The Pathophysiology of Concussion

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Abstract: Concussion is defined as a biomechanically induced brain injury characterized by the absence of gross anatomic lesions. Early and late clinical symptoms, including impairments of memory and attention, headache, and alteration of mental status, are the result of neuronal dysfunction mostly caused by functional rather than structural abnormalities. The mechanical insult initiates a complex cascade of metabolic events leading to perturbation of delicate neuronal homeostatic balances. Starting from neurotoxicity, energetic metabolism disturbance caused by the initial mitochondrial dysfunction seems to be the main biochemical explanation for most postconcussive signs and symptoms. Furthermore, concussed cells enter a peculiar state of vulnerability, and if a second concussion is sustained while they are in this state, they may be irreversibly damaged by the occurrence of swelling. This condition of concussion-induced brain vulnerability is the basic pathophysiology of the second impact syndrome. *N*-acetylaspartate, a brain-specific compound representative of neuronal metabolic wellness, is proving a valid surrogate marker of the post-traumatic biochemical damage, and its utility in monitoring the recovery of the aforementioned "functional" disturbance as a concussion marker is emerging, because it is easily detectable through proton magnetic resonance spectroscopy.

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INTRODUCTION

Concussion is the most common form of traumatic brain injury (TBI) worldwide [1,2]. In European countries, approximately 235 people per 100,000 are admitted annually to the hospital after TBI, 80% of which are classified as belonging in the mild TBI (mTBI) category [3,4]. This phenomenon mirrors U.S. figures, in which approximately 1.5-8 million people experience a TBI each year and, among those requiring hospitalization, a proportion ranging from 75%-90% are classified as "mildly" injured or "concussed" [1]. Although the incidence of mTBI is relatively high, death from this type of trauma appears to be very low (6-10 per 100,000/year), and only 0.2% of all patients with mTBI who visit emergency departments (EDs) will die as a direct result of this injury [4].

Supported by the absence of structural lesions on traditional neuroimaging, a general and broadly accepted view is that mTBI is indeed a very frequent entity but is not a very serious injury, leading only to transient disturbances, and that no intervention other than observation typically is required [5-10]. However, according to a recent report revealing that the diagnosis of an intracranial hematoma in such patients was made with a median delay of 18 hours [11], the quality of the "observation" that mildly injured patients receive while in the hospital is of utmost concern. In the United States, it has been found that neurological observations were documented in only 50% of patients admitted with a mild head injury [11], and in Europe, patients with mTBI historically have been observed on nonspecialist wards by nurses and doctors not experienced in neurological observation. The issue of whether to perform imaging tests, observe, or discharge a patient with mTBI is one of the many challenges of the concussive injury, whose early and late symptoms and sequelae may be under-reported by patients and underestimated by physicians [6-11].

The label "mild" in mTBI does not reflect the severity of the underlying metabolic and physiologic processes, if not even the potential clinical manifestations. The word "mild" implies the general absence of overt structural brain damage. However, long beyond the typically reported recovery interval of 1 week to 3 months, at least 15% of persons with a

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history of mTBI continue to see their primary care physician because of persistent problems [12-16]. In addition, various health care professionals frequently become involved in the care of persons with mTBI, including family practice physicians, behavioral psychologists, clinical psychologists, neuropsychologists, neurologists, psychiatrists, neuro-ophthalmologists, neurosurgeons, physiatrists, nurses, occupational therapists, and physical therapists.

Awareness of the potential of a high level of disability after mTBI is increasing. The provision of comprehensive diagnostic and treatment services could bring great benefits to patients who otherwise would spend prolonged periods off work or dependent on others. Yet considerable confusion and inconsistency still exists in defining and understanding the pathophysiology of this type of trauma [17,18]. The following review represents the authors' effort to piece together the current concepts and the most recent findings about the complex basic physiology underlying the injury processes of this particular type of brain trauma and to emphasize the nuances involved in conducting research in this area.

DEFINING CONCUSSION

Although concussion certainly is blended into the vast world of mTBI, by definition, concussion should be considered a discrete and distinct entity because not all cases of mTBI are truly "concussive"; thus the 2 terms refer to different constructs and should not be used interchangeably [19]. That being said, the authors understand the common synonymous acceptance of mTBI and concussion. During the past decade, concussion has been considered by 3 international consensus conferences in which it has been defined and redefined by a panel of experts, until finally and unanimously the following statement was reached: "Concussion is a complex pathophysiological process affecting the brain, induced by traumatic biomechanical forces" [19-21].

Given this general and propaedeutical definition, several common features were added by the consensus panel to better explain the nature of this peculiar brain injury [19]. In brief, concussion typically results in the rapid onset of short-lived impairment of neurologic function, which resolves spontaneously. Postconcussive symptoms may be prolonged in a small percentage of cases, but the acute clinical symptoms largely reflect a functional disturbance rather than a structural injury, which usually is confirmed by the absence of abnormalities on standard neuroimaging studies. Finally, concussion may or may not involve loss of consciousness.

Notwithstanding such a comprehensive, well-designed, and multifunctional definition, a certain degree of confusion still exists regarding the compelling pathomechanisms that are triggered by the mechanical insult and that unfold thereafter. The dominant theory that diffuse axonal injury (DAI) is the main neuropathological process behind concussion is proving to be weak or, at best, inconclusive, given the current literature and the fact that neuronal injury inherent to mTBI improves with few lasting clinical sequelae in the vast majority of patients. Clinically, concussion still can be considered as the mildest form of the spectrum continuum that is DAI. A large body of clinical and experimental evidence suggests that such a distinctive course based on temporal neuronal dysfunction is an inevitable consequence of complex biochemical and neurochemical cascade mechanisms that are directly and immediately triggered by traumatic insult.

The distinction between DAI and concussion is not merely theoretical, and from a biomechanical perspective, it is well acknowledged. According to Ommaya et al [22], rotational head acceleration must exceed the threshold of 12,500 rad/s^2 to cause a mild DAI, whereas for moderate and severe DAI, 15,500 rad/s^2 and 18,000 rad/s^2 are required, respectively. Although other authors determined that a rotational acceleration of 9000 rad/s^2 is capable of generating DAI [23], it recently has been reported that much smaller head acceleration values ranging from 4500 to 5500 rad/s^2 are needed to provoke a concussion [24].

THE MECHANICAL INSULT AND THE "IGNITION" OF THE NEUROCHEMICAL CASCADES

Concussive head injury causes the brain to experience a mechanical "shake," by virtue of the action of the acceleration and deceleration forces transmitted to the head immediately after the impact, initiating a complex cascade of subsequent neurochemical and neurometabolic events.

The sudden stretching of the neuronal and axonal membranes initiates an indiscriminate flux of ions through previously regulated ion channels and transient physical membrane defects [25,26]. This process is followed by a widespread release of a multitude of neurotransmitters, particularly excitatory amino acids (EAAs) [27,28], resulting in further changes of neuronal ionic homeostasis. Among the EAAs, glutamate plays the pivotal role by binding to the kainite, *N*-methyl-d-aspartate, and D-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ionic channels. *N*-methyl-d-aspartate receptor activation is responsible for a further depolarization, ultimately causing an influx of calcium ions into the cells.

The essential point of this post-traumatic ionic cellular derangement is mitochondrial calcium overloading [29-31], which is responsible for inducing changes of inner membrane permeability with consequent malfunctioning, uncoupling of oxidative phosphorylation, and finally, organelle swelling [32,33]. As suggested by experiments in which the mitochondrial capacity to catalyze the tetravalent reduction of molecular oxygen through the electron transport chain appears compromised, dysfunctional mitochondria become the main intracellular source of reactive oxygen species (ROS) [34-36], inducing a phenomenon known as oxidative stress. The occurrence of an overproduction of ROS beyond the physiologic capacity of the cell to scavenge them progres-

sively leads to a decrease of antioxidant cell defenses with consequent irreversible modification of biologically important macromolecules. ROS-mediated damage, mainly characterized by the onset of lipid peroxidation, is revealed by measuring tissue malondialdehyde, a compound undetectable during normal conditions [37].

As clearly demonstrated in bench studies, this event occurs very rapidly, starting 1 minute after trauma and persisting for 24-48 hours after injury [37]. Once the lipid peroxidation reaction chain is initiated, it spontaneously propagates and causes significant ascorbate depletion, explained either by the direct oxidizing action of ROS on ascorbate or by its use in the redox cycling of α-tocopherol (vitamin E), which represents the only membrane-bound lipid soluble compound capable of breaking lipid peroxidation reaction chain [38].

Although no full explanation has been found, a collateral phenomenon occurring during the onset of oxidative stress is the significant depletion of the nicotinic coenzyme pool [39-41], a condition that jeopardizes all the oxidoreductive reactions, including those related to the cell energy supply. Possible mechanisms for this phenomenon are the hydroxyl radical–induced hydrolysis of the N-glycosidic bond of reduced nicotinamide adenine dinucleotide (phosphate) and the activation of the oxidized form of the enzyme nicotinamide adenine dinucleotide glycohydrolase [42]. Both mechanisms cause the hydrolysis of nicotinic coenzymes and give rise to the same end products, that is, adenosine diphosphate (ADP)-ribose(P) and nicotinamide. Experimental evidence showed that methylenetetrahydrofolate reductase can be subject to direct ROS attack and subsequent irreversible degradation of a consistent amount of the reduced coenzymes [43,44].

To re-establish pretrauma ionic balance, the Na1/K1 adenosine triphosphate (ATP)-dependent pumps must work at their maximal capacities, and a high level of glucose oxidation is urgently required to satisfy this sudden increased energy demand. Under normal aerobic conditions and correct mitochondrial functioning, most of glucose consumption is coupled to oxygen consumption, thus optimizing ATP generation. However, damaged by the calcium overloading and under multiple attacks from ROS, most of these oxidoreductive reactions are impaired, and the mitochondria cannot maintain the correct phosphorylating capacity. This scenario results in a rapid net decrease of all metabolites representative of the cell energy state, such as high-energy phosphates (eg, ATP and guanosine triphosphate). This phenomenon is mirrored by a proportional increase of their dephosphorylated products (ie, ADP, adenosine monophosphate, guanosine diphosphate, guanosine monophosphate, nucleosides, and oxypurines). Particularly interesting are the increases of xanthine (5×) and uric acid (7×), which strongly suggests the activation of xanthine oxidase, a phenomenon that perpetuates the vicious cycle of ROS production via the catalytic mechanism of this enzyme [45].

Thus it happens that during the time of maximum energy request, the concussion-induced transient mitochondrial malfunctioning causes an imbalance between ATP consumption and production, a condition that obligates neurons to work overtime via the more rapid, but less efficient, oxygen-independent glycolysis. The uncoupling between oxygen and glucose consumption and the yet unfulfilled energy requirement explain the paradoxical temporary increase in neuronal glucose consumption, notwithstanding a period of general metabolic depression. In fact, local cerebral metabolic rates for glucose are documented to increase by 46% above control levels within the first 30 minutes after injury and may last from 30 minutes to 4 hours [46-50].

The overall evidence from these studies demonstrates that the traumatic insult is directly responsible for sudden biochemical changes, beginning immediately after injury, and leads to subsequent depression of brain energy metabolism. Even if it is considered a "mild" form of TBI, concussion is able to cause profound biochemical changes, with the only difference being that the described modifications are fully reversible [51]. As recently reported, the metabolic derangement and the post-mTBI "energy crisis" are considered chiefly responsible for the compromised synaptic plasticity and the subsequent cognitive deficits [52].

A SURROGATE MARKER OF POSTCONCUSSIVE BRAIN DAMAGE: N-ACETYLASPARTATE

When proton magnetic resonance spectroscopy (^{1}H-MRS) was applied to the human brain, it was evident that the most prominent proton signal, detectable after having suppressed the proton signal of water, was that of a metabolite known as *N*-acetylaspartate (NAA). Subsequently, NAA became the most reliable molecular marker for the imaging of several pathologies in brain ^{1}H-MRS studies. These findings captured the attention of the general neurosciences, dramatically accelerating the pace of research into the neurochemistry and neurobiology of a molecule indeed definable as "unique" [53].

Although the exact biochemical role of this compound remains to be fully established, brain NAA was found in concentrations hundreds-fold higher than in non-nervous system tissues and therefore was considered a brain-specific metabolite and an in vivo marker of neuronal density [53,54]. A decrease in NAA has been observed in many neurological diseases that cause neuronal and axonal degeneration, such as tumors, epilepsy, dementia, stroke, hypoxia, multiple sclerosis, and many leukoencephalopathies. Conversely, the only known pathologic state characterized by a dramatic increase in cerebral NAA is an autosomal-recessive genetic leukodystrophy (Canavan disease) caused by the synthesis of a defective form of the enzyme responsible for the NAA degradation (*N*-acetyl-asparto-acylase [ASPA]). More generally, any major central nervous system disease involving either direct neuronal and/or axonal damage, secondary hypoxic-ischemic, or toxic insult will result in abnor-

malities of NAA homeostasis. In the field of TBI, however, a very innovative hypothesis seemed more fascinating among others, according to which NAA reduction was believed to be proportional to the severity of trauma [55].

By measuring whole-brain levels of NAA via high-performance liquid chromatography [56] in 3 different levels of experimental, closed, and diffuse TBI (mild, moderate, and severe), it was clearly demonstrated that at 48 hours after injury, a reduction in NAA correlated to the severity of the insult, revealing spontaneous recovery with lower levels of trauma and irreversible decrease in the others [57]. The findings also were consistent with long-term behavioral observation in animals injured with the same model of mTBI, showing only slight differences with sham-injured animals, with the main differences being present 1 day after injury and showing consistent improvement over time [58]. All these bench data strongly supported the indication for a potential role of NAA in quantifying neuronal damage and predicting neuropsychological outcome after TBI [59] and being of high clinical relevance since the use of ^{1}H-MRS allow to measure NAA noninvasively in vivo [59,60].

The finding of recovery in the "concussed" animals implied that the process leading to the reversible NAA reduction was attributable to transient biochemical changes and not simply to cell death. Similar to the previously described biochemical changes, the striking finding was again the rapidity of the onset of significant NAA reduction, identified as early as 2 hours after injury, with the lowest values recorded at 15 hours after impact (−46% compared with control values). Spontaneous recovery was observed to occur within 48-96 hours, but that took place only in mildly injured rats. Beyond showing the profound TBI-induced modification in NAA homeostasis, this finding clearly demonstrated that different levels of "physical" injury correlated with different levels and kinetics of "biochemical" damage, which are reversible in mTBI and irreversible in severe TBI (sTBI) [51].

Substantial evidence exists that NAA synthesis takes place exclusively in neuronal mitochondria, that it is strictly tied to neuronal energy metabolism, and that the distribution pattern of NAA closely parallels the distribution of "respiratory activity." For an overview of the data supporting a bioenergetic role for NAA in neurons, see Moffet et al [53].

A close linear relationship has been demonstrated between the efficacy of ATP synthesis and the ability to synthesize NAA [60,61]. NAA synthesis is indeed an energy-requiring process dependent on the availability and the energy of hydrolysis of acetyl coenzyme A (CoA) used as the acetyl group donor in the acetylation reaction of aspartate catalyzed by aspartate-*N*-acetyltransferase. It is fundamental to understand that when acetyl CoA is used for NAA synthesis, there is an indirect high-energy cost to the cell. In fact, because acetyl CoA will not enter the citric acid cycle (Krebs' cycle), a decrease will occur in the production of reducing equivalents (3 reduced nicotinamide adenine dinucleotide and 1 reduced flavin adenine dinucleotide) as the fuel for the electron transport chain. It has been clearly evidenced that in the general post-traumatic metabolic derangement, acetyl CoA homeostasis is affected by graded head injury, following a pattern very similar to those observed for both ATP and NAA [62]. Therefore in metabolic conditions of low ATP availability, when all of the pathways and cycles devoted to energy supply are operating at their maximal activity with the aim of replenishing ATP levels, acetyl CoA will not be accessible for NAA synthesis. Only when the ATP deficiency is fully restored acetyl CoA will become available to be shifted into the NAA "production" pathway. It also should be recalled that high ATP concentrations are able to inhibit the activity of citrate synthase, which is the enzyme of the Krebs' cycle, using acetyl CoA to synthesize citric acid [63]. With this in mind, a low NAA concentration can be seen as an indirect marker of post-traumatic metabolic energy impairment.

According to these concepts, it is evident that if NAA is not recovering after an mTBI, the concussive biochemical derangement (involving more complex pathways than simple NAA homeostasis [64]) cannot be considered to be resolved. Thus NAA embodies a biochemical surrogate marker to monitor the overall cerebral metabolic status, and it appears that under conditions of reduced NAA, although the cells are functional, they are still experiencing energetic imbalance.

POSTCONCUSSIVE BRAIN VULNERABILITY

The basic pathophysiological paths explored thus far have clarified some aspects of this particular clinical entity, suggesting that even if concussion is considered a form of mTBI, it ought not to be considered as "mild" as the name would suggest. With the exception of the almost always punctual reversibility of all the modifications induced, it probably is not prudent to use the adjective "mild" when referring to a traumatic event that can have such consequences to the fundamental metabolic and energy states of neuronal cells. However, while all of these biochemical modifications are scientifically interesting, they might appear, at a first glance, of negligible clinical utility because they are all spontaneously and fully reversible.

Despite this reversibility, a reasonable body of evidence clearly demonstrates that the "concussed" brain cells undergo a peculiar state of "vulnerability," during which time if they sustain a second, typically nonlethal insult in a close temporal proximity, they would suffer irreversible damage and die [65,66]. In the preclinical setting, this period of time has been well defined in duration thanks to high reproducibility of the closed-head rat model of mTBI that has been used to demonstrate biochemically the concept of vulnerability [62,64], originally proposed by Hovda et al [65]. In other words, concussion-induced pathophysiologic conditions, mainly manifested by energetic metabolic perturbations, make the brain more susceptible to severe and irreversible cellular injury by a second impact of modest entity, creating a disproportion between the trauma severity and the subsequent cerebral damage.

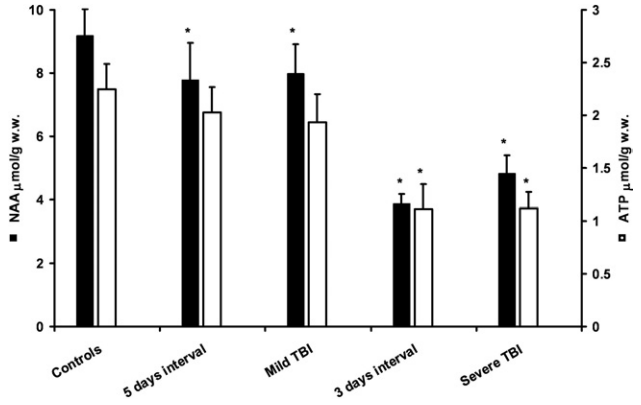

Figure 1. Concentrations of N-acetylaspartate (NAA) (left y-axis) and adenosine triphosphate (ATP) (right y-axis) as determined by high-performance liquid chromatography in the whole brains of rats subjected to repeat diffuse mild traumatic brain injuries (TBIs) (spaced by 3 or 5 days) or single diffuse TBI (mild TBI or severe TBI). Control subjects were sham-operated animals, ie, animals receiving anesthesia and surgical procedures out of injury. Each histogram is the mean of 6 animals. No significant differences were demonstrated when rats sustaining a single mild TBI and rats sustaining a repeat mild TBI spaced by 5 days were compared. Similarly, no differences were observed when rats sustaining a single severe TBI and rats sustaining a repeat mild TBI spaced by 3 days were compared. $^*P < .05$ versus control subjects.

Several studies in animals in which investigators focused on mTBI-induced dysfunction have been published, and current data support the concept of transient biochemical and physiologic alterations that may be exacerbated by repeated mild injuries within specific time windows of vulnerability [62,64,67]. In a rat weight-drop experiment performed by applying a new and easily reproducible protocol to simulate a "second impact" condition, it was clearly demonstrated that levels of NAA, ATP, and the ATP/ADP ratio decreased significantly when measured 2 days after repeated concussion (Figure 1). Maximal metabolic abnormalities were seen when the occurrence of 2 mild injuries were separated by a 3-day interval; in fact, the metabolic abnormalities in these animals were similar to those occurring after sTBI. In a follow-up study, similar perturbations were found to persist as late as 7 days after double impact, indicating prolonged metabolic effects from repeat mTBI in the same model [62]. Similar data were reported by Laurer et al [68] in a histopathology study in which they described the important cumulative effects of 2 episodes of mTBI (24 hours apart) in mice, which led to pronounced cellular damage compared with animals that sustained only a single trauma. The authors concluded that although the brain was not morphologically damaged after a single concussive insult, its vulnerability to a second concussive impact was dangerously increased.

According to Hovda et al [65] and Doberstein et al [69], metabolic alterations can persist for days after concussion, creating no morphological damage but representing the pathological basis of the brain's vulnerability. All these data provide the experimental demonstration of the exquisitely metabolic nature of "brain vulnerability" after mTBI and offer a unique contribution to the complex biochemical damage underlying the clinical scenario of a repeated concussive trauma, sometimes leading to catastrophic brain injury.

To explain the differences between the underlying metabolic dysfunction occurring after a concussion and those occurring after an sTBI, it is again necessary to use bench data and consider the degree of the NAA and ATP reduction, which is approximately 20% and 50%, respectively [57]. More importantly, the ADP concentration is only slightly increased after mTBI but is found to be substantially increased by 35% after sTBI [70]. Despite the significant ATP reduction by one fifth, if the insult is "mild," the mitochondria are not yet irreversibly damaged and still possess a sufficient phosphorylating capacity (ie, a modest decrease of the ATP/ADP ratio, which is a very good index to evaluate the mitochondrial phosphorylating activity) to allow spontaneous complete ATP restoration, which was fulfilled after approximately 5 days in the aforementioned experiment [70]. On the contrary, the 35% increase in ADP found after more severe levels of injury indicates a profoundly different situation with an altered capacity of mitochondria to support the cell energy requirements in terms of ATP synthesis (ie, profound decrease in the ATP/ADP ratio).

If after a first mild injury a second concussion finds the cells in the condition of recovering from the initial and still in perfectly reversible energetic failure, it will cause further mitochondrial malfunctioning, leading to the same irreversible energetic failure observed in severe injury. Thus 2 mTBIs that occur too close in temporal proximity can simulate the effects of a single severe injury. The key biochemical issue of the vulnerable brain lies in the incomplete resolution of the initially reversible energetic crisis triggered by the first insult.

The foremost clinical implication of these experimental data is that within days after injury, the metabolic effects of 2 concussions can be dangerously additive. This information might not be surprising; however, similar human data regarding brain metabolites currently are not available. The second clinical implication of this notion is again remarkable because it is very difficult to establish how long the aforementioned period of brain vulnerability will last and when the occurrence of a second trauma would be uneventful.

THE SECOND IMPACT SYNDROME AND THE HYPOTHESIS OF "THE PERFECT STORM"

A handful of previously published cases have reported on patients (mostly involved in sports-related activities) who, while still having symptoms from a previous head injury, experienced a second injury that unexpectedly and unpredictably led to sustained intracranial hypertension and catastrophic outcomes [71]. This entity, also known as the sec-

ond impact syndrome (SIS), is the occurrence of catastrophic cerebral edema after mTBI/concussion [72-77].

Skepticism about this entity notwithstanding [78], the major concern about SIS is that it is an exceedingly rare clinical condition when compared with the overall incidence of concussion, even though an elevated risk for subsequent mTBI exists among persons who are still recovering from a previous one [79-81]. The topic is further complicated by the fact that the resolution of clinical symptoms might not coincide with the "closure" of the temporal window of brain metabolic imbalance "opened" by the first trauma [82,83]. Thus the question of whether the brain had fully recovered from the first concussive injury while experiencing the second one remains unanswered.

Once again, laboratory data have provided clarification of some of these complex matters. In a recently published weight-drop experiment, rats were subjected to 2 diffuse mTBIs, with the second mTBI delivered after 1, 2, 3, 4, and 5 days, and then all animals were killed 48 hours after the last impact. Notably, mitochondrial-related changes progressively worsened with the time between concussions up to 3 days apart, when the metabolic abnormalities were similar to those occurring after a single sTBI [62]. In this model and with this experimental timeline, the third day after trauma was the point when the cell's energy-dependent recovery processes were at their maximal intensity. However, if the reproducibility of the model allowed to establish the duration of the window of vulnerability in the rat, this can not be affirmed in the case of human beings in which the very many uncontrolled variables render each impact different from another. This concept was clearly developed by Giza and Hovda [66], who showed that each physiologic parameter modified by a concussion has its own time frame, and each head injury can be very different from the next. Therefore, they concluded that it is difficult to definitively state the true duration of vulnerability to a second injury [66]. Results of our studies in concussed athletes strongly corroborated this knowledge [82,83]. In fact, while it was clear that none of our 50 concussed athletes recovered NAA concentration before 30 days post-impact, it was also evident that the time of NAA normalization was not identical in each subject, thus rendering impossible to define the time of brain vulnerability with the same degree of certainty found in animals [62,64]. On the other hand, it was also clearly demonstrated that none of our concussed patients had clearance of post-concussive clinical symptoms faster than NAA normalization, ie, disturbance of brain metabolism lasted much longer than gross clinical signs [82,83], when post-concussive symptoms are persistent for weeks after concussion. In an as yet unpublished article, we describe a group of 6 doubly concussed athletes in which the post-concussive syndrome persisted up to 2 months post-injury (Vagnozzi et al., 2011, submitted). Even in this restricted group of patients, recovery of NAA occurred much later (75 to 120 days), once again suggesting that rescue of brain metabolism does not correlate with self-reported clearance of post-concussive symptoms. Therefore a second impact occurring at this stage had the most profound effects because of the minimal "metabolic buffering capacity" to counteract the known early changes reinitiated by the new mTBI. With their biochemical homeostasis not yet re-established, the ionic imbalance will prevail and massive cerebral swelling will take place [84,85].

The reason why SIS is, fortunately, an extremely rare condition is probably because it represents a sort of "perfect storm," an extremely random and hardly predictable situation generated by the odd combination of the severity of the initial concussion, the time interval between the 2 traumas, and the metabolic state of the brain at the time of the second concussion.

The results of a recent pilot study performed in a cohort of singly and doubly concussed athletes who were examined by ^{1}H-MRS for their NAA cerebral content at different time points after concussive events demonstrated that the recovery of brain metabolism is not linearly related to time [82]. In this study, athletes who experienced a second concussion between the 10th and the 13th day after the first insult did not have SIS, nor did they demonstrate signs of sTBI; however, they all had a significant delay in both symptom resolution and NAA normalization [82]. In other words, the effects of the second concussion were not fatal, but they were somehow not proportionate to the entity of the concussive insult. Most likely, the second concussion occurred when the brain cells were completing recovery of impaired metabolic functions, and thus it only produced a limited cumulative effect with moderate worsening of the clinical pictures. Thus it is conceivable to infer that it is the time interval between the 2 concussions that drives the clinical and metabolic evolution.

It is our belief that SIS should not be solely considered as an "all-or-none" phenomenon and should not be limited to those instances that result in death from malignant swelling. The concept of SIS should be extended to include all the other occurrences in which a disproportion between the severity of the second injury and the concussive clinical features (ie, intensity and/or time of resolution) or cerebral metabolic changes (ie, extent of NAA decrease and/or delay in its normalization) is clearly observed. The degree of this type of SIS will depend on which phase of the metabolic recovery the brain is in at the time of the second concussion.

UNDERSTANDING THE DEGREE OF MILDNESS OF AN mTBI: CHANGES IN GENE EXPRESSION

With use of the same model of experimental repeat mTBI, studies from our laboratories [62] demonstrated that there was an effect of the time interval between concussions on *ASPA* gene expression. A progressive increase in the messen-

ger ribonucleic acid transcript of the *ASPA* gene was observed, again with a maximum 4-fold increase in animals that sustained the 2 injuries 3 days apart [62]. Animals reinjured past 5 days had values of messenger ribonucleic acid for ASPA comparable with those recorded in control animals. Based on these data, it appears that TBI-induced NAA variations may not be attributable simply to a decreased rate of NAA biosynthesis.

The aforementioned results allowed researchers to hypothesize that TBI-induced NAA decrease occurs in 2 distinct phases with 2 different mechanisms. Initially, independent from the severity of injury, a change in mitochondrial permeability [86] causes an increased velocity of NAA outflow from neurons to the extracellular space. Simultaneously, mitochondrial impairment causes a cell energy deficit with consequent diminution in NAA synthesis. In the case of reversible brain damage, such as single mTBI or repeat mTBI, in which the second impact occurs outside the brain's vulnerability "window," recovery of mitochondrial functions will allow restoration of ATP homeostasis and the subsequent normalization in the rate of NAA efflux and biosynthesis (ie, NAA levels close to those of control subjects with no increase in ASPA expression). In single sTBI or in repeat mTBI in which the second impact occurs within the brain vulnerability "window," the profound energy crisis caused by the steady mitochondrial malfunctioning induces a constant NAA outflow toward the oligodendrocytes, which, as an adaptive mechanism, increases the expression of ASPA. This phenomenon, combined with the decreased rate of NAA biosynthesis caused by persistent mitochondrial impairment, is ultimately responsible for the dramatic NAA depletion.

These results were immediately followed by a collaborative study on transcriptomics in which the authors studied the simultaneous expression of approximately 30,000 rat genes whose products are involved in a variety of cellular processes [87]. With the use of complementary deoxyribonucleic acid microarray technology, it was reported that after stretch injury to hippocampal slice cultures (as a suitable cell model to induce graded TBI), the expression of 999 genes was altered in mTBI compared with control patients. The altered genes in mTBI-stretched cells clustered in the so-called "biological process" group, which was shown to be involved in the structural damage of cellular architecture.

Most of these genes are indeed involved in signal transducer activity, regulation of transcription, and cell communication. This finding indicated that even after a mild stretch injury, as compared with a closed, diffuse mTBI, intense activity involving transcription and signaling exchange is initiated. In addition, it has been found that certain genes involved in the apoptotic process, such as voltage-dependent anion-selective channel protein 1 (ie, *VDAC1*), SH3-domain GRB2-like endophilin B1 (*SH3GLB1*), pleckstrin homology-like domain, family A, member 1 (*PHDLA1*), Rho-associated coiled-coil containing protein kinase 1 (*ROCK1*), and eukaryotic translation initiation factor 4 gamma, 2 (*EIF4G2*-predicted), were down-regulated. Furthermore, an up-regulation was seen in genes involved in the antiapoptotic process, such as chemokine (C-C motif) ligand 2 (*CCL2*), vascular endothelial growth factor A (*VEGFA*), baculoviral IAP repeat-containing 3 (*BIRC3*), TSC22 domain family, member 3 (*TSC22D3*), BCL2/adenovirus E1B 19-kD interacting protein 3 (*BNIP3*), and nuclear receptor subfamily 4, group A, member 1 (*NR4A1*).

Most of these expression changes were only found after mild stretch injury, indicating that these hippocampal cell cultures have activated protective and repair mechanisms. The most interesting finding was that more genes were differentially expressed after mild brain injury than after severe injury, further supporting the notion that even after mTBI, characterized by the absence of radiological and clinical abnormalities, a complex cellular response is initiated and distinct neuronal dysfunction occurs. This finding corroborates previous findings that these effects are "primary" cellular effects not determined by local blood flow or oxygen delivery or by any systemic factors [88].

The overall doubt that might be generated from the combination of these studies on gene expression with the biochemical works previously cited is that, apparently, not much rationale is left to justify the adjective "mild" when dealing with a concussive injury. It is undeniable that all the aforementioned changes are fully reversible, but it must be kept in mind that this reversibility is true only if a second "equally mild" TBI does not occur within the temporal window of metabolic brain vulnerability.

CLINICAL IMPLICATIONS

In the 18th century, Alexis Littre performed a famous postmortem examination providing evidence that concussion can occur without obvious anatomic damage to the brain. He performed an autopsy on one particular patient who had been rendered unconscious and died soon after his head hit a wall. Littre detected no cerebral injury, a finding consistent with the 16th-century Ambroise Pare's notion, according to which the symptoms of concussion ". . . reflected a functional disturbance rather than structural damage such as contusion, hemorrhage or laceration of the brain" [89].

More than half a millennium since Pare's first intuition, basic science data collected thus far have clarified only some of the many aspects of this particular clinical entity, suggesting that short-term as well as long-term consequences may very well be overcome simply by understanding the metabolic conditions of the injured brain cells.

Data reported in this summary strongly suggest that measuring NAA after an initial concussion and monitoring it until normalization might represent a significant step forward in quantifying the objective nature of postconcussive metabolic disturbances. Because of its high concentration within neurons

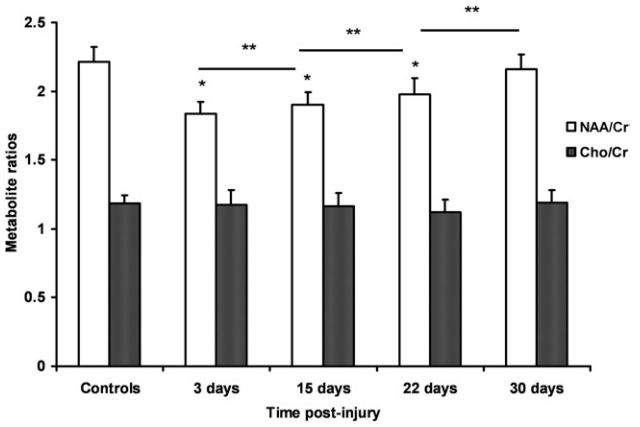

Figure 2. Metabolite ratios of N-acetylaspartate/creatine-containing compounds (NAA/Cr) and choline-containing compounds/creatine-containing compounds (Cho/Cr) in healthy control patients and concussed athletes. Histograms are the means of 30 healthy control patients and 40 concussed athletes. Standard deviations are represented by vertical bars. Data were collected in 3 different neuroradiological centers with the use of either the "single-voxel" mode through a 3-T apparatus, the "single-voxel" mode through a 1.5-T apparatus or the "multivoxel" mode through a 3-T apparatus. No differences were observed when data collected in the 3 neuroradiology centers were compared. At 3 days after injury the NAA/Cr ratio decreased by 17.6% and gradually recovered to complete normalization at 30 days. The Cho/Cr ratio did not show any significant variation. $*P < .01$ with respect to control patients. $**P < .01$ with respect to values determined at the previous time points.

(~10 mmol/L brain water), NAA levels are easily demonstrated by ^{1}H-MRS. This technique is based on the ability to localize the MR signal into a specific volume of tissue, thus providing a real-time "image" of the brain neurochemistry. At the present time, ^{1}H-MRS offers a unique opportunity to endeavor to "biologically" grade the "severity" of a concussion by quantifying the actual metabolic dysfunction, apart from signs and symptoms, because often clearance of clinical disturbances does not coincide with full cerebral metabolic recovery.

The results of a multicenter clinical trial [83] involving 40 concussed athletes and 30 healthy volunteers recently have been published and reveal that despite different combinations of magnetic field strengths (1.5 or 3.0 T) and modes of spectrum acquisition (single- or multi-voxel) among the MR scanners currently in use in most neuroradiology centers, NAA determination represents a quick (15-minute), easy to perform, noninvasive tool to accurately measure changes in cerebral biochemical damage that occur after a concussion. Patients exhibited the most significant alteration of metabolite ratios at day 3 after injury and showed a gradual recovery, initially in a slow fashion and, after day 15, more rapidly. At 30 days after the injury, all subjects exhibited complete recovery, that is, having metabolite ratios similar to values detected in control subjects (Figure 2).

Interestingly, patients self-declared a clearance of their symptoms between 3 and 15 days after concussion. To have a snapshot of the degree of energetic impairment and to monitor the eventual recovery curve might represent a useful strategy to avoid a second mTBI soon afterward that could lead to a more severe injury.

Finally, the combination of metabolic regional data obtained with longitudinal ^{1}H-MRS studies, serial neuropsychological evaluation, and diffusion tensor imaging studies to correlate metabolic alteration with possible white matter tract damage can minimize the risk of recurrent injury that may be responsible for the cumulative impairments of cerebral function and cognition, including early onset of memory disturbances, early depression, and even dementia.

CONCLUSIONS

Sudden and profound biochemical changes occur after a concussive trauma. These changes are activated by the mechanical insult itself and lead to ionic disturbance, EAA "neurotoxicity," initial mitochondrial dysfunction, ROS-mediated damage, energy metabolism depression, alteration of gene expression, and ultimately variation of NAA concentration, the "surrogate" marker of the dysfunctional neurons. This complex pathophysiology represents the modern explanation of the clinical presentation of concussion—a capricious combination of headache, dizziness, insomnia, fatigue, lethargy, uneven gait, nausea/vomiting, blurred vision, attention difficulty, concentration problems, memory problems, orientation problems, self-appraisal problems, expression and speech or language problems, irritability, depression, anxiety, sleep disturbance, problems with emotional control, loss of initiative, blunted affect, somatic preoccupation, hyperactivity, disinhibition, or problems related to employment, marriage, relationships, and home and or school management.

More problematically, within days after a simple blow to the head, this intricate biochemical derangement can result in a dangerous state for the brain, generating a situation of metabolic vulnerability to the point that if another equally "mild" injury were to occur, the 2 concussions would show the biochemical equivalence of a severe brain trauma. The immediate clinical implication derived from this evidence is that trials are warranted to investigate the application of ^{1}H-MRS for measurement of NAA and to monitor the full recovery of brain metabolic functions.

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