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Metabolism and inflammation: implications for traumatic brain injury therapeutics

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Metabolism and inflammation: Implications for traumatic brain injury therapeutics

ABSTRACT

Introduction: Traumatic Brain Injury (TBI) is a leading cause of death and disability in young people, affecting 69 million people annually, worldwide. The initial trauma disrupts brain homeostasis resulting in metabolic dysfunction and an inflammatory cascade, which can then promote further neurodegenerative effects for months or years, as a 'secondary' injury. Effective targeting of the cerebral inflammatory system is challenging due to its complex, pleiotropic nature. Cell metabolism plays a key role in many diseases, and increased disturbance in the TBI metabolic state is associated with poorer patient outcomes. Investigating critical metabolic pathways, and their links to inflammation, can potentially identify supplements which alter the brain's long-term response to TBI and improve recovery.

Areas covered: The authors provide an overview of literature on metabolism and inflammation following TBI, and from relevant pre-clinical and clinical studies, propose therapeutic strategies.

Expert commentary: There is still no specific active drug treatment for TBI. Changes in metabolic and inflammatory states have been reported after TBI and appear linked. Understanding more about abnormal cerebral metabolism following TBI, and its relationship with cerebral inflammation, will provide essential information for designing therapies, with implications for neurocritical care and for alleviating long-term disability and neurodegeneration in post-TBI patients.

KEYWORDS: Inflammation, Lactate Pyruvate Ratio (LPR), Metabolic Dysfunction, Metabolism, Supplementation, Traumatic brain injury (TBI).

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1.0 Traumatic brain injury

Traumatic Brain Injury (TBI) is the leading cause of death and disability for those under 40 years of age in the developed world [1,2]. While an estimated 69 million people are affected worldwide every year [3], there has been significant progress in the last century to combat the extent of such injuries. Public policies such as road safety measures and occupational health and safety regulations can decrease the incidence of TBI, and advanced neurocritical care has improved individual patient management. Multi-modality monitoring is one of such advancements which enables real-time observation of cerebral pressure and oxygenation. Clinicians can then attempt to resolve disturbances in these parameters of the brain using surgical and medical interventions. Despite the progress in these areas and our improved understanding of the brain in recent decades, there is still no standard effective drug treatment for TBI. There is a great opportunity for such treatment to mitigate the extent of tissue damage and cell death, however many clinical and pre-clinical trials that have seemed promising in earlier stages have failed to show significant benefit when implemented in patients [4]. This may be due to a number of things, including issues with animal model to human translation, lack of trial optimisation in humans, the heterogeneous nature of the injuries, shortcomings of the Glasgow Outcome Scale (GOS) in assessing smaller improvements, and the complexity of targeting the inflammatory system due to the pleiotropic nature of its components [5].

Initial or 'primary' trauma of the brain is caused by impact and physical movement of the brain, resulting in damage of blood vessels, damage to cell axons, shearing of tissue, and swelling. The 'secondary' injury of continued degeneration, however, is less well understood and can vary vastly between patients with similar injuries. TBI is a heterogenous disease ranging in severity; this review is focussed on severe TBI in the context of neurocritical care. Recent studies have highlighted the high occurrence of chronic traumatic encephalopathy in players of contact sports, and how multiple mild head injury events can lead to chronic traumatic encephalopathy with dementia and Parkinson's-like symptoms [6]. Mild TBI and multi-injury concussive brain injury patients differ from severe TBI patients in terms of pathology [7,8], inflammatory activation levels [9], and brain metabolic changes [10]. Caution and additional research must therefore be taken when applying treatments proposed for single-injury severe TBI patients in this review to other forms of TBI.

In the days following severe TBI, we see a prominent inflammatory cascade in the majority of patients [11], with subsets of patients experiencing a chronic inflammation for many months or even years post-injury, accompanied by cognitive neurodegeneration [12]. Mitochondrial dysfunction is also a hallmark of both the acute and post-acute stages of severe TBI, with changes in mitochondrial morphology [13], accumulation of mitochondria at injury sites, altered levels of metabolites and overall mitochondrial function [14,15]. There is increasing evidence that these two phenomena of inflammation and mitochondrial metabolism have significant cross-talk and impact upon one another in multiple other disease states of

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the brain, including multiple sclerosis [16], Parkinson's [17], Alzheimer's [18,19], and amyotrophic lateral sclerosis [20]. This relationship also warrants investigation in TBI, and any mechanistic links between metabolism and inflammation may be key for dual-acting therapies which can ameliorate the negative effects of both pathways.

This review will outline the critical roles and interaction of metabolism and inflammation in TBI, highlighting the implications of these interactions for potential therapeutic strategies. As this is an emerging field of interest, evidence of such interaction between the two processes in several disease models will be presented, along with the case for further research into the important relationship.

2.0 Brain Metabolism

2.1. Fundamentals of metabolism

The brain requires around 20% of the body's total energy output to send electrical signals and maintain homeostatic function. Cellular metabolism provides the energy that underlies all brain activity by processing substrates into ATP. Glucose is the main source of fuel for energy production in the brain, but the mechanism of its consumption varies in different environmental conditions and between different cell types, with downstream substrates such as lactate purported to play a key role [21].

Glycolysis, the initial breakdown of glucose molecules, produces a low yield of 2 molecules of ATP per molecule of glucose, and 2 pyruvate molecules, which can subsequently be used for further energy production (Figure 1). After glycolysis, the pyruvate molecules can go on to be converted to lactate with no additional ATP produced, or used in the high yield oxidative phosphorylation pathway, through the tricarboxylic acid (TCA) cycle and electron transport chain (ETC). The energy yield per molecule of glucose metabolised fully to CO_2 (by the combination of glycolysis, NADH shuttling and mitochondrial respiration) is theoretically 36–38 molecules of ATP, but the actual yield is considered somewhat lower [22,23].

The pentose phosphate pathway (PPP) is yet another potential fate of a glucose molecule, diverging from initial glycolysis steps, which produces no ATP but does produce molecules for nucleic acid sequences. The PPP is a complex biosynthetic network constituting a detour around several steps of glycolysis, generating many species including lactate and does not involve molecular oxygen. Its main function can be regarded as sacrificing some of the cells' supply of glucose molecules, which might otherwise have been used for ATP synthesis, for the sake of generating more reducing power (NADPH) and the ability to protect, repair, or build cells.

Another energetic pathway theory which is gaining in acceptance (although still debated) is the astrocyte-neuron lactate shuttle (ANLS) hypothesis [24,25]. In the ANLS theory, glycolysis - the conversion of glucose to pyruvate - occurs predominantly in astrocytes, followed by the subsequent conversion of pyruvate to

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lactate by lactate dehydrogenase (LDH). The lactate is exported from astrocytes then transported by monocarboxylate transport proteins (MCT) into neurons where it can be oxidized back to pyruvate for entry into the TCA cycle. The astrocyte-neuron lactate shuttle (ANLS) is depicted schematically in Figure 2 and explained further in the legend [26].

The advantages of this transfer of lactate include the ability to maintain brain function by supporting neurons at the energetic cost of the astrocytes. Neurons, for example, have reduced expression of enzymes that detoxify by-products of glycolysis (glyoxase 1 and 2) compared to astrocytes [26]. Also, in comparison to neurons, astrocytes have lower activity of pyruvate dehydrogenase, which processes pyruvate for use in the TCA cycle [26], and a higher concentration of lactate (considered as a reservoir) [27,28]. Further evidence to support specific cell type metabolic substrate preferences is detailed in neocortical cell culture models [29] and in positive implications from small studies of human patients [30]. However, findings of a kinetic modelling study in rats using labelled glucose [31] favoured an "independent" model in which neurons and astrocytes take up and oxidise glucose according to their respective energy needs, rather than the ANLS model. It still remains to be shown directly that there is net transfer of lactate from glia to neurons *in vivo*, nevertheless it is important to consider the potential energetic impacts of such processes in our evolving understanding of brain metabolism.

Whether glucose or lactate molecules are the primary source of fuel for neuronal cells, maintaining optimum energetic functioning of the brain can thus be achieved by utilising multiple metabolic substrates. The evidence of flexibility in the uptake and use of such molecules highlights a wider range of potential targets for energetic supplementation. Optimal energy processing in both homeostasis and injury is therefore an important aspect for further research as it may assist in recovery.

2.2 Metabolism in traumatic brain injury

Distinct changes in brain metabolism become apparent when brain cells are stressed by TBI [32]. Metabolic indicators of the glycolysis pathway are increased, as well as the presence of extracellular lactate, producing a high extracellular lactate to pyruvate ratio (LPR) in some TBI patients. LPR is often used as an indicator of the brain's overall metabolic state in TBI, with a high LPR reflecting mitochondrial dysfunction or a lack of oxygen supply [15]. Increased lactate production from pyruvate may also reduce the total pyruvate available for mitochondrial metabolism, and as such reduce overall ATP production.

Prior to surgical and medical interventions, a severe head injury or insult would likely cause a lack of oxygen to brain cells – hypoxia, so this switch to glycolysis may simply be an evolutionary coping mechanism, to ensure that energy production can still occur. Glycolysis and pyruvate to lactate conversion occurs without the need for

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oxygen, unlike mitochondrial energy processing, and as such, cerebral hypoxia in TBI patients is also accompanied by an increase in glutamate and lactate levels [33].

The pentose phosphate pathway (PPP) [34] also does not require oxygen and may be another source of increased lactate post-TBI. In a recent small study, several TBI patients had PPP-derived lactate elevation above “normal” (non-TBI) brain ranges, correlating with decreasing brain tissue oxygen concentrations, indicating shifting glucose metabolism from glycolysis towards PPP (although with glycolysis remaining dominant) [34].

These perturbation in brain metabolism however can still occur in TBI patients even when access to oxygen is well conserved by clinical management strategies. Despite modern neurocritical care supplying the brain with seemingly adequate levels of oxygen and nutrients (e.g. glucose), the injured brain cannot always use the fuels it receives. Such patients suffering metabolic dysfunction (characterised by a high LPR) had significantly poorer clinical outcomes in a large study (223 patients) [15]. The divergence from normal metabolic function despite adequate oxygen, may imply that the change in metabolism may be associated with additional functions than just that of producing energy, such as involvement in the other key mechanisms which cope with injury, like inflammation.

Reliance on the glycolysis pathway for energy production in TBI may also be due to damage sustained by the mitochondria [35]. Complex I of the mitochondrial electron transport chain is thought to be a likely site of impairment or alteration in activity in both TBI [36] and neurodegenerative disorders such as Parkinson’s [37], multiple sclerosis [38] and amyotrophic lateral sclerosis [39,40]. It has been demonstrated in animal models that the function of complex I decreases with age, and that it is particularly vulnerable to reactive oxygen species (ROS) which are produced adjacent to the complex [41]. The purported cause of this vulnerability is credited to the large proportion of mitochondrial rather than nuclear DNA subunits encoding complex I [42]. It is well established that mitochondrial DNA functionality decreases with age in various rodent tissues [43], accompanied by an increase in mitochondrial ROS production [41].

The fragile complex 1 may be too damaged post-TBI to utilize metabolic substrates, causing the observed extracellular accumulation and high LPR in patients [44]. Targeted supplementation of the brain’s mitochondria for rescuing the TCA cycle and sites of damage are therefore a prospective avenue for therapeutics. Recovering metabolic function may also contribute towards the whole brain response towards more reparative mechanisms such as inflammation, as detailed further in section 4.

3.0 Inflammation in TBI

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The mechanism by which the force and impact of a TBI stimulates cells to initiate inflammatory signals is not fully understood. The trauma results in cellular debris and Disease Associated Molecular Patterns (DAMPs) including free DNA, RNA and alarmins such as HMGB1[45,46]. This begins the sequence of the inflammatory cascade, which activates and recruits both resident and peripheral immune cells. The inflammatory cascade initiates for a number of beneficial functions, including the phagocytosis and clearing of dead cells, the isolation of healthy brain area by 'walls' of microglia and astrocytes, and protection against infection [47]. However, persistent or unregulated inflammation can inflict 'secondary insults' on the brain, and it is difficult to detect at what point this inflammation becomes maladaptive [48]. Inflammatory processes in combination with a cytotoxic environment may lead to the excessive pruning of still-functioning synapses and engulfing of viable cells. This chronic level of inflammation and activated immune cells, which have been identified up to 17 years post-injury in patients, may play a significant role in the decrease of white matter density and corresponding reduction in cognitive ability [12,49].

There is an opportunity to intervene with treatments to limit this chronic inflammatory process, however, it is difficult differentiate the beneficial inflammation from the harmful. Inflammation in the brain post-injury could potentially be modulated by the use of targeted anti-inflammatory treatments (e.g. IL-1ra), as these may reduce the negative effects of this cascade [50]. However, a refined approach is required to maintain the beneficial aspects of inflammation such as repair and promotion cell survival [11], as anti-inflammatory therapies may therefore also impact on these restorative processes.

In ideal circumstances acute inflammation in the brain is self-resolving, yet the exact processes that govern the cascade or how to 'switch' from encouraging cell apoptosis, clearance and stress signals, to promoting cell survival and repair *in vivo* remain uncharacterised. Thelin et al. [51] have further described the complexity of the post-TBI environment, detailing the interactions between cell types and highlighting key signalling molecules, shown schematically in Figure 3 and explained in the legend. The complement system, for example, may contribute to neurodegeneration in TBI, as it enhances the inflammatory cascade. Complement, (for example C1q, C3b, C3d, C5-9) is increased in the brain post-injury and in microglia specifically – evidence has shown that this increase may influence their activation state, stimulate accumulation of inflammatory cells and formation of brain edemas and further neuronal damage [52,53]. The complement system therefore provides another avenue for exploration into inhibiting exacerbation of chronic inflammation, and research into this area is promising for future potential therapies, as detailed further in the review by Hammand et al [54]. In addition to complement, a recent study of neurogenic inflammation mediator- substance P is also an important peptide neurotransmitter to note in TBI, and is further detailed in a review by Vink et al. [55].

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It has been established in rodent models of TBI that monocytes and other inflammatory cells such as neutrophils enter the brain via the bloodstream [56-58], however the literature is currently lacking in definitive confirmation of this process in human TBI patients. The recent discovery of microscopic 'tunnels' from both mouse and human skull bone marrow to the dura and meninges however illuminated additional avenues through which these inflammatory cells may invade the brain [59].

The level of blood brain barrier (BBB) dysfunction and the role it plays in regulating this infiltration is likely to differ with severity of injury, and as such is difficult to study in humans. Csuka et al. demonstrated that BBB dysfunction was independent of key cytokine levels, comparing markers of BBB dysfunction and IL-10 levels in TBI patient cerebral spinal fluid (CSF) samples [60]. While in rodent models, Semple et al. found that deficiency of a chemokine receptor, CXCR2 was able to attenuate neutrophil infiltration [61]. Invading inflammatory cells from the peripheral nervous system could be vital to recovery post-insult, or to the perpetuation of a chronic inflammatory state. Further research into the roles of these cells in injury would be of great benefit to our overall understanding of the intricate relationship between BBB functionality and inflammation in the brain.

3.1 Cytokines and glia

Cytokines and chemokines comprise a broad range of small cell signalling proteins that are autocrine and paracrine regulators of inflammation in the brain. They are released from activated, stressed or dying cells in order to promote survival, breakdown cellular membranes, recruit other cells and mark them for repair or phagocytosis [62]. Although cytokines are often classified as pro- or anti-inflammatory, many have been found to be involved in opposing roles, depending on the context of their environment and timing after injury [63].

The levels of numerous cytokines and chemokines found in TBI patients have been characterised in the days after injury in microdialysates. These studies have highlighted how specific signalling proteins can be associated with aspects of the timing and nature of the inflammatory state of the brain [11]. High IL-1β and TNFα are typically associated with the first 24 hours post-injury, related to pro-inflammatory mechanisms including apoptosis in many disease models [64-66], while high IL-10, and later IL-4 can be associated with the latter stages and are known for their roles in repair and promoting cell survival [63]. Also of note is IL-6, an signalling molecule which appears at high concentrations in the acute phase of injury, acting as a biomarker of inflammation [67].

Microglia are the resident inflammatory cells in the brain and are a key source and regulator of the cytokine cascade. When activated *in vitro*, they can be seen to

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produce profiles of cytokines which are also often classified as pro- or anti-inflammatory, namely the M1 and M2 phenotypes [68]. While this characterisation has been criticised for its simplicity in describing a complex phenomenon [69], and proposals have been made for revisions and alterations to the paradigm [70], they are nonetheless useful terms when attempting to understand and refer to the ways in which activated microglia and their cytokine profile can be associated with reparative, or destructive and phagocytic processes.

In a number of pathological conditions, such as neurodegenerative disorders, both M1 and M2 type macrophages have been found to coexist and also from other unique phenotypes, and it is likely that most cells sit on a spectrum of activation as opposed to these extreme polarised states [70,71]. It can also be difficult to distinguish infiltrating monocyte derived macrophages from activated microglia in the brain. Levels of 'CD' antigens such as CD11b and CD45 [72,73] have been used in multiple publications to differentiate these cell types in the past two decades, however newer stains and labelling systems such as TMEM119, a specific marker for microglia [74,75] are proving valuable. The impact of these infiltrating vs resident cells and their inherent neuroprotective or pro-inflammatory role are still being debated, and again can change dependant on the severity of injury [76-78].

Astrocytes have similarly been assigned A1 and A2 designations of activation in recent studies, denoting states of activation in these cells [79]. Their activation is closely linked to that of microglia; their role in TBI has been found to be important for tissue protection in mild TBI, but not severe TBI [80], and more research is required to understand their role in this specific disease context.

Oligodendrocytes also play a key role post-TBI in remyelination and repair of damaged axons, however excessive myelination by dysregulated cells may also lead to further damage [81,82]. Direct mechanistic links of oligodendrocytes to the inflammatory cascade however are not well defined, although some links have been made to metabolic dysfunction in the overall Wallerian degeneration of axons.

4. Synergistic inflammation and metabolic response

The relationship between inflammation and metabolism in TBI remains largely unexplored but may have substantial implications for treatment, given that metabolism plays a significant role in encouraging the reparative mechanisms of the brain.

Macrophages and other myeloid cells, as previously described in their involvement in the inflammatory response, have several metabolic characteristics which distinguish the M1 and M2 phenotypes *in vitro*. When these non-CNS macrophages are activated into classical M1 or alternative M2 states by LPS and inflammatory cytokines, there is a measured change in metabolism [83], including an increased

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glucose consumption and lactate production [84,85]. Blocking oxidative metabolism in these cells also blocks the M2 phenotype, and drives the macrophage into an M1 state. Similarly, forcing oxidative metabolism in an M1 macrophage potentiates the M2 phenotype [83].

The hypothesis for how the change in metabolism affects the inflammatory state, is via increased TCA cycle intermediates like succinate and malate. These intermediates are responsible for regulating HIF1α, which drives the sustained production of the pro-inflammatory cytokine IL-1β. Therefore, the change in metabolism and build-up of such intermediates caused more 'pro-inflammatory' cytokine IL-1β, and therefore an M1-like state.

Levels of oxygen are also theorised to play an important role in HIFα regulation, potentially in a similar way by blocking mitochondrial respiration and causing similar build-up of intermediates. Therefore hypoxia-like environments, where mitochondrial dysfunction occurs as in TBI, may similarly drive a M1 pro-inflammatory phenotype in infiltrating macrophages [83]. Microglia, as the resident macrophage cells of the brain also respond similarly to such metabolic impairments. The inhibition of mitochondrial respiratory chain complex I in microglia by the compound rotenone led to production of mitochondrial ROS and TNFα [86,87].

The separation of neuronal and glial cultures is important for investigation of metabolic and inflammatory pathways, as the interaction between different cell types in response to stress is complex. For example, neuronal death caused by addition of mitochondrial inhibitors to cell cultures was not observed unless microglia [88], or mixed glia [89] were also present in the culture. Gao et al attributed this phenomenon to the release of NADPH oxidase-derived superoxide from activated microglia [89], while Mount et al found that antibodies neutralizing the cytokine IFN-γ (produced by the microglia) improved neuronal survival of this mitochondrial stress [88]. Phagocytic activity of microglia was also found to be increased by mitochondrial inhibitors causing further neuronal loss in mixed neuronal/glial cultures [90]. Astrocytes also have different roles and associated response to stress and cytokines [91] and may also warrant individual investigation. Mixed cell cultures of neurons and glia are therefore necessary in order to gain a 'whole brain' perspective, while individual cell types in culture can elucidate cell-specific mechanisms.

Further evidence detailing the relationship between inflammation and metabolism is found in several disease models. Succinate produced by macrophages in an arthritis cell culture model exacerbated the inflammatory response, resulting in increased inflammatory cytokine IL-1β [92]. In atherosclerosis mouse models, pre-treatment of macrophages with mitochondrial inhibitors abolished the anti-inflammatory effects of cytokine IL-4. This indicates that anti-inflammatory mechanisms may be modulated by metabolism. However, inhibition of mitochondrial respiration had no effect on

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inflammatory activation of macrophages by proinflammatory cytokine IFN-γ and bacterial inflammatory response generator LPS [85].

More recently, in a Parkinson's disease model, transmembrane protein 173 or 'stimulator of interferon genes' (STING) was identified as the key signalling molecule activated during mitochondrial stress [17]. It also indicated the importance of proteins PINK1 and parkin, which aid in removing damaged mitochondria, in order to prevent this STING activation.

The relationship between inflammation and metabolism therefore has a strong evidence base and holds great potential for manipulation to improve injury and disease states.

5. Therapeutic avenues for TBI

5.1 Standard treatment and monitoring

Despite the progress in research in TBI, there is still no standard effective drug treatment for TBI which addresses metabolic dysfunction or the inflammatory cascade. Patients receive varying levels of other treatments and medication in accordance with their condition, for example antibiotics for pneumonia or infections, which may also impact on both their central and peripheral inflammatory state.

Multi-modality monitoring of patients enables real-time observation and intervention of disturbances in brain qualities such as pressure and oxygenation, managed both medically and surgically. Cerebral microdialysis also allows monitoring of brain glucose levels which can be controlled, as well as lactate and pyruvate as detailed further in Section 5.3, for indication of metabolic dysfunction. Monitoring of a number of biochemical compounds including cytokines have been investigated as potential biomarkers for brain injury and have been sparsely incorporated into clinical practice [11,93,94]. Ongoing biomarker analysis can assist in detection of secondary harmful events, however is not currently used for targeting therapeutics.

The majority of past TBI clinical drug studies focused on more multifunctional global brain approaches to influences hormones and steroids, including corticosteroids [95], progesterone [96,97], citicoline [98], magnesium sulphate [99], and statins [100] with limited success. This may not be due solely to poor efficacy of the therapies, as with the anti-inflammatory therapies mentioned below in in 5.2, but instead could be related to multiple factors such as issues with animal model to human translation, lack of trial optimisation in humans, the heterogeneous nature of TBIs, and difficulty in assessing minor improvements in patient outcome. Some illustrative studies of previous anti-inflammatory and metabolic therapeutics for TBI are summarised in Supplementary Table 1.

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5.2 Anti-inflammatory therapies

Anti-inflammatory therapies have been extensively studied in TBI, however have yet to demonstrate definitive clinical benefit. Wide ranging anti-inflammatory agents have been repurposed for studies in TBI, with many of the aforementioned multifunctional therapies in 5.1 and Supplementary Table 1, also having a potential anti-inflammatory effect. The broad immunosuppressant minocycline has been tested with limited success in rodent models [101], phase II human trials of spinal cord injury [102], and recently in TBI patients [103]. In Scott et al. 2018, minocycline treatment managed to reduce markers indicative of microglial activation, however also increased markers of neurodegeneration in TBI patients. Common anti-inflammatory therapeutic ibuprofen caused no significant effects in clinical studies [104] and deteriorated cognitive outcomes in rodent models of TBI [105].

Targeted anti-inflammatory therapies are also being explored in TBI. These inhibit the action of specific cytokines using antibodies and synthetic receptor antagonists. Tumour necrosis factor (TNF), inhibition has shown improvements across multiple clinical domains following treatment of both stroke and TBI patients in a large cohort study [106]. This study, however, had several limitations and a small TBI cohort, and hence a randomized, placebo-controlled trial is necessary to further characterise this outcome. The inhibition of cytokines IL-1α and IL-1ß have similarly been explored, due to their significant 'pro-inflammatory' effects with success in pre-clinical rodent models of TBI [107,108]. IL-1ra, the naturally occurring inhibitor of IL-1α and IL-1ß, when given subcutaneously also reduced markers of peripheral inflammation in subarachnoid haemorrhage patients [109]. Evidence of IL-1ra relationship with TBI patient outcome was established in a small study of 15 patients by Hutchinson et al. 2007, in which higher endogenous IL-1ra levels correlated to favourable outcomes [110]. Supplementing IL-1ra levels in the brain with Anakinra (recombinant IL-1ra) has also proven to be safe in TBI patients, however, initial findings suggest that it produces an increased 'pro-inflammatory' or 'M1' cytokine profile compared to untreated patients [50,111]. These effects will be further explored in a dose optimisation study of recombinant IL-1ra in a placebo controlled randomised trial by Helmy et al. (estimated completion 2023). The extent of the numerous novel pre-clinical anti-inflammatory studies are beyond the scope of this review, however is well detailed in other publications [112-114].

Nonspecific anti-inflammatories may be able to reduce the negative effects of an overwhelming cytotoxic cascade, however, they may also dampen repair and cell survival mechanisms. Equally, it can be difficult to produce a measurable effect in humans when targeting only a single cytokine or chemokine, as they act synergistically, and it can be difficult to tease out specific cytokine functions within a complex system [115]. Antibodies or increase of specific cytokines could also potentially trigger the opposite effect to that intended, depending on dose, timing,

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disease context and environmental factors. For example, cells may have their own feedback loop for limiting further transcription and production of cytokines [116,117], which anti-inflammatory therapies may interrupt.

Despite their targeting limitations, monitoring the effect that any potential TBI treatments may have on these signalling proteins is an important feature to investigate. As this review details, one potential avenue for anti-inflammatory action is through specific metabolic targeting as a dual action therapy.

5.3 Metabolic Supplementation

The brain's metabolic status post-TBI has been studied in great detail with spectral analysis techniques used noninvasively and also in the analysis of patient samples such as serum, CSF and microdialysis fluid. These techniques include; magnetic resonance spectroscopy (MRS) [118], mass spectrometry [119,120], and nuclear magnetic resonance (NMR) with both unlabelled [121] and ^{13}C labelled substrates [34,122]. The results of these studies highlight potential metabolic pathways which could be targeted by supplementation to improve brain energy status.

There is promising evidence currently emerging for metabolic supplementation, and its reinforcement of the 'normal' (non-trauma) homeostasis of the brain [123]. The impact of these substances on the inflammatory cascade, however, is yet to be demonstrated in most cases. Studies need to further investigate whether these agents will aid an overactive immune system perturbing the brain or encourage a more reparative environment.

5.3.1 Glucose

Brain glucose is of significant importance post-TBI, with patients often presenting with cerebral extracellular glucose levels outside the normal range, correlating with worse outcomes. High glucose levels identified in patient serum were associated poorer outcomes in patients [124] as were persistent low extracellular glucose levels in brain microdialysates [125]. Brain glucose is directly influenced by blood glucose levels and glutamate pathogenicity increases when blood glucose levels are low [126]. It is important to note, that glucose is of utmost importance for overall brain metabolism, and although additional substrates we are suggesting for supplementation may assist in brain metabolism in the acute phase post-TBI, glucose levels must first be corrected if at abnormal levels. The addition of glucose as metabolic rescue agent is therefore a critical factor in patient outcome, and many centres use glucose control in their standard TBI protocols, aiming for blood glucose levels of 4-7 mM [127]. However, controlling glucose levels too tightly in patients has

also been found to have no benefits in terms of decreased mortality [128] and increased incidences of metabolic crisis [129,130].

5.3.2 SuccinateSuccinate is another energetic molecule which has been explored as a therapeutic supplement in TBI. It acts at complex II of the Electron Transport Chain (ETC), in the mitochondria, located downstream potential sites of damage such as at complex I as previously described in 2.2. Succinate is converted into fumarate by the enzyme succinate dehydrogenase, as an integral part of the TCA cycle. Succinate, the anion of succinic acid, has multiple biological roles as a metabolic intermediate; as part of the production of ATP and as a signalling molecule of the cellular metabolic state [131].

It has been shown that succinate supplementation improves TBI brain chemistry, in a mixed glial model, indicated by biomarkers and reduced the LPR after exposure to stress by rotenone [132], highlighting its potential for use in TBI treatment. There is evidence to suggest, however, that succinate can have negative effects in hypoxic conditions, where it can build up in tissue, leading to the production of excessive reactive oxygen species (ROS) when oxygen is returned [131,133]. These studies however often used artificial dimethyl or diethyl succinate which is more cell permeable and may have further implications beyond that of natural metabolic utilisation, unlike the disodium salt. Ischemia reperfusion-induced injuries are also less common in TBI patients with modern neurocritical care that includes adequately managed brain oxygen supply as was the case in patients in a clinical study of succinate [123], and as such this succinate build up would be unlikely to occur.

Areas to be carefully considered during implementation of succinate as a therapeutic supplement therefore include ensuring adequate tissue oxygenation and reviewing the implications of using artificial features in such as methyl- and ethyl- groups on such molecules. Preliminary studies of disodium succinate use in TBI patients have provided a promising outlook for its use in delivery via microdialysis catheter, demonstrating effectiveness in lowering of the LPR [123] and improving the $NADH/NAD^+$ redox state of the brain [134]. In a patient with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes), oral succinate therapy (6 g/day, for more than 30 months) was associated with freedom from the stroke-like episodes and convulsions that had afflicted this patient prior to succinate therapy [135].

Succinate's effect on inflammation, however, requires further investigation as in mice and *in vitro* experiments succinate enhanced pro-inflammatory IL-1β production [131]. Succinate oxidation and subsequent ROS generation has also been linked to 'pro-inflammatory' macrophage activation [136]. However, in a recent paper using neural stem cells, when succinate was released from mononuclear phagocytes, it

initiated a chain of signalling resulting in anti-inflammatory effects [137], again highlighting the importance of context when assessing such complex systems.

5.3.3 Lactate

Mounting evidence indicates that lactate may be an efficient energy substrate for neurons and contribute to maintaining synaptic transmission, particularly during periods of intense activity [138] via the ANLS as previously described in 2.1. Its use as a supplement in TBI has been explored in both animal [139] and human models [27], although warrants additional clinical study in this context. The mechanism of lactate as a signalling molecule has also been explored with several different functionalities identified in depolarisation, currents and action potential activity [140]. Recent studies have also highlighted additional roles relevant to TBI, finding that L-lactate supplementation of cell culture model medium increased mRNA expression of genes regulating synaptic plasticity and neuroprotection [141].

High concentrations of lactate as a therapeutic agent would likely be tolerated well by human cells, as although non-wounded tissue in humans and rodents contain lactate at concentrations of 0.5–2 mM, wound levels can be at 5–15 mM or higher [142]. Lactate infusion studies in healthy patients which elevated blood plasma levels to 4 mM have also been conducted and shown a potential preference for lactate over glucose for brain cell metabolism [143].

Lactate's role in wound healing is one of particular interest to TBI- important in the regulation of VEGF and stimulating collagen deposition for formation of blood vessels and healing in cell culture models [142]. It was found that high lactate levels when accompanied by normoxic conditions, stimulated optimal blood vessel formation [142]. In subarachnoid haemorrhage (SAH), where blood vessel healing is critical, a microdialysis study in patients showed a pattern of elevated brain lactate and cerebral hyperglycolysis was associated with good recovery [144], while cerebral hypoxic lactate was associated with an increased mortality.

In early studies, Pellerin and Magistretti attributed brain lactate levels largely to astrocyte activity as blood-borne lactate does not easily cross the BBB and was therefore not a likely source in 'healthy' brain [24]. However, in brain perturbations such as TBI and SAH, periods of endogenous lactate import have been found to occur, which may help support injured brain [34,121,144].

In TBI, lactate infusions have been studied, not specifically for metabolic enhancement, but as an alternative for lowering intercranial pressure [145]. Metabolic inferences however have been made as patients who are initially more metabolically 'stressed' (i.e. an elevated LPR), had higher brain glucose concentrations after receiving hypertonic lactate infusion, than patients with lower

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initial LPR [146]. This decreased glucose consumption indicates a preferential use of lactate in these elevated LPR cases, which could be beneficial for assisting energy production in more perturbed brains. The high lactate concentrations may also have a self-regulating effect, driving less lactate production by cells overall. This hyperosmolar nature of the infusion however could also be playing a key role in creating a more favourable environment for the brain and also reducing stress on brain cells [147]. Therefore, additional studies on non-hypertonic lactate would assist in better describing its individual role in these cases. This addition of lactate to the system would artificially increase the L/P ratio - a measure typically associated with worse outcome, and other biomarker measures would also have to be used for such studies.

5.3.4 Acetate

Acetate is another simple metabolite that is transported across the cellular membrane to be used in the TCA cycle and in the production of phospholipids. It is estimated that circulating acetate levels may contribute up to 10%–15% of the basal energy demands of astrocytes [148]. This increased acetate processing (also upregulated in tumour growth [149]) may be important for meeting the bioenergetic demands in TBI. In addition to acetate's role as an energy substrate, it is noted to increase during stress, hypoxia exposure, and glucose deprivation. It has also been linked to HIF-2 signalling- required for lipid synthesis, proliferation, migration, and invasion in cancer cells *in vitro* [150].

Dichloroacetate (DCA) has been used to inhibit pyruvate dehydrogenase kinase. This decreases the conversion of pyruvate to lactate, promoting aerobic glycolysis in treatment of inherited mitochondrial disorders, pulmonary hypertension and solid tumours [151,152]. Several studies have been done on DCA and similar acetate-derived molecules to reduce lactate production *in vitro* [153,154], which may be applicable to TBI. Glyceryltriacetate (GTA), another form of bioavailable acetate, has similarly been used in rodent models of TBI, where it improved motor performance and increased ATP levels [155].

One difficulty with acetate supplementation in humans, however, is side effects such as alkalosis. Plasma acetate concentration in humans varies from 0.05 to 0.25 mM under resting conditions (and up to 1 mM after alcohol consumption), while the acetate concentration in mouse and rat plasma ranges from 0.20 to 0.30 mM, and as such rodents may tolerate supplementation better in pre-clinical studies [156]. Sodium acetate infusion in healthy human subjects at up to 2 mM in plasma produced a significant rise in plasma pyruvate, lactate, and α-hydroxybutyrate concentrations, indicating metabolic use, however this occurred along with temporary alkalosis [157]. High concentration daily infusions of DCA in humans was also associated with peripheral neuropathy [158,159].

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Investigating the role of acetate in the metabolic and inflammatory response to TBI models could highlight another pathway in which we could attempt to regulate these processes, however its use as a supplement is currently limited in terms of concentration due to side-effects (see above).

5.3.5 Other metabolic supplementation pathways for consideration

Pyruvate is another candidate that has been clearly identified as a potential supplement for increasing metabolic substrate availability, which would effectively impact LPR. Pyruvate would also bypass the glycolysis step and potential diversion to lactate that can occur with glucose, however pyruvate may need to be given in a semi-altered form (e.g. ethyl pyruvate [160]) as pyruvate in solution can self-react, forming dimers (e.g. parapyruvate) that could inhibit the TCA cycle [161]. Ethyl pyruvate has been tested in pre-clinical rodent models of TBI, and has been found to improve cognitive function [162] and decrease neuronal loss [163], while sodium pyruvate can also decrease neuronal loss and attenuate metabolic dysfunction [164,165]. HMGB1, an inflammatory protein which further increases release of cytokines, was also found to be reduced by ethyl pyruvate in a study of TBI in rats [166]. Human cell culture studies have also identified pyruvate as a possible therapeutic scavenger for free radicals created during NOS activity [167].

Ascorbic acid is another molecule warranting further investigation as a metabolic supplement, due to its potential role as a ‘switch’ in metabolic molecule uptake in neurons from glucose to lactate and ability to scavenge ROS [168,169].

The brain-gut axis is another pathway that is increasingly represented in the literature and may also need to be taken into consideration due its effects on inflammation and neurodegenerative disease [170]. Supplementing microbiota could have indirect effects on inflammation and metabolism. The brain-gut axis is outside the scope of this review, however is recognised in multiple sclerosis [171], and some preliminary studies in TBI [172,173].

5.4 Metabolic attenuation

As well as metabolic supplementation, metabolic attenuation could also have potential to have therapeutic effects on the post-TBI brain by regulating excessive and potentially harmful cell processes.

5.4.1 Glutamate

Glutamate is the most common neurotransmitter released by neurons for cell signalling and is linked to multiple cell processes. The astrocyte-neuron lactate

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shuttle hypothesis links increased glutamate to stimulation of astrocyte metabolic pathways [24,138, 174], as previously described in Figure 2. In this hypothesis, astrocytes take up glutamate for processing, which also increases astrocytic glucose uptake.

In patients with TBI, increased glucose metabolism and LPR is associated with poorer outcomes, and increased glutamate may be contributing to this metabolic dysfunction via the ANLS pathway. Glutamate levels have been shown to rise in severe TBI cases, correlating with poorer outcomes [15]. Glutamate transporters have also been found to be decreased in astrocytes following TBI [175]. Acute glutamate release is also associated with post-traumatic epilepsy and subsequent neuronal cell death, established in both rodent models [176,177], and in approximately 20% of human closed head injury patients [178]. In TBI patients, these epilepsy-associated electrophysiological disturbances are also associated with metabolic disturbances in terms of increased LPR [179]. This increase in extracellular glutamate increases excitotoxicity [180] and further contributes to the brains pathological state.

In addition to metabolism, pathological glutamate release is also linked to inflammatory pathways, as the local levels of this molecule have been associated with pro- or anti-inflammatory activation of cultured microglial cells [181]. This is hypothesised to act through glutamate's effect on the production of free radicals from nitric oxide synthase (NOS) activity [182].

Attenuating glutamate release to inhibit post-TBI damage and epilepsy is currently in early stages of investigation. Overexpression of the glutamate transporter, GLT-1, in mouse models significantly reduces ischemia-induced glutamate overflow, resulting in decreased cell death and improved recovery [183]. The impact of such experiments attenuating glutamate on the brain's metabolic status and potential inflammatory implications would also be of great interest. Similarly, the effect of any other supplementation on glutamate levels would be important to consider in clinical studies.

5.4.2 Cyclosporine

Cyclosporine is used in patients receiving transplants due to its immunosuppressive properties, however has since been explored further for drug repurposing. It was discovered to have additional metabolic functions, inhibiting mitochondrial membrane permeability and excessive ROS production. Cyclosporine was recently shown to exhibit these metabolic effects in pre-clinical rat [184] and porcine [185] models of TBI, reducing injury lesion volume and improving mitochondrial function. Previous studies in TBI patients have also found cyclosporine to decrease LPR [186]. Cyclosporine's safety for use in TBI patients have been shown in phase II clinical

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data [187,188] with no significant difference in adverse effects compared to controls [189]. However, improved neuroprotection or cognitive outcome in humans is yet to be confirmed. This effect may be largely due to cyclosporine treatment corresponding to an increase in glucose levels detected in the microdialysates [162]. The combination of metabolic and anti-inflammatory actions marks cyclosporine a very noteworthy dual-acting therapy for future studies.

6. Conclusion and future potential: metabolic modulation as a dual-action anti-inflammatory therapy

There is a clearly emerging field of work highlighting the relationship between metabolism and inflammation with implications for many disease states. The diverging evidence for use of metabolic intermediates such as glucose and succinate as supplements highlights the importance of the disease context and brain environment in their application, and also their significant impact on brain homeostasis and patient outcome. Further studies of new metabolic intermediates supplements, or new drugs for attenuation of adverse metabolic tendencies, and how these candidates may alter inflammatory signalling are needed. This emerging field is of particular interest for their use in acute injury and recovery, as there is still no effective neuroprotective drug treatment for TBI. Investigation into these links and pathways are still in their early stages, however have great potential for new treatment avenues for further focus in neurological injury and disease.

7. Expert commentary

Understanding the nature of the abnormal cerebral metabolism following TBI, and its relationship with brain inflammation, will provide essential information for designing therapies. The results would have implications not only for neurocritical care but also for the long-term disability and accelerated onset of neurodegenerative diseases (e.g. dementia and Parkinson's) in post-TBI patients. Studies suggest that after the initial few days post-TBI there may still be an on-going and persistent inflammatory process. TBI patients show varying degrees of disability months or even years post-injury, and chronic low-grade brain inflammation may conceivably play a role. Mitochondrial function is known to play a key role in *in vitro* repolarization of such M1 cells to an M2 phenotype. Research in inflammation in TBI animal models has received considerable success and attention yet there is lack of evidence for benefit in human TBI patients. In general, TBI research suffers from a "failure to translate" whereby drugs that looked promising in animals have failed in clinical trials. Modern clinical technologies such as microdialysis and advanced scanning, in combination with laboratory-based instrumentation e.g. multiplex immuno-analysers for cytokine profiling and metabolic flux analysers for real-time measurements on cells, provide us with the means to further study TBI in patients, and should in turn inform design of

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appropriate therapies. In particular, the possibility of improving mitochondrial function, promoting efficient oxidative metabolism, which in turn ameliorates brain inflammation, is an considerable therapeutic goal.

8. Five year view

The prospect of using metabolic supplementation to ameliorate secondary injury including inflammation after TBI merits further exploration in model systems, and in carefully controlled clinical studies in TBI and non-TBI individuals. Phase III studies of clinical outcomes are costly, and demand time, resources and large numbers of patients. It is therefore important that adequate Phase I and Phase II clinical studies are performed employing appropriate measurements and biomarkers. These include multiplex immunoassays, metabolic monitoring techniques such as microdialysis and ex-vivo analysis e.g. ISCUSflex (a bedside clinical microdialysis analyser that performs enzymatic colorimetric assays for glucose, lactate, pyruvate, glutamate and glycerol), NMR, mass spectrometry, and in-vivo scanning (e.g. MRI and MRS).

Metabolic therapy for TBI patients is an exciting prospect for neurocritical care and for alleviating long-term disability and neurodegeneration in post-TBI patients. Moreover, metabolic therapy may also have potential for treating other conditions where evidence suggests that mitochondrial function is disrupted, including “ageing-related” diseases such as dementia and Parkinson’s, which often occur at an earlier age in brain injury survivors compared to those without any previous brain injury.

Key Issues

- Traumatic Brain Injury patients have no standard therapies to address the metabolic or inflammatory effects of their disease in the short or long term.
- Metabolic dysfunction- identified by a high lactate/pyruvate ratio despite adequate brain glucose and oxygenation, has been characterised as occurring in a subset of patients.
- Inflammatory system activation can continue for months or years post-TBI, needing both acute and long-term pharmacological interventions. Inflammation immediately post-TBI is difficult to manage due to complex pleotropic nature of the signalling molecules, cytokines and chemokines.
- Links between inflammatory cell actions and their metabolism have been found in cell culture models of neuronal and non-neuronal cells. The further study of these links in both single cell and ‘whole brain’ is needed to tease out the specific metabolism/inflammation pathways and links.
- Metabolic supplementation is a potential to impact on inflammation and solve the energy crisis in the brain. Glucose control is important for clinical outcome,

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although strict control of such levels increase metabolic crisis. Succinate has potential to ameliorate metabolic stress, however, only in adequately oxygenated environments. Other supplementation candidates are also emerging, yet further research is needed.

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Figure Legends

Figure 1. Schematic of glucose energetic metabolism pathways in the cell. Glycolysis, which produces two molecules of ATP per molecule of glucose, is followed by oxidative phosphorylation of pyruvate (in the mitochondrion, through the tricarboxylic acid (TCA) cycle and electron transport chain (ETC), which produces 34 molecules of ATP, or LDH processing of pyruvate to lactate, which produces no molecules of ATP. Diagram copyright © 2018 Monica J. Killen, reproduced here with her permission.

Figure 2. The astrocyte-neuron lactate shuttle (ANLS). Glucose is delivered via the brain vasculature and can be taken up by both neurons and astrocytes for processing. Both cell types are able to process this glucose to pyruvate, which can then be delivered for subsequent ATP generation in the mitochondria. Astrocytes, however, can also supplement neuronal energy production by converting pyruvate to lactate and transporting it to the neurons via monocarboxylate transporters (MCT). Neurons then process the lactate back to pyruvate for mitochondrial processing. This process is also linked to the recycling of synaptic glutamate and sodium uptake in astrocytes, which in turn stimulates the need for increased glucose import. Reproduced from [14]: Magistretti, P.J. and Allaman I., A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. Neuron, 2015. 86(4): p. 883-901. Copyright © 2015 Elsevier, reproduced with permission from Elsevier.

Figure 3. The phased cell-mediated response to acute brain injury. A complex inflammatory cascade develops through interaction between several physiological compartments [cerebrospinal fluid (CSF), brain parenchyma, and vascular]. The initial response is first triggered by signalling molecules known as danger-associated molecular patterns (DAMPs) which are released into the extracellular environment post-injury. This stimulates resident microglia and astrocytes to produce cytokines, chemokines, and other potent chemotactic substances which promote the recruitment of peripheral nervous system cells. Monocytes and neutrophils enter the brain parenchyma through the permeable blood–brain barrier (BBB), and can further intensify the cytotoxic environment with their own secretion of signalling proteins. An ongoing cytokine release initiates a long-term inflammatory process, where typically we see an increase in M2 ‘reparative’ cell phenotype, as opposed to the initial pro-inflammatory M1 activated cells. However, chronic inflammation and overactivation of this system can lead to injurious outcomes, such as epilepsy, depression, and auto-immunization toward brain-enriched antigens. Reproduced from [33]: Thelin, E.P., et al., Monitoring the Neuroinflammatory Response Following Acute Brain Injury. Frontiers in Neurology, 2017. 8: p. 351. Copyright © 2017 Thelin, Tajsic, Zeiler, Menon, Hutchinson, Carpenter, Morganti-Kossmann and Helmy, published open-access under a Creative Commons Attribution License (CC-BY).

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Supplementary Table 1. Inflammatory and Metabolic Neurotherapeutics in TBI						
Treatment	**Effect**	**Author**	**Journal**	**Type**	**Cohort/Model**	**Outcome**
Perispinal Etanercept (PSE)	TNF inhibition	Tobinick et al. 2012[106]	CNS Drugs, 26(12): p. 1051-1070.	Clinical	617 stroke, 12 TBI patients.	In the TBI cohort, motor impairment and spasticity were statistically significantly reduced. in the stroke group, improvements in motor impairment, spasticity, sensory impairment, cognition, psychological/ behavioural function, aphasia and pain.
Minocycline	Reduced inflammation and mitochondrial stabilisation. Inhibition of microglial activation and proliferation, reduced excitotoxicity, reduced neuronal and glial apoptosis, neutralization of oxygen radicals, nitric oxide synthase inhibition, metalloproteinase inhibition.	Scott et al. 2018 [103]	Brain, 141(2): p. 459-471.	Clinical	15 patients >6 months post-TBI	Reduces chronic microglial activation after brain trauma but increases markers of neurodegeneration.
		Casha et al. 2005 [102]	Brain, 135(4): p. 1224-1236.	Clinical	Spinal cord Injury. 27 minocycline, 25 placebo control	Greater motor recovery in cervical but not thoracic injury.
		Ng et al. 2012 [101]	Journal of Neurotrauma, 34(7): p. 1410-1425.	Pre-Clinical	Mouse- controlled cortical impact	Reduced microglial activation and promoted early neurological recovery.
Anakinra (recombinant IL-1ra)	Inhibit IL-1ß and IL-1α function	Helmy et al. 2014 [50]	Journal of Cerebral Blood Flow & Metabolism, 34(5): p. 845-851.	Clinical	20 severe TBI patients (10 anakinra, 10 control)	Safety in patients, penetration of brain from initial subcutaneous injection. Difference in cytokine profiles, potential 'pro-inflammatory' effects.
		Newell et al. 2018 [108]	eNeuro, 5(2): p. 0385-17.2018.	Pre-Clinical	Mouse- fluid percussion injury	Improved learning/cognitive function.
Antibodies to IL-1ß and IL-1α	Decreased IL-1ß and IL-1α function	Lu et al. 2005 [107]	Journal of Neurotrauma, 22(8): p. 885-895.	Pre-Clinical	Rats- weight drop	Attenuated the TBI-induced loss of hippocampal neurons.
Ibuprofen	Antiplatelet effect, reversible cyclooxygenase inhibitor	Zangbar et al. 2015 [104]	The American Journal of Surgery, 209(6): p. 921-926.	Clinical	65 Ibuprofen, 130 control	No difference.
		Browne et al. 2006 [105]	Biochimica et Biophysica Acta - Molecular Cell Research, 1843(11): p. 2563-2582.	Pre-Clinical	Rat- fluid percussion injury	Worsened cognitive outcome, no effect on hippocampal and cortical tissue loss.
Cyclosporine	Preserve mitochondrial bioenergetic state, potential neuroprotective effect	Mazzeo et al. 2008 [186]	Acta Neurochirurgica, 150(10): p. 1019.	Clinical	50 severe TBI patients	Improved brain chemistry (lower LPR)

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Glucose	Glycaemic control	Clayton et al. 2004 [127]	British Journal of Anaesthesia, 93(6): p. 761-767.	Clinical	391 head injured patients before, 452 patients after protocol implemented	Relative risk reduction in intensive care mortality with introduction of tight glycaemic control (blood glucose 4-7 mmol litre−1)
Succinate	Supplement mitochondrial Complex II activity	Jalloh et al. 2017 [123]	Journal of Cerebral Blood Flow & Metabolism, 37(7): p. 2626-2638.	Clinical	9 TBI patients	Improved brain chemistry (lower LPR)
		Stovell et al. 2018 [134]	Scientific Reports, 8(1): p. 11140.	Clinical	8 TBI patients	Improved brain chemistry (lower LPR, improved NADH/NAD+ redox state, PCr/ATP ratio)
		Oguro et al. 2004 [135]	Internal Medicine, 43(5): p. 427-431.	Clinical	MELAS patient case study	Complete control of convulsions.
		Giorgi-Coll et al. 2017 [132]	Scientific Reports, 7(1): p. 1003.	Pre-Clinical	Rat cell culture	Improved metabolic performance (lower LPR, improved mitochondrial respiration)
Lactate	Metabolic supplement	Jalloh et al. 2018 [27]	Journal of Neurotrauma, 35(17): p. 2025-2035.	Clinical	9 severe TBI patients, 5 control	Lactate metabolised to pyruvate, before entering the TCA cycle, no difference between TBI and non-TBI metabolism.
Hypertonic Lactate	Reduce secondary intracranial hypertension, cerebral energetics	Quintard et al. 2016 [146]	Journal of Neurotrauma, 33(7): p. 681-687.	Clinical	24 severe TBI patients	Improvement of brain energetics.
		Ichai et al. 2009 [145]	Intensive Care Medicine, 35(3): p. 471-479.	Clinical	34 severe TBI patients	More effective reduction of ICP and better long-term outcome compared to mannitol.
Glyceryl triacetate (GTA)	Metabolic acetate supplementation	Arun et al. 2010 [155]	Journal of Neurotrauma, 27(1): p. 293-298.	Pre-Clinical	Rats- controlled cortical impact	increased the levels of both NAA and ATP and improved motor performance.
Sodium Pyruvate (SP) and Ethyl Pyruvate (EP)	Metabolic pyruvate supplementation	Moro et al. 2016 [164]	Brain Research, 1642(1): p. 270-277.	Pre-Clinical	Rats- controlled cortical impact	SP attenuates cerebral metabolic depression and both SP and EP decrease neuronal loss.
Ethyl Pyruvate	Neuroprotection via MMP-9 suppression and altered anti-inflammatory effects.	Shi et al. 2015 [162]	CNS Neuroscience and Therapeutics, 21(1): p.374-384.	Pre-Clinical	Rats- controlled cortical impact	Improved sensorimotor and cognitive functions, ameliorated brain tissue damage, attenuated BBB breach and brain edema.

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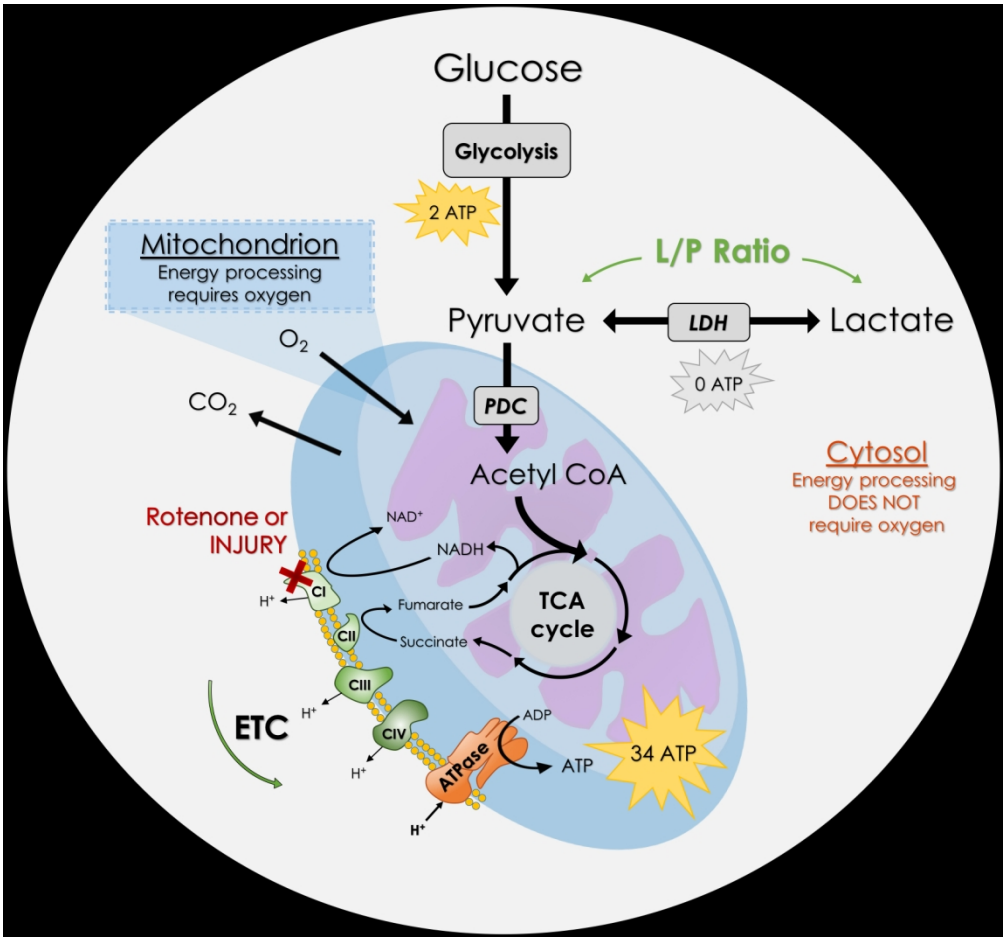

Figure 1. Schematic of glucose energetic metabolism pathways in the cell. Glycolysis, which produces two molecules of ATP per molecule of glucose, is followed by oxidative phosphorylation of pyruvate (in the mitochondrion, through the tricarboxylic acid (TCA) cycle and electron transport chain (ETC), which produces 34 molecules of ATP, or LDH processing of pyruvate to lactate, which produces no molecules of ATP. Diagram copyright © 2018 Monica Killen, reproduced here with her permission.

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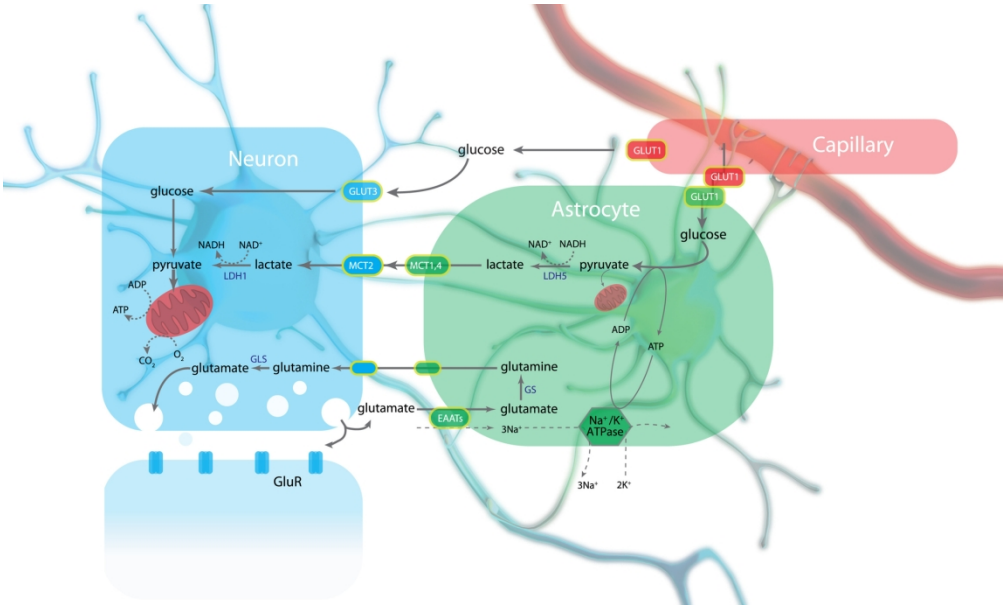

Figure 2. The astrocyte-neuron lactate shuttle (ANLS). Glucose is delivered via the brain vasculature and can be taken up by both neurons and astrocytes for processing. Both cell types are able to process this glucose to pyruvate, which can then be delivered for subsequent ATP generation in the mitochondria. Astrocytes, however, can also supplement neuronal energy production by converting pyruvate to lactate and transporting it to the neurons via monocarboxylate transporters (MCT). Neurons then process the lactate back to pyruvate for mitochondrial processing. This process is also linked to the recycling of synaptic glutamate and sodium uptake in astrocytes, which in turn stimulates the need for increased glucose import. Reproduced from [14]: Magistretti, P.J. and Allaman I., A Cellular Perspective on Brain Energy Metabolism and Functional Imaging. Neuron, 2015. 86(4): p. 883-901. DOI 10.1016/j.neuron.2015.03.035, Copyright © 2015 Elsevier, reproduced with permission from Elsevier (CC-BY-NC-ND).

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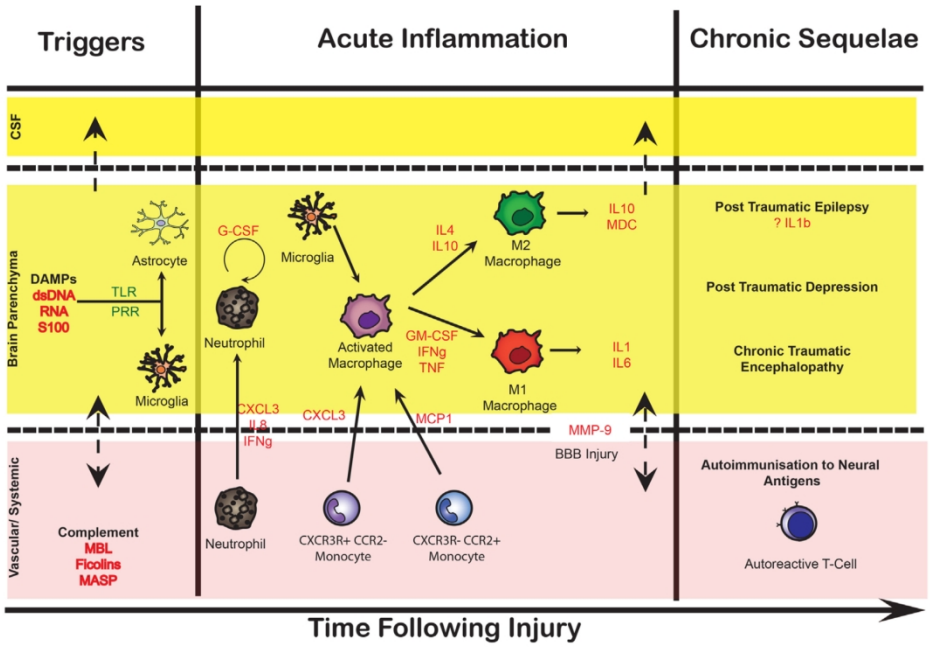

Figure 3. The phased cell-mediated response to acute brain injury. A complex inflammatory cascade develops through interaction between several physiological compartments [cerebrospinal fluid (CSF), brain parenchyma, and vascular]. The initial response is first triggered by signalling molecules known as danger-associated molecular patterns (DAMPs) which are released into the extracellular environment post-injury. This stimulates resident microglia and astrocytes to produce cytokines, chemokines, and other potent chemotactic substances which promote the recruitment of peripheral nervous system cells. Monocytes and neutrophils enter the brain parenchyma through the permeable blood–brain barrier (BBB), and can further intensify the cytotoxic environment with their own secretion of signalling proteins. An ongoing cytokine release initiates a long-term inflammatory process, where typically we see an increase in M2 'reparative' cell phenotype, as opposed to the initial pro-inflammatory M1 activated cells. However, chronic inflammation and overactivation of this system can lead to injurious outcomes, such as epilepsy, depression, and auto-immunization toward brain-enriched antigens. Reproduced from [33]: Thelin, E.P., et al., Monitoring the Neuroinflammatory Response Following Acute Brain Injury. Frontiers in Neurology, 2017. 8: p. 351. Copyright © 2017 Thelin, Tajsic, Zeiler, Menon, Hutchinson, Carpenter, Morganti-Kossmann and Helmy, published open-access under a Creative Commons Attribution License (CC-BY).

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Supplementary Table 1. Inflammatory and Metabolic Neurotherapeutics in TBI						
Treatment	**Effect**	**Author**	**Journal**	**Type**	**Cohort/Model**	**Outcome**
Perispinal Etanercept (PSE)	TNF inhibition	Tobinick et al. 2012 [106]	CNS Drugs, 26(12): p. 1051-1070.	Clinical	617 stroke, 12 TBI patients.	In the TBI cohort, motor impairment and spasticity were statistically significantly reduced. in the stroke group, improvements in motor impairment, spasticity, sensory impairment, cognition, psychological/ behavioural function, aphasia and pain.
Minocycline	Reduced inflammation and mitochondrial stabilisation. Inhibition of microglial activation and proliferation, reduced excitotoxicity, reduced neuronal and glial apoptosis, neutralization of oxygen radicals, nitric oxide synthase inhibition, metalloproteinase inhibition.	Scott et al. 2018 [103]	Brain, 141(2): p. 459-471.	Clinical	15 patients >6 months post-TBI	Reduces chronic microglial activation after brain trauma but increases markers of neurodegeneration.
		Casha et al. 2005 [102]	Brain, 135(4): p. 1224-1236.	Clinical	Spinal cord Injury. 27 minocycline, 25 placebo control	Greater motor recovery in cervical but not thoracic injury.
		Ng et al. 2012 [101]	Journal of Neurotrauma, 34(7): p. 1410-1425.	Pre-Clinical	Mouse- controlled cortical impact	Reduced microglial activation and promoted early neurological recovery.
Anakinra (recombinant IL-1ra)	Inhibit IL-1ß and IL-1α function	Helmy et al. 2014 [50]	Journal of Cerebral Blood Flow & Metabolism, 34(5): p. 845-851.	Clinical	20 severe TBI patients (10 anakinra, 10 control)	Safety in patients, penetration of brain from initial subcutaneous injection. Difference in cytokine profiles, potential 'pro-inflammatory' effects.
		Newell et al. 2018 [108]	eNeuro, 5(2): p. 0385-17.2018.	Pre-Clinical	Mouse- fluid percussion injury	Improved learning/cognitive function.
Antibodies to IL-1ß and IL-1α	Decreased IL-1ß and IL-1α function	Lu et al. 2005 [107]	Journal of Neurotrauma, 22(8): p. 885-895.	Pre-Clinical	Rats- weight drop	Attenuated the TBI-induced loss of hippocampal neurons.
Ibuprofen	Antiplatelet effect, reversible cyclooxygenase inhibitor	Zangbar et al. 2015 [104]	The American Journal of Surgery, 209(6): p. 921-926.	Clinical	65 Ibuprofen, 130 control	No difference.
		Browne et al. 2006 [105]	Biochimica et Biophysica Acta - Molecular Cell Research, 1843(11): p. 2563-2582.	Pre-Clinical	Rat- fluid percussion injury	Worsened cognitive outcome, no effect on hippocampal and cortical tissue loss.
Cyclosporine	Preserve mitochondrial bioenergetic state, potential neuroprotective effect	Mazzeo et al. 2008 [186]	Acta Neurochirurgica, 150(10): p. 1019.	Clinical	50 severe TBI patients	Improved brain chemistry (lower LPR)

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Glucose	Glycaemic control	Clayton et al. 2004 [127]	British Journal of Anaesthesia, 93(6): p. 761-767.	Clinical	391 head injured patients before, 452 patients after protocol implemented	Relative risk reduction in intensive care mortality with introduction of tight glycaemic control (blood glucose 4-7 mmol litre−1)
Succinate	Supplement mitochondrial Complex II activity	Jalloh et al. 2017 [123]	Journal of Cerebral Blood Flow & Metabolism, 37(7): p. 2626-2638.	Clinical	9 TBI patients	Improved brain chemistry (lower LPR)
		Stovell et al. 2018 [134]	Scientific Reports, 8(1): p. 11140.	Clinical	8 TBI patients	Improved brain chemistry (lower LPR, improved NADH/NAD+ redox state, PCr/ATP ratio)
		Oguro et al. 2004 [135]	Internal Medicine, 43(5): p. 427-431.	Clinical	MELAS patient case study	Complete control of convulsions.
		Giorgi-Coll et al. 2017 [132]	Scientific Reports, 7(1): p. 1003.	Pre-Clinical	Rat cell culture	Improved metabolic performance (lower LPR, improved mitochondrial respiration)
Lactate	Metabolic supplement	Jalloh et al. 2018 [27]	Journal of Neurotrauma, 35(17): p. 2025-2035.	Clinical	9 severe TBI patients, 5 control	Lactate metabolised to pyruvate, before entering the TCA cycle, no difference between TBI and non-TBI metabolism.
Hypertonic Lactate	Reduce secondary intracranial hypertension, cerebral energetics	Quintard et al. 2016 [146]	Journal of Neurotrauma, 33(7): p. 681-687.	Clinical	24 severe TBI patients	Improvement of brain energetics.
		Ichai et al. 2009 [145]	Intensive Care Medicine, 35(3): p. 471-479.	Clinical	34 severe TBI patients	More effective reduction of ICP and better long-term outcome compared to mannitol.
Glyceryl triacetate (GTA)	Metabolic acetate supplementation	Arun et al. 2010 [155]	Journal of Neurotrauma, 27(1): p. 293-298.	Pre-Clinical	Rats- controlled cortical impact	increased the levels of both NAA and ATP and improved motor performance.
Sodium Pyruvate (SP) and Ethyl Pyruvate (EP)	Metabolic pyruvate supplementation	Moro et al. 2016 [164]	Brain Research, 1642(1): p. 270-277.	Pre-Clinical	Rats- controlled cortical impact	SP attenuates cerebral metabolic depression and both SP and EP decrease neuronal loss.
Ethyl Pyruvate	Neuroprotection via MMP-9 suppression and altered anti-inflammatory effects.	Shi et al. 2015 [162]	CNS Neuroscience and Therapeutics, 21(1): p.374-384.	Pre-Clinical	Rats- controlled cortical impact	Improved sensorimotor and cognitive functions, ameliorated brain tissue damage, attenuated BBB breach and brain edema.

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