also reported significant losses in cardiolipins rich in polyunsaturated fatty acids in the cortical contusional zone at 1, 4, and 24 hours post-CCI in PND17 rats, with the highest decrease at 4 hours [95]. Due to their unsaturation, polyunsaturated fatty acids are prone to oxidation. Indeed, imaging mass spectrometry identified significant losses in polyunsaturated cardiolipins in the ipsilateral contusional cortex at 3 hours post-injury, and additionally cardiolipin decreased in the CA3 region of the hippocampus and thalamus on the ipsilateral side [39]. Levels of oxidised cardiolipins peaked at 1 hour post-injury (>3.5-fold) in the study by Chao and colleagues, and elevated levels were still noted at 4 and 24 hours post-injury [95]. These lower oxidised cardiolipin levels at later time points were mainly due to decreases in oxidised cardiolipins containing polyunsaturated fatty acids. Monolysocardiolipins slightly increased at 1 hour post-injury and further increased at 4 and 24 hours. A steady increase in monolysocardiolipins with less than seven double bonds was noted at later time points, likely reflecting hydrolysis of cardiolipins. It was noted that the early oxidation of cardiolipin was specific and not just caused by the high amount of cardiolipin containing DHA [76]. The PND17 rat CCI model showed oxidation of polyunsaturated cardiolipins at 3 hours post-injury, whereas other PL were not oxidised. Therefore, cardiolipin was specifically oxidised early on after TBI, and additionally, in the TBI group, apoptotic markers were increased at 24 hours post-injury. Oxidised cardiolipin has been described to be essential for the release of proapoptotic factors [96]. Similarly, Ji and colleagues reported oxidation of cardiolipins in the whole brain after CCI, using two-dimensional liquid chromatography mass spectrometry [63]. They identified that the number of oxidised cardiolipins increased from 10 in naïve brains to 166, 30 minutes after TBI. The oxidation was linked

to cell death and lesion volume, and inhibition of oxidation by applying a brain-permeable mitochondria-targeted electron scavenger led to improved outcomes.

5. Discussion

The studies reviewed here show a complex pattern of changes in various lipids, in blood, in CSF and in brain tissue. Key changes in major species in blood and CSF are summarized in Figure 2. The timeline of the various studies spans a period starting in the first hours after injury and up to several months or years after injury. Some lipid species show a monophasic change (decrease or increase as a function of time post-trauma), whereas other species show a biphasic change. There are several questions which are raised by these observations, around the lipid dynamics in the peripheral vs. the central compartment, how the changes in the two compartments could be linked and what avenues they open to improve diagnosis and prognosis in TBI.

a) *What underlies the dynamic alterations in lipids, from the acute to the chronic period?* It could be hypothesized that changes in the acute period (less than 24 hours after injury), both in blood and brain tissue, reflect the initial damage to tissue, and the primary injury-induced rapid demise of various structural components, including membrane lipids. The decrease in tissue PL is a consequence of the activation of enzymes such as the phospholipases which increase their activity in less than an hour following injury [47, 97]. However, as time progresses towards the subacute phase, the injury element is likely to be complemented by the emergence of a pro-repair response, and gradually the balance of processes becomes more heavily weighted towards neurorepair and regeneration. Bahado-Singh *et al.* [69] identified lipids as part of a complex serum metabolomic panel, in a closed TBI mouse model, and showed that certain lipids such as PC 34:4 are part of a metabolomic set that differentiates TBI from control animals, whereas SM 18:0 and 18:1 were specifically part of a set that reflected the evolution of the injury from 4 hours to 24 hours. In a study on serum neuroproteomics in rat CCI, Kobeissy and collaborators [98], also illustrated these shifts in panel composition between the acute (1 day) and subacute period (7 days) post-injury, with proteins involved in neurorepair, axon growth and neuroregeneration being upregulated at the subacute time after injury.

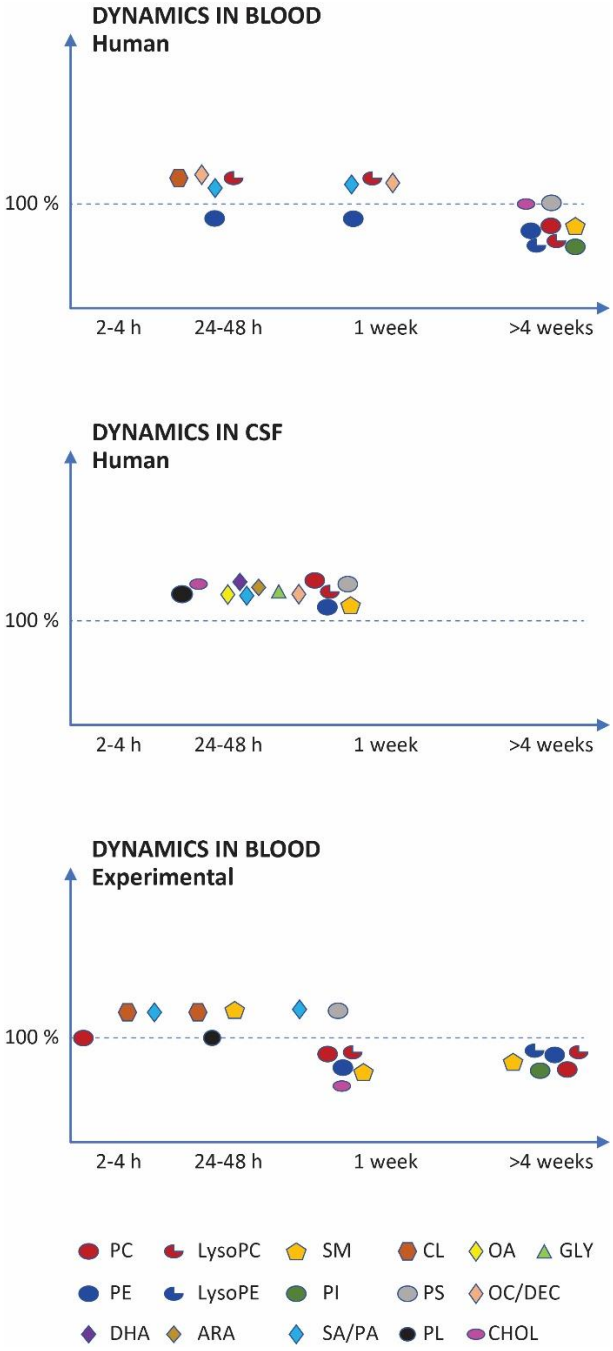

Figure 2: Summary of dynamic changes in lipids from various lipid classes in human blood (plasma, serum) and human CSF in TBI clinical studies, and blood (plasma, serum) in experimental models of TBI. *Increased values vs. respective controls are represented above the line, and decreased values below the line. PC (phosphatidylcholine), PE (phosphatidyl-ethanolamine), lysoPC (lysophosphatidylcholine), lysoPE (lysophosphatidylethanolamine); SM (sphingomyelin), CL (cardiolipin), PI (phosphatidylinositol), PS (phosphatidylserine), OA (oleic acid), DHA (docosahexaenoic acid), ARA (arachidonic acid), PA (palmitic acid), SA (stearic acid), OC (octanoic acid), DEC (decanoic acid), PL (total phospholipids), CHOL (cholesterol), GLY (glycerol).*

b) *How are changes in the peripheral compartment linked to the changes detected in the injured brain tissue?* There are several possible mechanisms responsible for the transfer of substances from the central compartment into the peripheral compartment: mechanical BBB disruption [99], passive efflux from brain/CSF [100], and also glymphatic transport [101]. The disruption of the BBB is maximal within a few hours post-trauma [102, 103], therefore it is very likely that in the acute period the transfer is mainly a consequence of the BBB being damaged by injury. In support of this hypothesis, Orešič *et al.* [53] showed that there is a similarity between the changes seen in serum and in the brain microdialysate from TBI patients, in the acute phase. However, it is also interesting to note that Glushakova *et al.* [104] showed that some of the changes in BBB can be protracted, and may be directly linked to pathological processes such as white matter injury post-trauma, which unfold over a longer time.

c) *What is the correlation between the changes seen in specific lipids and neurological outcome post-TBI?* Only a few studies have so far explored this aspect. Thus, Pilitsis *et al.* [58] clearly showed that the high levels of fatty acids in the CSF post-TBI, correlated with poorer outcome. Orešič *et al.* [53] also reported that high levels of medium-chain fatty acids, linked to tissue energy metabolism disruption, are correlated with poorer outcome. Yi *et al.* [105] explored potential markers of TBI-induced cognitive impairment in patients with moderate to severe TBI; their study provided evidence that changes in certain fatty acids, i.e. palmitic acid (decrease), linoleic acid and ARA (increase) are linked to TBI with poor cognitive outcome, as compared to TBI devoid of this complication.

d) *How specific are lipid changes reported in traumatic injury vs. an injury of the brain of vascular origin, such as stroke?* Pilitsis *et al.* [106] have reported similar observations on high fatty acid (such as ARA and DHA) levels in CSF following stroke, with higher levels being predictors of poorer outcome. Furthermore, Liu *et al.* [107] have shown that high levels of lysoPC 18:2 in serum could have prognostic value for the development of post-stroke cognitive impairment. Stroke activates phospholipases such as $cPLA_2$ [108], and this could explain the PL changes similar to those reported in TBI. Therefore, it appears that stroke also leads to changes in specific lipid species, in the central or peripheral compartment.

e) *Can the changes seen suggest new therapeutic solutions in TBI, targeting lipid disruption*? The decline in certain lipids, such as DHA, in brain tissue, suggests that supplementation with this fatty acid may have beneficial effects after brain injury. Interestingly, in athletes who are at risk of repeated brain concussion during a football season, the treatment with moderate doses of DHA may have protective effects [109]. There is also evidence that providing animals after

a CCI injury with a multi-nutrient containing PL biosynthetic precursors, significantly improves outcome [110].

a) *Are there specific lipids which appear to be the most sensitive biomarkers of TBI?* The data so far has not enabled the identification of a uniquely sensitive and specific lipid biomarker (or biomarker panel) for TBI, applicable across the whole spectrum of this condition. However, there are interesting observations in recent studies on specific CNS lipids such as brain-specific cardiolipin, associated with mitochondrial damage [38], or oxidized PL species, e.g. hydroperoxy-arachidonoyl- and adrenoyl-PE, associated with the ferroptosis process triggered by TBI [111, 112]. Furthermore, there is still limited information on the levels of eicosanoids and docosanoids after TBI, in human studies and animal models. New insights are likely to be generated in future studies focused on these lipid mediator species.

6. Conclusion

The concerted effort in the biomarker field over the last decade has led to identification of a variety of biomarkers of TBI, reflecting neuronal or astrocytic injury, BBB disruption or the immune response triggered by neurotrauma [21, 113]. Biomarker measurements in blood are less invasive, therefore likely to be more easily implemented in the clinic. There are already strong candidates such as neurofilament L, the protein tau (total t-tau or the phosphorylated form p-tau), glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase L1 (UCHL1), S100 calcium-binding protein B (S100B) and spectrin breakdown products (SBDP). A recent study showed that in the first 2 weeks after TBI, GFAP and NFL levels added the most independent information to improve prediction of outcome [114]. It is likely that the field of TBI biomarkers will evolve towards use of various panels of biomarkers, suited to specific questions, for example, the panel used to predict within the first 24 hours whether a mild TBI patient requires a scan will be different from that used to predict over the first 3-7 days the outcome after a severe TBI - as recently discussed by Gan and collaborators [21]. Therefore, future efforts in lipidomics may follow a similar path, and focus on specific severities and stages of the injury in order to better personalize the management of this complex condition. There is still an open question whether TBI studies (including biomarker research) in rodents, with a much lower white matter to grey matter ratio than humans [115, 116], are the most informative. Furthermore, before moving from research evidence to clinical practice in TBI, there are still unresolved issues in the field, linked to the design, analysis and reporting of biomarker studies, and the analytical rigour and reproducibility of the data [117, 118]. It is to be hoped that the combination of different types of biomarkers, including specific lipids, and reflecting different cellular origins and pathophysiological processes, will become a valuable tool for improved patient stratification in future TBI clinical studies.

Declaration of Interest:

The authors declare no conflict of interest.

Acknowledgements:

Isabell Nessel was supported by the Bernice Hazle Charitable Trust. The funders had no involvement in the writing of this manuscript.

References

[1] A.I.R. Maas, D.K. Menon, P.D. Adelson, N. Andelic, M.J. Bell, A. Belli, P. Bragge, A. Brazinova, A. Buki, R.M. Chesnut, G. Citerio, M. Coburn, D.J. Cooper, A.T. Crowder, E. Czeiter, M. Czosnyka, R. Diaz-Arrastia, J.P. Dreier, A.C. Duhaime, A. Ercole, T.A. van Essen, V.L. Feigin, G. Gao, J. Giacino, L.E. Gonzalez-Lara, R.L. Gruen, D. Gupta, J.A. Hartings, S. Hill, J.Y. Jiang, N. Ketharanathan, E.J.O. Kompanje, L. Lanyon, S. Laureys, F. Lecky, H. Levin, H.F. Lingsma, M. Maegele, M. Majdan, G. Manley, J. Marsteller, L. Mascia, C. McFadyen, S. Mondello, V. Newcombe, A. Palotie, P.M. Parizel, W. Peul, J. Piercy, S. Polinder, L. Puybasset, T.E. Rasmussen, R. Rossaint, P. Smielewski, J. Soderberg, S.J. Stanworth, M.B. Stein, N. von Steinbuchel, W. Stewart, E.W. Steyerberg, N. Stocchetti, A. Synnot, B. Te Ao, O. Tenovuo, A. Theadom, D. Tibboel, W. Videtta, K.K.W. Wang, W.H. Williams, L. Wilson, K. Yaffe, T.P. In, Investigators, Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research, Lancet Neurol 16(12) (2017) 987-1048.

[2] S.L. James, A. Theadom, R.G. Ellenbogen, M.S. Bannick, W. Montjoy-Venning, L.R. Lucchesi, N. Abbasi, R. Abdulkader, H.N. Abraha, J.C. Adsuar, M. Afarideh, S. Agrawal, A. Ahmadi, M.B. Ahmed, A.N. Aichour, I. Aichour, M.T.E. Aichour, R.O. Akinyemi, N. Akseer, F. Alahdab, A. Alebel, S.A. Alghnam, B.A. Ali, U. Alsharif, K. Altirkawi, C.L. Andrei, M. Anjomshoa, H. Ansari, M.G. Ansha, C.A.T. Antonio, S.C.Y. Appiah, F. Ariani, N.G. Asefa, S.W. Asgedom, S. Atique, A. Awasthi, B.P. Ayala Quintanilla, T.B. Ayuk, P.S. Azzopardi, H. Badali, A. Badawi, S. Balalla, A. Banstola, S.L. Barker-Collo, T.W. Bärnighausen, N. Bedi, M. Behzadifar, M. Behzadifar, B.B. Bekele, A.B. Belachew, Y.A. Belay, D.A. Bennett, I.M. Bensenor, A. Berhane, M. Beuran, A. Bhalla, S. Bhaumik, Z.A. Bhutta, B. Biadgo, M. Biffino, A. Bijani, N. Bililign, C. Birungi, S. Boufous, A. Brazinova, A.W. Brown, M. Car, R. Cárdenas, J.J. Carrero, F. Carvalho, C.A. Castañeda-Orjuela, F. Catalá-López, Y. Chaiah, A.P. Champs, J.-C. Chang, J.-Y.J. Choi, D.J. Christopher, C. Cooper, C.S. Crowe, L. Dandona, R. Dandona, A. Daryani, D.V. Davitoiu, M.G. Degefa, G.T. Demoz, K. Deribe, S. Djalalinia, H.P. Do, D.T. Doku, T.M. Drake, M. Dubey, E. Dubljanin, Z. El-Khatib, R. Ofori-Asenso, S. Eskandarieh, A. Esteghamati, S. Esteghamati, A. Faro, F. Farzadfar, M.H. Farzaei, S.-M. Fereshtehnejad, E. Fernandes, G.T. Feyissa, I. Filip, F. Fischer, T. Fukumoto, M. Ganji, F.G. Gankpe, A.K. Gebre, T.T. Gebrehiwot, K.E. Gezae, G. Gopalkrishna, A.C. Goulart, J.A. Haagsma, A. Haj-Mirzaian, A. Haj-Mirzaian, R.R. Hamadeh, S. Hamidi, J.M. Haro, H. Hassankhani, H.Y. Hassen, R. Havmoeller, C. Hawley, S.I. Hay, M.I. Hegazy, D. Hendrie, A. Henok, D.T. Hibstu, H.J. Hoffman, M.K. Hole, E. Homaie Rad, S.M. Hosseini, S. Hostiuc, G. Hu, M.A. Hussen, O.S. Ilesanmi, S.S.N. Irvani, M. Jakovljevic, S. Jayaraman, R.P. Jha, J.B. Jonas, K.M. Jones, Z. Jorjoran Shushtari, J.J. Jozwiak, M. Jürisson, A. Kabir, A. Kahsay, M. Kahssay, R. Kalani, A. Karch, A. Kasaeian, G.M. Kassa, T.D. Kassa, Z.Y. Kassa, A.P. Kengne, Y.S. Khader, M.A. Khafaie, N. Khalid, I. Khalil, E.A. Khan, M.S. Khan, Y.-H. Khang, H. Khazaie, A.T. Khoja, J. Khubchandani, A.A. Kiadaliri, D. Kim, Y.-E. Kim, A. Kisa, A. Koyanagi, K.J. Krohn, B. Kuate Defo, B. Kucuk Bicer, G.A. Kumar, M. Kumar, R. Lalloo, F.H. Lami, V.C. Lansingh, D.O. Laryea, A. Latifi, C.T. Leshargie, M. Levi, S. Li, M.L. Liben, P.A. Lotufo, R. Lunevicius, N.B. Mahotra, M. Majdan, A. Majeed, R. Malekzadeh, A.-L. Manda, M.A. Mansournia, B.B. Massenburg, K.K.V. Mate, M.M. Mehndiratta, V. Mehta, H. Meles, A. Melese, P.T.N. Memiah, W. Mendoza, G. Mengistu, A. Meretoja, T.J. Meretoja, T. Mestrovic, T. Miazgowski, T.R. Miller, G.K. Mini, A. Mirica, E.M. Mirrakhimov, B. Moazen, M. Mohammadi, M. Molokhia, L. Monasta, S. Mondello, M. Moosazadeh, G. Moradi, M. Moradi, M. Moradi-Lakeh, M. Moradinazar, S.D. Morrison, M.M. Moschos, S.M. Mousavi, S. Murthy, K.I. Musa, G. Mustafa, M. Naghavi, G. Naik, F. Najafi, V. Nangia, B.R. Nascimento, I. Negoi, T.H. Nguyen, E. Nichols, D.N.A. Ningrum, Y.L. Nirayo, P.S. Nyasulu, F.A. Ogbo, I.-H. Oh, A. Okoro, A.T. Olagunju, T.O. Olagunju, P.R. Olivares, S.S. Otstavnov, M.O. Owolabi, M. P A, S. Pakhale, A.R. Pandey, K. Pesudovs, G.D. Pinilla-Monsalve, S. Polinder, H. Poustchi, S. Prakash, M. Qorbani, A. Radfar, A. Rafay, A. Rafiei, A. Rahimi-Movaghar, V. Rahimi-Movaghar, M. Rahman, M.A. Rahman, R.K. Rai, F. Rajati, U. Ram, D.L. Rawaf, S. Rawaf, R.C. Reiner, C. Reis, A.M.N. Renzaho, S. Resnikoff, S. Rezaei, S. Rezaeian, L. Roever, L. Ronfani, G. Roshandel, N. Roy, G.M. Ruhago, B. Saddik, H. Safari, S. Safiri, M.A. Sahraian, P. Salamati, R.d.F. Saldanha, A.M. Samy, J. Sanabria, J.V. Santos, M.M.M. Santric Milicevic, B. Sartorius, M. Satpathy, K. Savuon, I.J.C. Schneider, D.C. Schwebel, S.G. Sepanlou, H. Shabaninejad, M.A.A. Shaikh, M. Shams-

Beyranvand, M. Sharif, M. Sharif-Alhoseini, S.M. Shariful Islam, J. She, A. Sheikh, J. Shen, K.N. Sheth, K. Shibuya, M.S. Shiferaw, M. Shigematsu, R. Shiri, I. Shiue, H. Shoman, S. Siabani, T.J. Siddiqi, J.P. Silva, D.G.A. Silveira, D.N. Sinha, M. Smith, A.M. Soares Filho, S. Sobhani, M. Soofi, J.B. Soriano, I.N. Soyiri, D.J. Stein, M.A. Stokes, M.a.B. Sufiyan, B.F. Sunguya, J.E. Sunshine, B.L. Sykes, C.E.I. Szoeke, R. Tabarés-Seisdedos, B.J. Te Ao, A. Tehrani-Banihashemi, M.G. Tekle, M.-H. Temsah, O. Temsah, R. Topor-Madry, M. Tortajada-Girbés, B.X. Tran, K.B. Tran, L. Tudor Car, K.N. Ukwaja, I. Ullah, M.S. Usman, O.A. Uthman, P.R. Valdez, T.J. Vasankari, N. Venketasubramanian, F.S. Violante, F.W.S. Wagnew, Y. Waheed, Y.-P. Wang, K.G. Weldegwergs, A. Werdecker, T. Wijeratne, A.S. Winkler, G.M.A. Wyper, Y. Yano, M. Yaseri, Y.J. Yasin, P. Ye, E.M. Yimer, P. Yip, E. Yisma, N. Yonemoto, S.-J. Yoon, M.G. Yost, M.Z. Younis, M. Yousefifard, C. Yu, Z. Zaidi, S.B. Zaman, M. Zamani, Z.M. Zenebe, S. Zodpey, V.L. Feigin, T. Vos, C.J.L. Murray, Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016, The Lancet Neurology 18(1) (2019) 56-87.

[3] K.R. Walker, G. Tesco, Molecular mechanisms of cognitive dysfunction following traumatic brain injury, Frontiers in aging neuroscience 5 (2013) 29.

[4] A.R. Rabinowitz, H.S. Levin, Cognitive sequelae of traumatic brain injury, Psychiatr Clin North Am 37(1) (2014) 1-11.

[5] V.E. Johnson, W. Stewart, D.H. Smith, Widespread tau and amyloid-beta pathology many years after a single traumatic brain injury in humans, Brain Pathol 22(2) (2012) 142-9.

[6] H.K. Wang, S.H. Lin, P.S. Sung, M.H. Wu, K.W. Hung, L.C. Wang, C.Y. Huang, K. Lu, H.J. Chen, K.J. Tsai, Population based study on patients with traumatic brain injury suggests increased risk of dementia, J Neurol Neurosurg Psychiatry 83(11) (2012) 1080-5.

[7] D.E. Barnes, A. Kaup, K.A. Kirby, A.L. Byers, R. Diaz-Arrastia, K. Yaffe, Traumatic brain injury and risk of dementia in older veterans, Neurology 83(4) (2014) 312-9.

[8] P.M. Washington, S. Villapol, M.P. Burns, Polypathology and dementia after brain trauma: Does brain injury trigger distinct neurodegenerative diseases, or should they be classified together as traumatic encephalopathy?, Experimental neurology 275 Pt 3 (2016) 381-388.

[9] M.C. Dewan, A. Rattani, S. Gupta, R.E. Baticulon, Y.C. Hung, M. Punchak, A. Agrawal, A.O. Adeleye, M.G. Shrime, A.M. Rubiano, J.V. Rosenfeld, K.B. Park, Estimating the global incidence of traumatic brain injury, J Neurosurg (2018) 1-18.

[10] A.I. Maas, D.K. Menon, E.W. Steyerberg, G. Citerio, F. Lecky, G.T. Manley, S. Hill, V. Legrand, A. Sorgner, C.-T. Participants, Investigators, Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI): a prospective longitudinal observational study, Neurosurgery 76(1) (2015) 67-80.

[11] Y.G. Bodien, M. McCrea, S. Dikmen, N. Temkin, K. Boase, J. Machamer, S.R. Taylor, M. Sherer, H. Levin, J.H. Kramer, J.D. Corrigan, T.W. McAllister, J. Whyte, G.T. Manley, J.T. Giacino, T.-T. Investigators, Optimizing Outcome Assessment in Multicenter TBI Trials: Perspectives From TRACK-TBI and the TBI Endpoints Development Initiative, J Head Trauma Rehabil 33(3) (2018) 147-157.

[12] K.E. Saatman, A.C. Duhaime, R. Bullock, A.I. Maas, A. Valadka, G.T. Manley, T. Workshop Scientific, M. Advisory Panel, Classification of traumatic brain injury for targeted therapies, Journal of neurotrauma 25(7) (2008) 719-38.

[13] J.J. Kim, A.D. Gean, Imaging for the diagnosis and management of traumatic brain injury, Neurotherapeutics 8(1) (2011) 39-53.

[14] L.G.F. Smith, E. Milliron, M.L. Ho, H.H. Hu, J. Rusin, J. Leonard, E.A. Sribnick, Advanced neuroimaging in traumatic brain injury: an overview, Neurosurg Focus 47(6) (2019) E17.

[15] J. Puig, M.J. Ellis, J. Kornelsen, T.D. Figley, C.R. Figley, I.E.P. Daunis, W.A.C. Mutch, M. Essig, Magnetic Resonance Imaging Biomarkers of Brain Connectivity in Predicting Outcome after Mild Traumatic Brain Injury: A Systematic Review, Journal of neurotrauma (2020).

[16] M.S. Davitz, O. Gonen, A. Tal, J.S. Babb, Y.W. Lui, Kirov, II, Quantitative multivoxel proton MR spectroscopy for the identification of white matter abnormalities in mild traumatic brain injury:

Comparison between regional and global analysis, Journal of magnetic resonance imaging : JMRI 50(5) (2019) 1424-1432.
[17] R. Vagnozzi, S. Signoretti, L. Cristofori, F. Alessandrini, R. Floris, E. Isgro, A. Ria, S. Marziali, G. Zoccatelli, B. Tavazzi, F. Del Bolgia, R. Sorge, S.P. Broglio, T.K. McIntosh, G. Lazzarino, Assessment of metabolic brain damage and recovery following mild traumatic brain injury: a multicentre, proton magnetic resonance spectroscopic study in concussed patients, Brain 133(11) (2010) 3232-42.
[18] E. Toman, S. Harrisson, T. Belli, Biomarkers in traumatic brain injury: a review, J R Army Med Corps 162(2) (2016) 103-8.
[19] D. Slavoaca, D. Muresanu, C. Birle, O.V. Rosu, I. Chirila, I. Dobra, N. Jemna, S. Strilciuc, P. Vos, Biomarkers in traumatic brain injury: new concepts, Neurol Sci (2020).
[20] B.I. Martinez, S.E. Stabenfeldt, Current trends in biomarker discovery and analysis tools for traumatic brain injury, J Biol Eng 13 (2019) 16.
[21] Z.S. Gan, S.C. Stein, R. Swanson, S. Guan, L. Garcia, D. Mehta, D.H. Smith, Blood Biomarkers for Traumatic Brain Injury: A Quantitative Assessment of Diagnostic and Prognostic Accuracy, Front Neurol 10 (2019) 446.
[22] M. Bou Khalil, W. Hou, H. Zhou, F. Elisma, L.A. Swayne, A.P. Blanchard, Z. Yao, S.A. Bennett, D. Figeys, Lipidomics era: accomplishments and challenges, Mass Spectrom Rev 29(6) (2010) 877-929.
[23] M.R. Wenk, Lipidomics: new tools and applications, Cell 143(6) (2010) 888-95.
[24] O. Quehenberger, A.M. Armando, A.H. Brown, S.B. Milne, D.S. Myers, A.H. Merrill, S. Bandyopadhyay, K.N. Jones, S. Kelly, R.L. Shaner, C.M. Sullards, E. Wang, R.C. Murphy, R.M. Barkley, T.J. Leiker, C.R. Raetz, Z. Guan, G.M. Laird, D.A. Six, D.W. Russell, J.G. McDonald, S. Subramaniam, E. Fahy, E.A. Dennis, Lipidomics reveals a remarkable diversity of lipids in human plasma, Journal of lipid research 51(11) (2010) 3299-305.
[25] D. Piomelli, G. Astarita, R. Rapaka, A neuroscientist's guide to lipidomics, Nat Rev Neurosci 8(10) (2007) 743-54.
[26] K. Bozek, Y. Wei, Z. Yan, X. Liu, J. Xiong, M. Sugimoto, M. Tomita, S. Paabo, C.C. Sherwood, P.R. Hof, J.J. Ely, Y. Li, D. Steinhauser, L. Willmitzer, P. Giavalisco, P. Khaitovich, Organization and evolution of brain lipidome revealed by large-scale analysis of human, chimpanzee, macaque, and mouse tissues, Neuron 85(4) (2015) 695-702.
[27] S.A. Bennett, N. Valenzuela, H. Xu, B. Franko, S. Fai, D. Figeys, Using neurolipidomics to identify phospholipid mediators of synaptic (dys)function in Alzheimer's Disease, Front Physiol 4 (2013) 168.
[28] M. Sud, E. Fahy, D. Cotter, A. Brown, E.A. Dennis, C.K. Glass, A.H. Merrill, Jr., R.C. Murphy, C.R. Raetz, D.W. Russell, S. Subramaniam, LMSD: LIPID MAPS structure database, Nucleic acids research 35(Database issue) (2007) D527-32.
[29] R.A. Colas, M. Shinohara, J. Dalli, N. Chiang, C.N. Serhan, Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue, Am J Physiol Cell Physiol 307(1) (2014) C39-54.
[30] E.A. Dennis, P.C. Norris, Eicosanoid storm in infection and inflammation, Nat Rev Immunol 15(8) (2015) 511-23.
[31] H. Watson, Biological membranes, Essays Biochem 59 (2015) 43-69.
[32] N.U. Olsson, A.J. Harding, C. Harper, N. Salem, High-performance liquid chromatography method with light-scattering detection for measurements of lipid class composition: analysis of brains from alcoholics, Journal of Chromatography B: Biomedical Sciences and Applications 681(2) (1996) 213-218.
[33] J.W. Choi, J. Chun, Lysophospholipids and their receptors in the central nervous system, Biochim Biophys Acta 1831(1) (2013) 20-32.
[34] S.S. Ousman, S. David, Lysophosphatidylcholine induces rapid recruitment and activation of macrophages in the adult mouse spinal cord, Glia 30(1) (2000) 92-104.
[35] 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, Int J Mol Sci 20(5) (2019).
[36] D. Ogasawara, H. Deng, A. Viader, M.P. Baggelaar, A. Breman, H. den Dulk, A.M. van den Nieuwendijk, M. Soethoudt, T. van der Wel, J. Zhou, H.S. Overkleeft, M. Sanchez-Alavez, S. Mori, W.

857 Nguyen, B. Conti, X. Liu, Y. Chen, Q.S. Liu, B.F. Cravatt, M. van der Stelt, Rapid and profound rewiring
858 of brain lipid signaling networks by acute diacylglycerol lipase inhibition, Proc Natl Acad Sci U S A 113(1)
859 (2016) 26-33.
860 [37] M. Orth, S. Bellosta, Cholesterol: its regulation and role in central nervous system disorders,
861 Cholesterol 2012 (2012) 292598.
862 [38] T.S. Anthonymuthu, E.M. Kenny, Z.E. Hier, R.S.B. Clark, P.M. Kochanek, V.E. Kagan, H. Bayır,
863 Detection of brain specific cardiolipins in plasma after experimental pediatric head injury, Exp Neurol
864 316 (2019) 63-73.
865 [39] H. Tian, L.J. Sparvero, A.A. Amoscato, A. Bloom, H. Bayır, V.E. Kagan, N. Winograd, Gas Cluster Ion
866 Beam Time-of-Flight Secondary Ion Mass Spectrometry High-Resolution Imaging of Cardiolipin
867 Speciation in the Brain: Identification of Molecular Losses after Traumatic Injury, Anal Chem 89(8)
868 (2017) 4611-4619.
869 [40] T.S. Anthonymuthu, E.M. Kenny, A.M. Lamade, H. Gidwani, N.M. Krehel, A. Misse, X. Gao, A.A.
870 Amoscato, A.C. Straub, V.E. Kagan, C. Dezfulian, H. Bayır, Lipidomics Detection of Brain Cardiolipins in
871 Plasma Is Associated With Outcome After Cardiac Arrest, Crit Care Med 47(4) (2019) e292-e300.
872 [41] Y. Xiong, A. Mahmood, M. Chopp, Animal models of traumatic brain injury, Nat Rev Neurosci 14(2)
873 (2013) 128-42.
874 [42] H. Algattas, J.H. Huang, Traumatic Brain Injury pathophysiology and treatments: early,
875 intermediate, and late phases post-injury, Int J Mol Sci 15(1) (2013) 309-41.
876 [43] H.M. Bramlett, W.D. Dietrich, Long-Term Consequences of Traumatic Brain Injury: Current Status
877 of Potential Mechanisms of Injury and Neurological Outcomes, Journal of neurotrauma 32(23) (2015)
878 1834-48.
879 [44] V. Di Pietro, G. Lazzarino, A.M. Amorini, S. Signoretti, L.J. Hill, E. Porto, B. Tavazzi, G. Lazzarino, A.
880 Belli, Fusion or Fission: The Destiny of Mitochondria In Traumatic Brain Injury of Different Severities,
881 Scientific reports 7(1) (2017) 9189.
882 [45] E.A. Dennis, Introduction to Thematic Review Series: Phospholipases: Central Role in Lipid
883 Signaling and Disease, Journal of lipid research 56(7) (2015) 1245-7.
884 [46] M. Joensuu, T.P. Wallis, S.H. Saber, F.A. Meunier, Phospholipases in neuronal function: A role in
885 learning and memory?, Journal of neurochemistry 153(3) (2020) 300-333.
886 [47] E. Shohami, Y. Shapira, G. Yadid, N. Reisfeld, S. Yedgar, Brain phospholipase A2 is activated after
887 experimental closed head injury in the rat, Journal of neurochemistry 53(5) (1989) 1541-6.
888 [48] W. Heller, P. Oldenkott, C. Stolz, J. Hausdorfer, Metabolic changes in the blood of patients with
889 brain injury and hypoxia, Resuscitation 3(3) (1974) 215-22.
890 [49] M. Daley, G. Dekaban, R. Bartha, A. Brown, T.C. Stewart, T. Doherty, L. Fischer, J. Holmes, R.S.
891 Menon, C.A. Rupar, J.K. Shoemaker, D.D. Fraser, Metabolomics profiling of concussion in adolescent
892 male hockey players: a novel diagnostic method, Metabolomics 12(12) (2016) 185.
893 [50] T. Emmerich, L. Abdullah, G. Crynen, M. Dretsch, J. Evans, G. Ait-Ghezala, J. Reed, H. Montague,
894 H. Chaytow, V. Mathura, J. Martin, R. Pelot, S. Ferguson, A. Bishop, J. Phillips, M. Mullan, F. Crawford,
895 Plasma Lipidomic Profiling in a Military Population of Mild Traumatic Brain Injury and Post-Traumatic
896 Stress Disorder with Apolipoprotein E ε4-Dependent Effect, J Neurotrauma 33(14) (2016) 1331-48.
897 [51] C.J.C. Huguenard, A. Cseresznye, J.E. Evans, S. Oberlin, H. Langlois, S. Ferguson, T. Darcey, A.
898 Nkiliza, M. Dretsch, M. Mullan, F. Crawford, L. Abdullah, Plasma Lipidomic Analyses in Cohorts With
899 mTBI and/or PTSD Reveal Lipids Differentially Associated With Diagnosis and, Front Physiol 11 (2020)
900 12.
901 [52] M.S. Fiandaca, M. Mapstone, A. Mahmoodi, T. Gross, F. Macciardi, A.K. Cheema, K. Merchant-
902 Borna, J. Bazarian, H.J. Federoff, Plasma metabolomic biomarkers accurately classify acute mild
903 traumatic brain injury from controls, PloS one 13(4) (2018) e0195318.
904 [53] M. Oresic, J.P. Posti, M.H. Kamstrup-Nielsen, R.S.K. Takala, H.F. Lingsma, I. Mattila, S. Jantti, A.J.
905 Katila, K.L.H. Carpenter, H. Ala-Seppala, A. Kyllonen, H.R. Maanpaa, J. Tallus, J.P. Coles, I. Heino, J.
906 Frantzen, P.J. Hutchinson, D.K. Menon, O. Tenovuo, T. Hyotylainen, Human Serum Metabolites

907 Associate With Severity and Patient Outcomes in Traumatic Brain Injury, EBioMedicine 12 (2016) 118-
908 126.
909 [54] I. Thomas, A.M. Dickens, J.P. Posti, M. Mohammadian, C. Ledig, R.S.K. Takala, T. Hyotylainen, O.
910 Tenovuo, M. Oresic, Integrative Analysis of Circulating Metabolite Profiles and Magnetic Resonance
911 Imaging Metrics in Patients with Traumatic Brain Injury, Int J Mol Sci 21(4) (2020).
912 [55] A.D. Kay, S.P. Day, M. Kerr, J.A. Nicoll, C.J. Packard, M.J. Caslake, Remodeling of cerebrospinal
913 fluid lipoprotein particles after human traumatic brain injury, J Neurotrauma 20(8) (2003) 717-23.
914 [56] A.E. Pasvogel, P. Miketova, I.M. Moore, Cerebrospinal fluid phospholipid changes following
915 traumatic brain injury, Biol Res Nurs 10(2) (2008) 113-20.
916 [57] A.E. Pasvogel, P. Miketova, I.M. Moore, Differences in CSF phospholipid concentration by
917 traumatic brain injury outcome, Biol Res Nurs 11(4) (2010) 325-31.
918 [58] J.G. Pilitsis, W.M. Coplin, M.H. O'Regan, J.M. Wellwood, F.G. Diaz, M.R. Fairfax, D.B. Michael, J.W.
919 Phillis, Free fatty acids in cerebrospinal fluids from patients with traumatic brain injury, Neurosci Lett
920 349(2) (2003) 136-8.
921 [59] J.G. Pilitsis, W.M. Coplin, M.H. O'Regan, J.M. Wellwood, F.G. Diaz, M.R. Fairfax, D.B. Michael, J.W.
922 Phillis, Free fatty acids in human cerebrospinal fluid following subarachnoid hemorrhage and their
923 potential role in vasospasm: a preliminary observation, J Neurosurg 97(2) (2002) 272-9.
924 [60] S.M. Peerdeman, A.R. Girbes, K.H. Polderman, W.P. Vandertop, Changes in cerebral interstitial
925 glycerol concentration in head-injured patients; correlation with secondary events, Intensive Care
926 Med 29(10) (2003) 1825-8.
927 [61] A. Karathanou, K. Paterakis, M. Pakopoulou, A. Tasiou, G. Hadjigeorgiou, A. Chovas, G. Paraforos,
928 K. Fountas, A. Komnos, Biochemical markers analyzed using microdialysis and traumatic brain injury
929 outcomes, J Neurosurg Sci 55(3) (2011) 173-7.
930 [62] I. Timofeev, K.L. Carpenter, J. Nortje, P.G. Al-Rawi, M.T. O'Connell, M. Czosnyka, P. Smielewski,
931 J.D. Pickard, D.K. Menon, P.J. Kirkpatrick, A.K. Gupta, P.J. Hutchinson, Cerebral extracellular chemistry
932 and outcome following traumatic brain injury: a microdialysis study of 223 patients, Brain 134(Pt 2)
933 (2011) 484-94.
934 [63] J. Ji, A.E. Kline, A. Amoscato, A.K. Samhan-Arias, L.J. Sparvero, V.A. Tyurin, Y.Y. Tyurina, B. Fink,
935 M.D. Manole, A.M. Puccio, D.O. Okonkwo, J.P. Cheng, H. Alexander, R.S. Clark, P.M. Kochanek, P. Wipf,
936 V.E. Kagan, H. Bayır, Lipidomics identifies cardiolipin oxidation as a mitochondrial target for redox
937 therapy of brain injury, Nat Neurosci 15(10) (2012) 1407-13.
938 [64] H. Chao, C. Lin, Q. Zuo, Y. Liu, M. Xiao, X. Xu, Z. Li, Z. Bao, H. Chen, Y. You, P.M. Kochanek, H. Yin,
939 N. Liu, V.E. Kagan, H. Bayır, J. Ji, Cardiolipin-Dependent Mitophagy Guides Outcome after Traumatic
940 Brain Injury, J Neurosci 39(10) (2019) 1930-1943.
941 [65] C.T. Chu, J. Ji, R.K. Dagda, J.F. Jiang, Y.Y. Tyurina, A.A. Kapralov, V.A. Tyurin, N. Yanamala, I.H.
942 Shrivastava, D. Mohammadyani, K.Z.Q. Wang, J. Zhu, J. Klein-Seetharaman, K. Balasubramanian, A.A.
943 Amoscato, G. Borisenko, Z. Huang, A.M. Gusdon, A. Cheikhi, E.K. Steer, R. Wang, C. Baty, S. Watkins, I.
944 Bahar, H. Bayir, V.E. Kagan, Cardiolipin externalization to the outer mitochondrial membrane acts as
945 an elimination signal for mitophagy in neuronal cells, Nat Cell Biol 15(10) (2013) 1197-1205.
946 [66] L. Abdullah, J.E. Evans, S. Ferguson, B. Mouzon, H. Montague, J. Reed, G. Crynen, T. Emmerich, M.
947 Crocker, R. Pelot, M. Mullan, F. Crawford, Lipidomic analyses identify injury-specific phospholipid
948 changes 3 mo after traumatic brain injury, FASEB J 28(12) (2014) 5311-21.
949 [67] T. Emmerich, L. Abdullah, J. Ojo, B. Mouzon, T. Nguyen, G.S. Laco, G. Crynen, J.E. Evans, J. Reed,
950 M. Mullan, F. Crawford, Mild TBI Results in a Long-Term Decrease in Circulating Phospholipids in a
951 Mouse Model of Injury, Neuromolecular Med 19(1) (2017) 122-135.
952 [68] S.R. Hogan, J.H. Phan, M. Alvarado-Velez, M.D. Wang, R.V. Bellamkonda, F.M. Fernández, M.C.
953 LaPlaca, Discovery of Lipidome Alterations Following Traumatic Brain Injury via High-Resolution
954 Metabolomics, J Proteome Res 17(6) (2018) 2131-2143.
955 [69] R.O. Bahado-Singh, S.F. Graham, B. Han, O. Turkoglu, J. Ziadeh, R. Mandal, A. Er, D.S. Wishart, P.L.
956 Stahel, Serum metabolomic markers for traumatic brain injury: a mouse model, Metabolomics 12(6)
957 (2016) 100.

[70] M.R. Viant, B.G. Lyeth, M.G. Miller, R.F. Berman, An NMR metabolomic investigation of early metabolic disturbances following traumatic brain injury in a mammalian model, NMR Biomed 18(8) (2005) 507-16.
[71] E.W. Baker, W.M. Henderson, H.A. Kinder, J.M. Hutcheson, S.R. Platt, F.D. West, Scaled traumatic brain injury results in unique metabolomic signatures between gray matter, white matter, and serum in a piglet model, PloS one 13(10) (2018) e0206481.
[72] S.A. Sheth, A.T. Iavarone, D.S. Liebeskind, S.J. Won, R.A. Swanson, Targeted Lipid Profiling Discovers Plasma Biomarkers of Acute Brain Injury, PLoS One 10(6) (2015) e0129735.
[73] V.S.S.S. Sajja, A. Jablonska, N. Haughey, J.W.M. Bulte, R.D. Stevens, J.B. Long, P. Walczak, M. Janowski, Sphingolipids and microRNA Changes in Blood following Blast Traumatic Brain Injury: An Exploratory Study, J Neurotrauma 35(2) (2018) 353-361.
[74] A. Baethmann, K. Maier-Hauff, L. Schurer, M. Lange, C. Guggenbichler, W. Vogt, K. Jacob, O. Kempski, Release of glutamate and of free fatty acids in vasogenic brain edema, J Neurosurg 70(4) (1989) 578-91.
[75] P. Demediuk, A.I. Faden, R. Romhanyi, R. Vink, T.K. McIntosh, Traumatic brain injury in the rat: effects on lipid metabolism, tissue magnesium, and water content, Journal of neurotrauma 5(2) (1988) 105-19.
[76] H. Bayir, V.A. Tyurin, Y.Y. Tyurina, R. Viner, V. Ritov, A.A. Amoscato, Q. Zhao, X.J. Zhang, K.L. Janesko-Feldman, H. Alexander, L.V. Basova, R.S. Clark, P.M. Kochanek, V.E. Kagan, Selective early cardiolipin peroxidation after traumatic brain injury: an oxidative lipidomics analysis, Ann Neurol 62(2) (2007) 154-69.
[77] S. Signoretti, V. Di Pietro, R. Vagnozzi, G. Lazzarino, A.M. Amorini, A. Belli, S. D'Urso, B. Tavazzi, Transient alterations of creatine, creatine phosphate, N-acetylaspartate and high-energy phosphates after mild traumatic brain injury in the rat, Mol Cell Biochem 333(1-2) (2010) 269-77.
[78] J.A. Mitamura, M.L. Seligman, J.J. Solomon, E.S. Flamm, H.B. Demopoulos, J. Ransohoff, Loss of essential membrane lipids and ascorbic acid from rat brain following cryogenic injury and protection by methylprednisolone, Neurol Res 3(4) (1981) 329-44.
[79] J. Chitturi, Y. Li, V. Santhakumar, S.S. Kannurpatti, Early behavioral and metabolomic change after mild to moderate traumatic brain injury in the developing brain, Neurochem Int 120 (2018) 75-86.
[80] S.A. Novgorodov, C.L. Riley, J. Yu, K.T. Borg, Y.A. Hannun, R.L. Proia, M.S. Kindy, T.I. Gudz, Essential roles of neutral ceramidase and sphingosine in mitochondrial dysfunction due to traumatic brain injury, J Biol Chem 289(19) (2014) 13142-54.
[81] F. Zheng, Y.T. Zhou, D.D. Feng, P.F. Li, T. Tang, J.K. Luo, Y. Wang, Metabolomics analysis of the hippocampus in a rat model of traumatic brain injury during the acute phase, Brain Behav 10(2) (2020) e01520.
[82] J.O. Ojo, M. Algamal, P. Leary, L. Abdullah, B. Mouzon, J.E. Evans, M. Mullan, F. Crawford, Converging and Differential Brain Phospholipid Dysregulation in the Pathogenesis of Repetitive Mild Traumatic Brain Injury and Alzheimer's Disease, Front Neurosci 13 (2019) 103.
[83] P. Muza, C. Bachmeier, B. Mouzon, M. Algamal, N.G. Rafi, C. Lungmus, L. Abdullah, J.E. Evans, S. Ferguson, M. Mullan, F. Crawford, J.O. Ojo, APOE Genotype Specific Effects on the Early Neurodegenerative Sequelae Following Chronic Repeated Mild Traumatic Brain Injury, Neuroscience 404 (2019) 297-313.
[84] A. Roux, L. Muller, S.N. Jackson, J. Post, K. Baldwin, B. Hoffer, C.D. Balaban, D. Barbacci, J.A. Schultz, S. Gouty, B.M. Cox, A.S. Woods, Mass spectrometry imaging of rat brain lipid profile changes over time following traumatic brain injury, J Neurosci Methods 272 (2016) 19-32.
[85] C. Han, S. Li, Q. Yue, N. Li, H. Yang, Z. Zhao, Polydopamine-capped AgNPs as a novel matrix overcoming the ion suppression of phosphatidylcholine for MALDI MS comprehensive imaging of glycerophospholipids and sphingolipids in impact-induced injured brain, Analyst 144(21) (2019) 6304-6312.

[86] N. Li, P. Wang, X. Liu, C. Han, W. Ren, T. Li, X. Li, F. Tao, Z. Zhao, Developing IR-780 as a Novel Matrix for Enhanced MALDI MS Imaging of Endogenous High-Molecular-Weight Lipids in Brain Tissues, Anal Chem 91(24) (2019) 15873-15882.
[87] L.J. Sparvero, A.A. Amoscato, A.B. Fink, T. Anthonymuthu, L.A. New, P.M. Kochanek, S. Watkins, V.E. Kagan, H. Bayır, Imaging mass spectrometry reveals loss of polyunsaturated cardiolipins in the cortical contusion, hippocampus, and thalamus after traumatic brain injury, J Neurochem 139(4) (2016) 659-675.
[88] K. Mallah, J. Quanico, D. Trede, F. Kobeissy, K. Zibara, M. Salzet, I. Fournier, Lipid Changes Associated with Traumatic Brain Injury Revealed by 3D MALDI-MSI, Anal Chem 90(17) (2018) 10568-10576.
[89] S. Guo, D. Zhou, M. Zhang, T. Li, Y. Liu, Y. Xu, T. Chen, Z. Li, Monitoring changes of docosahexaenoic acid-containing lipids during the recovery process of traumatic brain injury in rat using mass spectrometry imaging, Sci Rep 7(1) (2017) 5054.
[90] B.G. Lyeth, Q.-Z. Gong, H.S. Dhillon, M.R. Prasad, Effects of muscarinic receptor antagonism on the phosphatidylinositol bisphosphate signal transduction pathway after experimental brain injury, Brain Research 742(1) (1996) 63-70.
[91] H.S. Dhillon, D. Donaldson, R.J. Dempsey, M.R. Prasad, Regional levels of free fatty acids and Evans blue extravasation after experimental brain injury, Journal of neurotrauma 11(4) (1994) 405-15.
[92] S.W. Scheff, H.S. Dhillon, Creatine-enhanced diet alters levels of lactate and free fatty acids after experimental brain injury, Neurochemical research 29(2) (2004) 469-79.
[93] P. Homayoun, E.B. Rodriguez de Turco, N.E. Parkins, D.C. Lane, J. Soblosky, M.E. Carey, N.G. Bazan, Delayed phospholipid degradation in rat brain after traumatic brain injury, J Neurochem 69(1) (1997) 199-205.
[94] T.S. Anthonymuthu, E.M. Kenny, A.A. Amoscato, J. Lewis, P.M. Kochanek, V.E. Kagan, H. Bayir, Global assessment of oxidized free fatty acids in brain reveals an enzymatic predominance to oxidative signaling after trauma, Biochim Biophys Acta Mol Basis Dis 1863(10 Pt B) (2017) 2601-2613.
[95] H. Chao, T.S. Anthonymuthu, E.M. Kenny, A.A. Amoscato, L.K. Cole, G.M. Hatch, J. Ji, V.E. Kagan, H. Bayır, Disentangling oxidation/hydrolysis reactions of brain mitochondrial cardiolipins in pathogenesis of traumatic injury, JCI Insight 3(21) (2018).
[96] V.E. Kagan, V.A. Tyurin, J. Jiang, Y.Y. Tyurina, V.B. Ritov, A.A. Amoscato, A.N. Osipov, N.A. Belikova, A.A. Kapralov, V. Kini, I.I. Vlasova, Q. Zhao, M. Zou, P. Di, D.A. Svistunenko, I.V. Kurnikov, G.G. Borisenko, Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors, Nat Chem Biol 1(4) (2005) 223-32.
[97] H.S. Dhillon, T. Carbary, J. Dose, R.J. Dempsey, M. Renuka Prasad, Activation of phosphatidylinositol bisphosphate signal transduction pathway after experimental brain injury: a lipid study, Brain Research 698(1) (1995) 100-106.
[98] F.H. Kobeissy, J.D. Guingab-Cagmat, Z. Zhang, A. Moghieb, O.Y. Glushakova, S. Mondello, A.M. Boutte, J. Anagli, R. Rubenstein, H. Bahmad, A.K. Wagner, R.L. Hayes, K.K. Wang, Neuroproteomics and Systems Biology Approach to Identify Temporal Biomarker Changes Post Experimental Traumatic Brain Injury in Rats, Front Neurol 7 (2016) 198.
[99] A.A. Kanner, N. Marchi, V. Fazio, M.R. Mayberg, M.T. Koltz, V. Siomin, G.H. Stevens, T. Masaryk, B. Aumayr, M.A. Vogelbaum, G.H. Barnett, D. Janigro, Serum S100beta: a noninvasive marker of blood-brain barrier function and brain lesions, Cancer 97(11) (2003) 2806-13.
[100] A. Kleindienst, C. Schmidt, H. Parsch, I. Emtmann, Y. Xu, M. Buchfelder, The Passage of S100B from Brain to Blood Is Not Specifically Related to the Blood-Brain Barrier Integrity, Cardiovasc Psychiatry Neurol 2010 (2010) 801295.
[101] B.A. Plog, M.L. Dashnaw, E. Hitomi, W. Peng, Y. Liao, N. Lou, R. Deane, M. Nedergaard, Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system, The Journal of neuroscience : the official journal of the Society for Neuroscience 35(2) (2015) 518-26.
[102] H. Alluri, K. Wiggins-Dohlvik, M.L. Davis, J.H. Huang, B. Tharakan, Blood-brain barrier dysfunction following traumatic brain injury, Metab Brain Dis 30(5) (2015) 1093-104.

[103] D. Shlosberg, M. Benifla, D. Kaufer, A. Friedman, Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury, Nat Rev Neurol 6(7) (2010) 393-403.
[104] O.Y. Glushakova, D. Johnson, R.L. Hayes, Delayed increases in microvascular pathology after experimental traumatic brain injury are associated with prolonged inflammation, blood-brain barrier disruption, and progressive white matter damage, Journal of neurotrauma 31(13) (2014) 1180-93.
[105] L. Yi, S. Shi, Y. Wang, W. Huang, Z.A. Xia, Z. Xing, W. Peng, Z. Wang, Serum Metabolic Profiling Reveals Altered Metabolic Pathways in Patients with Post-traumatic Cognitive Impairments, Scientific reports 6 (2016) 21320.
[106] J.G. Pilitsis, W.M. Coplin, M.H. O'Regan, J.M. Wellwood, F.G. Diaz, M.R. Fairfax, D.B. Michael, J.W. Phillis, Measurement of free fatty acids in cerebrospinal fluid from patients with hemorrhagic and ischemic stroke, Brain Research 985(2) (2003) 198-201.
[107] 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, Mol Biosyst 11(12) (2015) 3287-96.
[108] I. Saluja, M.H. O'Regan, D. Song, J.W. Phillis, Activation of cPLA2, PKC, and ERKs in the rat cerebral cortex during ischemia/reperfusion, Neurochemical research 24(5) (1999) 669-77.
[109] J.M. Oliver, M.T. Jones, K.M. Kirk, D.A. Gable, J.T. Repshas, T.A. Johnson, U. Andreasson, N. Norgren, K. Blennow, H. Zetterberg, Effect of Docosahexaenoic Acid on a Biomarker of Head Trauma in American Football, Med Sci Sports Exerc 48(6) (2016) 974-82.
[110] O. Thau-Zuchman, R.N. Gomes, S.C. Dyall, M. Davies, J.V. Priestley, M. Groenendijk, M.C. De Wilde, J.L. Tremoleda, A.T. Michael-Titus, Brain Phospholipid Precursors Administered Post-Injury Reduce Tissue Damage and Improve Neurological Outcome in Experimental Traumatic Brain Injury, Journal of neurotrauma 36(1) (2019) 25-42.
[111] T.S. Anthonymuthu, E.M. Kenny, A.M. Lamade, V.E. Kagan, H. Bayir, Oxidized phospholipid signaling in traumatic brain injury, Free radical biology & medicine 124 (2018) 493-503.
[112] E.M. Kenny, E. Fidan, Q. Yang, T.S. Anthonymuthu, L.A. New, E.A. Meyer, H. Wang, P.M. Kochanek, C.E. Dixon, V.E. Kagan, H. Bayir, Ferroptosis Contributes to Neuronal Death and Functional Outcome After Traumatic Brain Injury, Crit Care Med 47(3) (2019) 410-418.
[113] K. Blennow, D.L. Brody, P.M. Kochanek, H. Levin, A. McKee, G.M. Ribbers, K. Yaffe, H. Zetterberg, Traumatic brain injuries, Nat Rev Dis Primers 2 (2016) 16084.
[114] E. Thelin, F. Al Nimer, A. Frostell, H. Zetterberg, K. Blennow, H. Nystrom, M. Svensson, B.M. Bellander, F. Piehl, D.W. Nelson, A Serum Protein Biomarker Panel Improves Outcome Prediction in Human Traumatic Brain Injury, Journal of neurotrauma 36(20) (2019) 2850-2862.
[115] K. Zhang, T.J. Sejnowski, A universal scaling law between gray matter and white matter of cerebral cortex, Proc Natl Acad Sci U S A 97(10) (2000) 5621-6.
[116] B. Mota, S.E. Dos Santos, L. Ventura-Antunes, D. Jardim-Messeder, K. Neves, R.S. Kazu, S. Noctor, K. Lambert, M.F. Bertelsen, P.R. Manger, C.C. Sherwood, J.H. Kaas, S. Herculano-Houzel, White matter volume and white/gray matter ratio in mammalian species as a consequence of the universal scaling of cortical folding, Proc Natl Acad Sci U S A 116(30) (2019) 15253-15261.
[117] S. Mondello, A. Sorinola, E. Czeiter, Z. Vamos, K. Amrein, A. Synnot, E. Donoghue, J. Sandor, K.K.W. Wang, R. Diaz-Arrastia, E.W. Steyerberg, D.K. Menon, A.I.R. Maas, A. Buki, Blood-Based Protein Biomarkers for the Management of Traumatic Brain Injuries in Adults Presenting to Emergency Departments with Mild Brain Injury: A Living Systematic Review and Meta-Analysis, Journal of neurotrauma (2018).
[118] J.R. Huie, S. Mondello, C.J. Lindsell, L. Antiga, E.L. Yuh, E.R. Zanier, S. Masson, B.L. Rosario, A.R. Ferguson, R. Transforming, T.T.R. Clinical Knowledge in Traumatic Brain Injury Investigators, C.E.N.E.R.i.T.B.I.P. Clinical Knowledge in Traumatic Brain Injury Investigators, C.E.N.E.R.i.T.B.I.P. Investigators, Investigators, Biomarkers for Traumatic Brain Injury: Data Standards and Statistical Considerations, Journal of neurotrauma (2020).