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Microstructural and metabolic changes in the brains of concussed athletes

Par

Luke C. Henry

Département de psychologie

Faculté des Arts et Sciences

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Microstructural and metabolic changes in the brains of concussed athletes

présentée par :

Luke C. Henry

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Dr. Franco Lepore, président-rapporteur

Dr. Maryse Lassonde, directrice de recherche

Dr. Dave Ellemberg, co-directeur de recherche

Dr. Sarah Lippé, membre du jury

Dr. J. Scott Delaney, examinateur externe

Dr. Gilles Bibeau, représentant du doyen de la FES

Résumé

Les commotions cérébrales ont longtemps été considérées comme une blessure ne comportant que peu ou pas de conséquences. Cependant, la mise à la retraite forcée de plusieurs athlètes de haut niveau, liée au fait d'avoir subi des commotions cérébrales multiples, a porté cette question au premier plan de la culture scientifique et sportive. Malgré la sensibilisation croissante du public et la compréhension scientifique accrue des commotions cérébrales, il reste encore beaucoup d'inconnus au sujet de ces blessures. En effet, il est difficile de comprendre comment cette atteinte peut avoir des effets si profonds malgré le fait qu'elle n'entraîne apparemment pas de conséquences physiques apparentes lorsque les techniques traditionnelles d'imagerie cérébrale sont utilisées.

Les techniques de neuroimagerie fonctionnelle ont cependant contribué à répondre aux nombreuses questions entourant les conséquences des commotions cérébrales ainsi qu'à accroître la compréhension générale de la physiopathologie de commotions cérébrales. Bien que les techniques de base telles que l'imagerie structurelle comme les scans TC et IRM soient incapables de détecter des changements structurels dans la grande majorité des cas (Ellemberg, Henry, Macciocchi, Guskiewicz, & Broglio, 2009; Johnston, Ptito, Chankowsky, & Chen, 2001), d'autres techniques plus précises et plus sensibles ont été en mesure de détecter avec succès des changements dans le cerveau commotionné. Des études d'IRM fonctionelle ont entre autres établi une solide relation entre les altérations fonctionnelles et les symptômes post-commotionels (Chen, Johnston, Collie, McCrory, & Ptito, 2007; Chen et al., 2004; Chen, Johnston, Petrides, & Ptito,

2008; Fazio, Lovell, Pardini, & Collins, 2007). Les mesures électrophysiologiques telles que les potentiels évoqués cognitifs (ERP) (Gaetz, Goodman, & Weinberg, 2000; Gaetz & Weinberg, 2000; Theriault, De Beaumont, Gosselin, Filipinni, & Lassonde, 2009; Theriault, De Beaumont, Tremblay, Lassonde, & Jolicoeur, 2010) et la stimulation magnétique transcrânienne ou SMT (De Beaumont, Brisson, Lassonde, & Jolicoeur, 2007; De Beaumont, Lassonde, Leclerc, & Theoret, 2007; De Beaumont et al., 2009) ont systématiquement démontré des altérations fonctionnelles chez les athlètes commotionnés. Cependant, très peu de recherches ont tenté d'explorer davantage certaines conséquences spécifiques des commotions cérébrales, entre autres sur les plans structural et métabolique.

La première étude de cette thèse a évalué les changements structurels chez les athlètes commotionnés à l'aide de l'imagerie en tenseur de diffusion (DTI) qui mesure la diffusion de l'eau dans la matière blanche, permettant ainsi de visualiser des altérations des fibres nerveuses. Nous avons comparé les athlètes commotionnés à des athlètes de contrôle non-commotionnés quelques jours après la commotion et de nouveau six mois plus tard. Nos résultats indiquent un patron constant de diffusion accrue le long des voies cortico-spinales et dans la partie du corps calleux reliant les régions motrices. De plus, ces changements étaient encore présents six mois après la commotion, ce qui suggère que les effets de la commotion cérébrale persistent bien après la phase aiguë.

Les deuxième et troisième études ont employé la spectroscopie par résonance magnétique afin d'étudier les changements neurométaboliques qui se produisent dans le

cerveau commotionné. La première de ces études a évalué les changements neurométaboliques, les aspects neuropsychologiques, et la symptomatologie dans la phase aiguë post-commotion. Bien que les tests neuropsychologiques aient été incapables de démontrer des différences entre les athlètes commotionnés et non-commotionnés, des altérations neurométaboliques ont été notées dans le cortex préfrontal dorsolatéral ainsi que dans le cortex moteur primaire, lesquelles se sont avérées corréler avec les symptômes rapportés. La deuxième de ces études a comparé les changements neurométaboliques immédiatement après une commotion cérébrale et de nouveau six mois après l'atteinte. Les résultats ont démontré des altérations dans le cortex préfrontal dorsolatéral et moteur primaire dans la phase aiguë post-traumatique, mais seules les altérations du cortex moteur primaire ont persisté six mois après la commotion.

Ces résultats indiquent que les commotions cérébrales peuvent affecter les propriétés physiques du cerveau, spécialement au niveau moteur. Il importe donc de mener davantage de recherches afin de mieux caractériser les effets moteurs des commotions cérébrales sur le plan fonctionnel.

Mots-clès : Commotion cérébrale, neuro-imagerie, traumatisme axonal, neurometabolism

Abstract

Concussions had long been considered an injury of little to no consequence. However, the forced retirement of several high profile athletes due to the impact of having suffered multiple concussions has pushed the issue to the forefront of scientific and sports culture alike. Despite the growing public awareness and the ever-expanding scientific understanding of concussions there is still much that remains unknown about these injuries. Indeed, understanding how an injury can have such profound effects, though mostly transient, without any apparent physical consequence continues to confound how concussions are conceptualized in research.

Neuroimaging techniques have helped answer many of the questions surrounding the physical consequences of concussions on the brain as well as increasing the general understanding of the pathophysiology of concussions. While basic structural imaging techniques such as CT scans and MRI are unable to detect any structural changes in the vast majority of cases (Ellemberg, et al., 2009; Johnston, et al., 2001), other more precise and sensitive techniques have been able to successfully detect changes in the concussed brain. Functional MRI studies have further established a strong relationship between functional alterations and post-concussion symptoms (Chen, et al., 2007; Chen, et al., 2004; Chen, et al., 2008; Fazio, et al., 2007). Electrophysiological measures such as ERP (Gaetz, et al., 2000; Gaetz & Weinberg, 2000; Theriault, et al., 2009; Theriault, et al., 2010) and TMS (De Beaumont, Brisson, et al., 2007; De Beaumont, Lassonde, et al., 2007; De Beaumont, et al., 2009) have consistently demonstrated alterations in concussed

athletes. However, there has been very little research that has attempted to further explore the specific structural and metabolic aspects of concussion.

The first study assessed structural changes in concussed athletes using diffusion tensor imaging which measures water diffusion in white matter. We compared concussed athletes with non-concussed control athletes in the days immediately after injury and again six months later. Our results indicated a consistent pattern of increased diffusion along neural tracts of the cortical spinal tract and in the corpus callosum underlying motor cortex. Furthermore, these changes were still present six months after injury suggesting that the effects of concussion are persistent past the acute phase.

The second and third studies employed magnetic resonance spectroscopy as a means of investigating the neurometabolic changes that occur in the concussed brain. The first of these studies investigated the neurometabolic changes, neuropsychological aspects, and symptomatology in the acute post-injury phase. While neuropsychological testing was unable to show differences between concussed and non-concussed athletes, neurometabolic alterations were noted in the dorsal lateral prefrontal cortex as well as in primary motor cortex which correlated with reported symptoms. The second study investigated neurometabolic changes immediately after concussion and again six months after injury. Results indicated alterations in the dorsolateral prefrontal and primary motor cortices in the acute post-injury phase, but only those in primary motor cortex persisted to the six month time point.

Keywords: Concussion, neuroimaging, traumatic axonal injury, neurometabolism

Table of Contents

List of Figures

List of Symbols and Abbreviations

AD: axial diffusivity

ALS: amyotrophic lateral sclerosis

AMPA: 2-amino-3-5-methyl-3-oxo-1,2- oxazol-4-yl propanoic acid

AQ: absolute quantitation

ARAS: ascending reticular activating system

Asp-NAT: Aspartate N-acetyltransferase

ATP: adenosine triphosphate

BOLD: blood oxygen level dependent

BSRF: brainstem reticular formation

Ca^{2+}: calcium

CBF: cerebral blood flow

CK: creatine kinase

Cl^{-}: chloride

CoA: coenzyme A

Cr: creatine

CSP: cortical silent period

CST: cortical spinal tract

CT: computer tomography

CTBI: chronic traumatic brain injury

CTE: chronic traumatic encephalopathy

DAI: diffuse axonal injury

DLPFC: dorsal lateral prefrontal cortex

DTI: diffusion tensor imaging

EAA: excitatory amino acids

EEG: electroencephalography

FA: fractional anisotropy

fMRI: functional magnetic resonance imaging

GABA: γ-Aminobutyric acid

GCS: Glasgow coma scale

Glu: glutamate

GOAT: Galveston orientation and amnesia test

H^+: Hydrogen

LoC: loss of consciousness

M1: primary motor cortex

MD: mean diffusivity

Mg^{2+}: Magnesium

mI: myo-inositol

MR: magnetic resonance

MRI: magnetic resonance imaging

MRS: magnetic resonance spectroscopy

MTBI: mild traumatic brain injury

NAA: N-acetyl aspartate

NAAG: *N*-Acetylaspartylglutamic acid

NFL: National Football League

NHL: National Hockey League

NMDA: N-methyl D-aspartate

NMDAr: N-methyl D-aspartate receptor

NSE: neuron-specific enolase

PCS: post-concussion syndrome

PCr: phosphocreatine

PET: positron emission tomography

rCBF: regional cerebral blood flow

RQ: relative quantitation

SPECT: single photon emission tomography

TAI: traumatic axonal injury

TBI: traumatic brain injury

WHO: World Health Organization

Dedication

For my mother, my brother and my sister-

Thank you from the very marrow of my bones.

For my Uncle Bill- your resolve reminds me to never shrug.

And for my father-

If you could see me now…

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General Introduction

Theoretical Considerations

Historical perspectives, Definition, and Symptomatology

Concussions [1] have been described and documented throughout history, spanning across many cultures (see Echemendia, 2006; Shaw, 2002; Verjaal & van 'T Hooft, 1975 for a complete review). The term itself which means "to shake violently," was not used to refer to brain injury until the 16th century. The earliest descriptions of concussive injuries date back as far as 3000 B.C.E. The Edwin Smith papyrus, 1700 B.C.E., catalogs 10 cases of traumatic brain injury, nine of which detail penetrating or fracturing head injuries; however, one case depicts a patient who received a blow to the head and suffered subsequent neurological change without visible injury (Verjaal & van 'T Hooft, 1975). The author of the ancient text explained that,

> "It can hardly be devoted that the items of the diagnosis are indications of causes evidently concerning the shoulder, the hand, and the foot of the side affected. Apparently the condition of the patient is similar to that resulting from some other injury or disease as the surgeon is warned against false conclusions arising from this similarity" (as cited in Verjaal & van 'T Hooft, 1975).

Biblical texts recount an apparent concussion in the fabled story of David and Goliath. In Greek texts Hippocrates wrote of a "shaking or concussion of the brain produced by any cause [that] inevitably leaves the patient with an instantaneous loss of voice

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[1] Officially according to the CDC and WHO the term "concussion" has been replaced by the term mild traumatic brain injury (MTBI), but "concussion" is still used ubiquitously in the sports concussion literature. Consequently, the current thesis uses the term concussion where it is understood to be a specific kind of MTBI (Gerberding & Binder, 2003; Holm, Cassidy, Carroll, & Borg, 2005).

(unconsciousness)" dating back as far as 415 B.C.E. (Verjaal & van 'T Hooft, 1975). Some 1200 years later (832-929 C.E.), Rhazes, a Persian physician, observed that brain injury could occur without skull fracture or gross pathology, a fact that still confounds the general understanding of concussion.

The Renaissance brought several physicians who further described concussion including Lafranchi of Milan (1315) who taught that concussive blows jolt and agitate the brain, and Jacopo Berengario da Carpi (1470-1550) who penned one of the original pathophysiological theories of concussion claiming that small intracerebral hemorrhages caused the loss of consciousness (LoC) (Muller, 1975). A contemporary of da Carpi, the French physician Ambroise Paré (1510-1590), is credited with popularizing the term "concussion" when he wrote about the "commotion or shaking of the brain" in injured soldiers (Verjaal & van 'T Hooft, 1975). A century later Jean-Louis Petit (1674-1750) distinguished between the immediate post-concussion changes in arousal and sensibility and the slower, gradual decline associated with hemorrhage. He also acknowledged that there are "degrees" of concussion, and they are not simply all-or-none (as cited in Verjaal & van 'T Hooft, 1975). Alexis Littré performed the first documented post-mortem on a closed-head injury patient, a prisoner who was sentenced to be broken on the wheel. To escape the horrific torture to which he had been sentenced, the man rushed head-long into a wall (Feinsod, 2002). Upon examination, Littré found no visible pathology or injury, consistent with Paré's original assertion that concussions create a functional rather than a structural deficit such as a lesion (Muller, 1975; Shaw, 2002).

Reconciling the neural effects of concussion with the apparent absence of visible damage was a point of emphasis in the 19th century. The French military surgeon Jean-Pierre Gama proffered in 1835 that the "delicate" nature of neural tissue left it particularly vulnerable to stretching and breaking when the head is injured (Strich, 1970), a phenomenon now understood to be *diffuse axonal injury*, though the term *traumatic axonal injury* is now more favored (see section on *Biomechanics*). The autonomic nervous system symptom cluster of concussion including headache, nausea, and dizziness was described by Victor Von Bruns (1811-1883) and Ernst Von Bergmann (1836-1907) (Muller, 1975). The 20th century was dominated by the introduction of experimental animal models of concussion, understanding the biomechanical mechanisms that precipitate concussion, and pathophysiology theories that will be reviewed later.

Defining concussion is complex and fraught with controversy. There remains much contention over which symptoms are necessary for the diagnosis and the relative weight of each as they pertain to severity. Benjamin Bell (1749-1806) defined concussion in 1787 as

> "every affection of the head attended with stupefaction, when it appears as the immediate consequence of external violence, and when no mark or injury is discovered, is in general supposed to proceed from commotion or concussion of the brain, by which is meant such a derangement of this organ as obstructs its usual functions to render it capable of having its real nature ascertained by dissection" (as cited in Peerless & Rewcastle, 1967).

This definition, though pioneering, is inadequate. It does key in on the presence of a confused state (stupefaction) and that there is an injury for which there is no discernable

outward sign, but misses out on several other signs and symptoms. Granted, it was an important step in even acknowledging that there was indeed an injury, but it had little value otherwise. Furthermore, this and other contemporary definitions of concussion fail to account for the long term consequences and the effects of multiple blows to the head. While historically LoC was the defining characteristic (Muller, 1975; Ward, 1964) and is still considered by some to be indicative of severity and integral to diagnosis (Ropper & Gorson, 2007), all of the most recent consensus statements overwhelmingly state that it is not necessary to define or diagnose concussion (Aubry et al., 2002; Cantu, 2001; McCrory et al., 2005; McCrory et al., 2009; Webbe & Barth, 2003), nor is it associated with injury severity (Henry & Lassonde, 2009; McCrory, et al., 2009). The transient nature of the LoC associated with concussion also led to speculation that there was no structural damage because concussions were purely physiological events and therefore without physical consequence (Denny-Brown & Russell, 1940). Holbourn contested the notion that concussions were not accompanied by physical damage though he lacked the tools to prove his assertion (1943, 1945). Peerless and Rewcastle (1967) further specified the nature of these physical injuries as being shear strain to white matter or traumatic axonal injury (see *Biomechanics*) (Gentry, 1994). While gross imaging techniques rarely reveal structural damage to the concussed brain, it is now largely understood that concussions do result in at least transient structural damage (Gentry, 1994; Johnston, et al., 2001) and that physiological and morphological changes may continue for days or weeks (Giza & Hovda, 2001). Considering all these issues, concussion will be defined in the current thesis as *a closed head injury due to a direct blow to the head or shaking of*

the head from an impulsive force resulting in a transient alteration in mental status and brain processes that may include loss of consciousness, memory dysfunction (retrograde or anterograde amnesia), impairment of reflex activity, and/or disorientation. Although for the majority of cases most symptoms resolve within 7-10 days, physical symptoms such as headache, cognitive symptoms including memory dysfunction, and neurological disturbances like sleep disturbance may persist indefinitely (Binder, 1986; Echemendia, 2006; Quality Standards Subcommittee & Neurology, 1997) *and may be worsened when multiple impacts are sustained* (De Beaumont, Lassonde, et al., 2007; Guskiewicz et al., 2003; Theriault, et al., 2010).

Concussions can vary in severity across a number of different factors including the force-mass combination, force vector, and individual differences. Because of this variability, categorizing all concussions as a unitary phenomenon is impractical and incorrect. Concussions had been categorized as either simple or complex based on the amount of time it takes a patient to recover (McCrory, et al., 2005): Simple (type 1) concussions resolve without complication 7-10 days after the injury whereas Complex concussions (type 2) include all cases where the patient suffers persistent symptoms, prolonged LoC, or prolonged cognitive impairment and are typically incurred by people who have had multiple concussions, or have lowered impact tolerance (McCrory, et al., 2005). However, such a distinction is extremely limited both clinically and experimentally as it is a retrospective classification with no bearing on early management, only altering later treatment course. The most recent consensus statement has nixed this distinction with the retention of the basic concept that most concussions

resolve within 7-10 days in most people, somewhat longer in children and adolescents, and even more protracted in a minority of people (McCrory, et al., 2009). Many grading systems have been employed (see Cantu, 2001 for a thorough review), but the practice of grading concussions has been abandoned for the most part. Instead, the severity of the injury is often determined after all of the symptoms have resolved. Specifically, the severity relates to the nature, burden, and duration of symptoms (Cantu, 2001; McCrory, et al., 2009).

Persons suffering from a concussion often appear dazed, forgetful, display inappropriate affect, and report experiencing headache, nausea, double vision, increased emotionality, changes in sleep patterns, and express increased sensitivity to light and noise (McCrory, et al., 2009; Quality Standards Subcommittee & Neurology, 1997). Further, enduring problems typical of more severe concussions include headaches, irritability, noise and light sensitivity, fatigue, anxiety, and impaired concentration and memory dysfunction. The presence of one or more of the above mentioned symptoms after the occurrence of a head injury is termed Post Concussion Syndrome (PCS). These symptoms typically resolve within 30 days, but persist for some patients (Eyres, Carey, Gilworth, Neumann, & Tennant, 2005; King, 1997; King, 1996) in whom the remaining symptom cluster including physical, cognitive, and behavioral symptoms is referred to as persistent PCS which can last well past 30 days from months to years after the injury incident suggesting at least persistent functional alterations and likely damage (Ryan & Warden, 2003).

Epidemiology

The prevailing attitude in sports culture had been rather dismissive of concussions, where they once were considered as a minor injury without consequence, even comical in instances where a player struggled to regain his balance or could not find his way back to the bench (Echlin, 2010; Zillmer & Spiers, 2001). In the recent past, sportscasters humorously spoke of how a struck player was "seeing stars," had his "bell rung," or even how on particularly devastating hits may be unable to return to the bench or sideline (Echlin, 2010; Zillmer & Spiers, 2001). This has continued to a lesser degree as hard hits are still rewarded and reinforced within sports media. Athletes compound the indifference with bravado and an understandable, though misguided desire to continue playing. The seriousness of concussion is slowly coming to the forefront in the athletic community and the public at large as the documented short and long term symptoms/effects continue to be investigated due to the forced retirement of several high profile athletes including former NFL quarterbacks Steve Young and Troy Aikman and NHL star Eric Lindros to name a few. Of the 51 cases of neuropathologically confirmed chronic TBI (CTBI) which can only be done by autopsy, 90% have occurred in athletes. The gross pathology of CTBI is obviously not uniform across all cases. However, common elements include 1) reduced brain weight particularly in the frontal and temporal lobes that typically extends to other structures such as the hippocampus, entorhinal cortex and amygdala as severity increases, 2) enlarged lateral and third ventricles, 3) volume loss in the corpus callosum, 4) cavum septum pellucidum in almost 70% of cases and with fenestrations in nearly 50% of those cases, and 5) scarring and

neuronal loss of the cerebellar tonsils (Corsellis, Bruton, & Freeman-Browne, 1973). Other common gross features include pallor of the substantia nigra and locus coeruleus, atrophy of the olfactory bulbs, thalamus, mammillary bodies, brainstem, and cerebellum (Corsellis, et al., 1973). More recent examination of five case studies revealed very similar gross anatomical findings (McKee et al., 2009).

Given the controversy in defining concussions as well as the largely uninformed social attitudes toward them, diagnosing concussions is an even more imprecise and daunting task. It has been conservatively estimated that over 2 million sports related concussions occur annually in the United States, (Reddy, Collins, & Gioia, 2008). These figures are only estimations as they do not account for the large number of unreported and undiagnosed incidences, leaving a potentially large gap between the numbers of athletes who report having sustained a concussive injury and those who actually sustained a concussive injury. Indeed, recent work points to a lack of knowledge and education about concussion in coaches, trainers and athletes as a crucial factor in diagnosing and reporting such injuries (Cusimano, 2009; Echlin et al., 2010). Consequently, identifying a concussion is not simply a matter of refining diagnostic techniques and heightening clinical vigilance. It is also incumbent upon the athletes themselves to recognize and report post-concussion symptoms (Delaney, Lacroix, Leclerc, & Johnston, 2002; Echlin, 2010). A three-year prospective study among intercollegiate athletes reported that of all sports related injuries, 6.2% were concussive (Covassin, Swanik, & Sachs, 2003) with rates differing across sports where boxing leads the way by a significant amount (Echemendia & Julian, 2001). Further, concussion incidence increases from practices to

games in all sports (Echemendia, 2006). A systematic literature review spanning from 1985 to 2000 found the highest incidence of concussion for high school, college, and amateur athletes in hockey and rugby, while soccer had the lowest (Koh, Cassidy, & Watkinson, 2003). Annual football concussion rates including recreational, high school, collegiate, and professional levels indicate that players have an annual concussion incidence estimated to be 4–20% (Bailes & Cantu, 2001) and that they are three times more likely to sustain a second concussion in the same season (Guskiewicz, Weaver, Padua, & Garrett, 2000). However, Delaney et al. (2002) reported that 70.4% of football players and 62.7% of soccer players reported having experienced concussion symptoms during the previous year, but only 23.4% of concussed football players and 19.8% of concussed soccer players realized they had suffered a concussion.

The concussion rate is even more staggering when multiple concussions (84.6% for football players and 81.7% for soccer players) are considered. At the mild end of the spectrum, the harmful consequences of multiple concussions were originally introduced by Quigley in 1945 who anecdotally determined with his infamous *three-strike rule* that an athlete who had experienced three concussions in a season was to be held out for the remainder of that season. Multiple concussions were referenced just seven years later without any more rigorous research on the matter (Thorndike, 1952). Some two decades later, Gronwall described the cumulative effects of mild closed head injuries in a series of papers covering a range of neuropsychological domains including memory, information processing, perception, and motor skills (Gronwall & Wrightson, 1974, 1975, 1981; Gronwall, 1977). Though important in documenting the cumulative effects of multiple

mild head injuries, none of these studies were done with athletes where the nature of the injury and the resultant management can differ from a non-sports related injury, namely that athletes may tend to "fake good" in order to return to play before the signs and symptoms have resolved (Echemendia, 2006). At the other end of the spectrum, severe manifestations of the effects of repeated blows to the head in boxers were first documented and dubbed "punch drunk syndrome" by pathologist Harrison Martland (1928), and later termed Dementia Pugilistica due to its infamy amongst boxers (Millspaugh, 1937). The term chronic traumatic brain injury is now used to describe the condition which first begins as cognitive deteriorations, followed by degrading executive functions, motor impairments, and dementia in the bleakest of cases.

Elucidating the temporal effects of concussion is also important as this informs management and return to play decisions. The acute post-injury phase, approximately three months post-concussion, has been the most heavily documented in terms of the neuropsychological effects of concussion (Barth et al., 1989; Boutin, Lassonde, Robert, Vanassing, & Ellemberg, 2008; Chen, et al., 2007; Collins et al., 2003; Collins et al., 1999; Fazio, et al., 2007; Iverson, Brooks, Collins, & Lovell, 2006). The neuropsychological assessment of athletes outside of the acute post-injury phase has revealed mixed results. Iverson, Brooks, Lovell, and Collins (2006) found that high school athletes with a history of one or two prior concussions did not demonstrate any neuropsychological deficits six months after injury relative to athletes who had not suffered any concussions. Similarly, a study of collegiate athletes at least six months removed from their last injury did not demonstrate any detectable enduring

neuropsychological changes (Bruce & Echemendia, 2009). Understanding the similarities and differences between the acute and chronic post injury phases is thus crucial to determining how the brain changes, how it recovers, and/or how it remains altered due to a concussion.

The aggregate of the data detailing the effects of repeated blows to the head is beginning to paint a very complex, yet consistent picture. What seems apparent in understanding the neuropsychological sequelae of concussions, particularly after repeated injuries, is that the resultant consequences are not binary in nature. That is to say, they are not all or nothing, but rather they progress in a semi-stepwise manner. Indeed, the evidence suggests that there is a continuum of potential outcomes ranging from no consequences to a second or third injury (Iverson, Brooks, Lovell, et al., 2006; McCrory, 2001) to subclinical eletrophysiological changes (De Beaumont, Lassonde, et al., 2007; Gaetz, et al., 2000; Theriault, et al., 2009; Theriault, et al., 2010) to more subtle cognitive and motor changes (De Beaumont, et al., 2009), progressing toward mood and cognitive symptoms (Guskiewicz et al., 2005; Guskiewicz, Marshall, et al., 2007) and finally to severe injuries such as CTBI (Corsellis, et al., 1973; Heilbronner et al., 2009; Jordan, 1990; Roberts, Allsop, & Bruton, 1990) that have accompanying neurohistopathological correlates (Corsellis, et al., 1973; Geddes, Vowles, Nicoll, & Revesz, 1999; Geddes, Vowles, Robinson, & Sutcliffe, 1996; Hof et al., 1992; McKee, et al., 2009). Though these changes can take a career's worth to accrue, rare though severe acute cases can result in immediate disability or even death in the uncommon second-impact syndrome (Bey & Ostick, 2009; Cantu, 1995; Cantu, 1996). Our clinical and scientific

understanding of the effects of sustaining impacts to the head has increased dramatically, but it is clear that there are many more questions than answers at the present time.

Pathophysiology theories

Currently there is no unified pathophysiology theory to explain the underlying mechanisms of concussion (McCrory, et al., 2005). Historically researchers focused theories around explaining loss of consciousness, usually considered to be exclusively a brainstem phenomenon, and memory dysfunction widely understood to be a cortical phenomenon. Though many theories have been put forward, current research in the field has generally shifted its attention from finding a mechanism toward describing and treating concussion. The five most historically prominent theories include the vascular theory, the centripetal theory, the reticular theory, the pontine cholinergic theory, and the convulsive theory.

The vascular theory, the oldest pathophysiology theory, is generally considered to be outdated and insufficient to explain concussion. Its basic assumption is that LoC and other transient neural dysfunctions are caused by brief episodes of cerebral ischemia (Nilsson & Ponten, 1977; Verjaal & van 'T Hooft, 1975). The cause of the ischemic events is attributed to a number of pathophysiologies including vasospasm, vasoparalysis, expulsion of blood from capillaries, reflex stimulation, and most prominently an obstruction of blood flow due to compression of the brain (Echemendia, 2006; Shaw, 2002). However, membrane potential and neuronal functioning are not lost immediately after brief ischemic events (hemorrhages, strokes, etc) (Lipton, 1999) as proposed by the vascular theory (Shaw, 2002). Theoretically, the vascular theory is logical in that brain

functions are disturbed after ischemic events, but the effects of such injuries are not as immediate and abrupt as they are in concussion. Experimental proofs against the vascular theory using a heartless frog model demonstrated that vascular involvement is not necessary for a concussion to occur (Denny-Brown & Russell, 1940); furthermore, the predicted energy production deficiency is not necessary to observe a concussed state (Nilsson & Ponten, 1977).

The centripetal theory was championed by Ommaya and Gennarelli (1974). According to this theory, concussions occur across a graded set of clinical syndromes caused by mechanical stresses and strains that inevitably affect the structure and function of the brain beginning at the surface (cortex) in less severe injuries extending to the diencephalic-mesencephalic juncture for the most severe of injuries (Ommaya & Gennarelli, 1974) where rotational force vectors (see *Biomechanics*) were shown to be responsible for the majority of concussions. The heuristic value of this theory, especially as it pertains to biomechanics is still valuable, though its bias toward LoC as being associated with more severe injuries proves to be its undoing as there are many clinical cases where patients sustained LoC without the predicted cognitive after-effects (Echemendia, 2006).

The reticular theory has easily been given the most precedence in understanding concussion as it explains a number of the symptoms associated with concussion. Nuclei within the brainstem are associated with the control of reflex activity and the maintenance of autonomic activity. Injury or disruption of these nuclei could account for

LoC, changes in blood pressure, slowing heart rate, altered cerebral blood flow, and the disruption of various reflexes, loss of equilibrium and muscle tone, respiratory arrest, nausea/vomiting, and pupil dilation (Shaw, 2002). Theoretically, the basic principle posits that a concussive blow temporarily disrupts (paralyzes/depresses) polysynaptic pathways within the brainstem reticular formation (BSRF)/ ascending reticular activating system (ARAS). Recovery within the BSRF stimulates the ARAS to become operational again thus allowing the cortex to be reactivated again via thalamocortical pathways prompting a quick recovery of consciousness. The source of brainstem injury is postulated to be the flexion of brainstem structures during the peak rotational acceleration/deceleration at the cervicomedullary junction causing stretching and possible shearing leading to massive functional failures (Shaw, 2002). Experimental work in rats (Povlishock, Becker, Cheng, & Vaughan, 1983), cats (Povlishock, Becker, Miller, Jenkins, & Dietrich, 1979; Povlishock, Becker, Sullivan, & Miller, 1978), and monkeys (Gurdjian, 1972; Jane, Steward, & Gennarelli, 1985) supports the reticular theory showing traumatic chromatolytic changes (disintegration of the granule Nissl bodies in a nerve cell body, usually occurring after exhaustion of the cell or damage to its peripheral process) to neurons in the BRSF. Clinical observations demonstrating injury to the brainstem further support reticular involvement (Oppenheimer, 1968).

The reticular theory has endured in large part due to the ambiguous nature of the evidence used to support it. While evoked potentials have been used to champion the theory, these results neither prove nor disprove its basic tenets. Similarly, the reticular theory predicts that when a concussive injury incapacitates the ARAS, the subsequent

cortical EEG recording will be of low frequency and high voltage (Shaw, 2002). In actuality, immediate experimental post-concussion EEG patterns fall into two categories: 1) an almost complete attenuation of the EEG or 2) a brief period of excitation marked by both high frequency and high amplitude spiking (epileptiform). While these two patterns are in conflict with each other, neither matches the prediction of depressed reticular activity posited by this theory theory (Hayes, Katayama, Young, & Dunbar, 1988; Shaw, 2002). The neuropathological data is the most convincing evidence in favor of the reticular theory. However, this data is limited in its applicability to a general pathophysiological understanding of concussion because experimentally it is specific to certain methodologies and selective in terms of the anecdotal accounts citing only those cases where the impact was to the back of the head (Shaw, 2002).

Disruption of BSRF activity explains muscle flaccidity and reflex suppression following cerebral concussion, but it cannot explain the convulsive movements occasionally seen experimentally in animal studies (Govons, Govons, VanHuss, & Heusner, 1972; Nilsson, Ponten, & Voigt, 1977; Nilsson et al., 1994) and anecdotally reported clinical accounts (Chadwick, 1997a, 1997b; McCrory & Berkovic, 1998, 2000; Perron, Brady, & Huff, 2001; Ropper & Gorson, 2007). Even Andy (1989) who reported a concussive injury to the brainstem with subsequent convulsions implicated reticular formation discharges, in stark contrast to the depressed activity necessary for the reticular theory to hold water. Perhaps most damning to the reticular theory is the absence of an understanding of how the reticular formation would come to be depressed by a concussive injury leading to questions of whether LoC would necessarily occur even if

the BSRF was disrupted (Shaw, 2002). Also missing is a comprehensive account of how reticular functioning is depressed by a concussive insult or whether LoC is the necessary consequence of disrupted BSRF activity (Shaw, 2002). Furthermore, this theory fails to account for traumatic memory loss, one of the most frequently observed symptoms in concussion that is also more predictive of severity than LoC (Shaw, 2002).

The pontine-cholinergic theory is a modern neurochemical variant of the reticular theory. Where the reticular theory posits that the brain stem is deactivated because of the injury, the pontine-cholinergic theory contends that the brain insult activates an inhibitory/depressive system (Barr, 2005). The concussive injury sets in motion a cascade of events that excite an inhibitory cholinergic system in the dorsal pontine tegmentum, again supposing a pre-eminent role for the brainstem. The inhibition of the cholinergic system then reduces consciousness to variable levels, depending on the level of excitation of the inhibitory system. While this theory accounts for altered consciousness and LoC, the neurochemistry does not add up. Studies using an acetylcholine blocker to circumvent the inhibition of the cholinergic system failed to prevent or reduce concussive symptoms (Lyeth et al., 1988).

The symptoms of concussion bear remarkable similarity to those of a generalized seizure (Shaw, 2002). Earl Walker and colleagues (1944), who originally proposed the convulsive theory of concussion based their theory in part on experimental observations of tonic-clonic movements in concussed animals (Walker, et al., 1944). From these observations they concluded that the shaking or vibration of the brain resulted in a short

lasting widespread neuronal discharge followed by a long lasting period of neuronal exhaustion and/or inhibition that manifests itself in muscle relaxation, behavioral stupor, and depressed cortical rhythm (Walker, et al., 1944). Both conditions can be marked by a near instantaneous, though transient (seconds or minutes) LoC and an equally sudden return to consciousness commonly accompanied by a period of depressed and disoriented function. Reflex suppression (stretch, withdrawal, and corneal) is also common to both concussion and seizure. Physiological changes including increased heart rate, blood pressure, and transitory respiratory arrest are also common to both (Shaw, 2002). Parallel disturbances in autonomic function include giddiness and nausea. Post-incident headaches are commonly reported as are both anterograde and retrograde memory dysfunction. Residual symptoms of seizure and concussion share similarities as well including: irritability, depression, sleep dysfunction, anxiety, fatigue, persistent headaches, restlessness, dizziness, emotional lability, and slowed cognitive processing (Shaw, 2002).

The convulsive theory is well supported by electrophysiological, neurochemical, and ultrastructural data, but it does not readily account for LoC. It is best explained by cortico-cortical and cortico-reticular theories where it is posited that hypersynchronous cortical epileptiform activity blocks sensory signals leaving the cerebral cortex functionally detached which renders the patient unresponsive and insensible. This account, though logical is only theoretical, thus leaving a significant knowledge gap in support of this theory. The most oft-cited criticism of the convulsive theory is the low frequency of tonic-clonic seizures in concussion, though many physicians do

acknowledge their rarefied occurrence resulting from concussion (Shaw, 2002). While the coupling of convulsions and concussions is rare, the primary issue is of mechanism, not manifestation. A convulsion is the shaking of the body due to rapid tonic-clonic muscle movements. A seizure on the other hand is the result of abnormal electrical activity in the brain that can result in a wide range of behavioral manifestations, including but not limited to convulsions. Indeed, there is an entire classification of seizures that are nonconvulsive (i.e. absence seizures), yet these seizures are equivalent to convulsive seizures in terms of what is happening at a neuronal level (Engel & Pedley, 1998). Therefore, the label "convulsive" is misleading as convulsive movements, if they occur, are a symptom not a mechanism. Rather, hyperexcitability and the ensuing excitotoxicity are the common mechanisms and should be reflected in the nomenclature of the pathophysiological theory. This is more than a trivial semantic argument as the basis chosen for comparison is reflected in the understanding of concussion which subsequently informs treatment and management.

Biomechanics

Despite the seeming lack of physical damage in concussive injuries, it is important to recognize that concussions are the result of the brain moving within the cranial vault, and that movement has physical consequences on the brain. Without the benefit of modern imaging technology, Brodie (1828) eloquently contended that, "If we consider that the ultimate structure of the brain is on so minute a scale that our senses are incapable of detecting it, it is evident that there may be changes and alterations of structure which our senses are incapable of detecting also." At about the same time, the

aforementioned French military surgeon J-P Gama was conducting what are widely accepted to be the first biomechanical investigations of concussion. His model was composed of a gelatinous substance meant to simulate the brain suspended by thin wires in a long-neck glass flask. By striking the walls of the flask he was able to document oscillatory and vibratory movements of the wires. Though rudimentary by current standards, this was the first effort to document the biomechanics of concussive injuries in general, and of *coup-contrecoup* injuries in particular (Gama, 1835). Our understanding of the biomechanics of concussion is now more sophisticated, understanding that they are predicated on the physiological composition of the brain and its position within the skull. The physical properties of nervous tissue make the brain relatively incompressible, but readily distortable (Barr, 2005; Holbourn, 1943, 1945; Kandel, Schwartz, & Jessell, 2000). Anatomically, the brain is suspended inside the skull by cerebrospinal fluid in the subarachnoid space allowing the brain to move within the skull. The brain's response to sudden changes in the velocity of the head is to vacillate, slide, or rotate within the skull (Holbourn, 1945; Shaw, 2002). The cerebrospinal fluid normally protects the brain from injury by cushioning it against the skull; however, when the velocity of the brain lags behind that of the skull (acceleration) or continues to move after the skull has stopped (deceleration), the brain comes into violent contact with the bones of the skull, particularly at the frontotemporal suture (Cantu, 1992). Compression of the skull without fracture may lead to a focal injury accompanied by an increase in intracranial volume as well as a probable increase in intracranial pressure (Gurdjian, 1972). The resulting pressure wave passes through the relatively closed system of the brain, meninges,

cerebrospinal fluid system, and vasculature causing general and local tissue deformation, thereby compromising the structural integrity of the neurons (Gurdjian, 1972).

Most concussions result from either a direct blow to the head or from an impulse insult to the head. Despite the absence of direct contact to the head, impulse injuries can be just as severe as impact injuries (Barth, Freeman, Broshek, & Varney, 2001). Regardless of the nature of the injury (direct or indirect), concussive effects are exerted via inertial loading (acceleration or deceleration) (Shaw, 2002). Impact injuries occur when an object of sufficient mass strikes the skull such that kinetic energy imparted to the skull is transferred to the brain (Echemendia, 2006). These types of injuries typically result in focal damage where the affected brain area is directly beneath the point on the skull where the impact occurred- a *coup* injury. When the acceleration of the brain is abruptly halted by the skull an injury opposite to the point of impact can occur: a *contre-coup* injury (Bailes & Cantu, 2001; Cantu, 1992; Gama, 1835). Impulse injuries result in abrupt inertia changes of the head without direct impact.

Concussions occur along one of two force vectors: Linear (translational) or rotational (angular). Linearly applied force is simply inertial force applied in a straight line. Typically linear forces result in a coup injury leading to the compression and possible stretching of neural tissue (Echemendia, 2006). However, because not all combinations of force and mass produce the same effects, injury outcomes vary. For example, a small object moving at high velocity, like a bullet, is more likely to penetrate the skull and create local damage along its trajectory. Similarly, larger objects moving at

slower speeds may crush the skull, but avoid any acceleration/deceleration forces on the brain thereby not resulting in a concussion. Concussions are more likely to result when there is an intermediary combination of mass and force, which is characteristic of the vast majority of head injuries (Holm, et al., 2005).

Rotational injuries result from an inertial force that produces an angular acceleration of the head around the midline axis (Echemendia, 2006; Holbourn, 1945). Because of the interconnectivity between the bones, muscles, and connective tissue of the head, neck, and upper torso rotational injuries are more likely to result in a concussion when the force is directed laterally, rotating around the axis created around the midline of the neck up through the top of the skull (Echemendia, 2006) around the fronto-parietal junction (Bayly et al., 2005). These injuries are more complex in terms of the effects on neural tissue including shearing/tearing or stretching. Injuries resulting from angular acceleration are most detectable at gray-white matter junctures beginning at the cortical surface and less detectable descending toward the brain stem (Echemendia, 2006). Rotational acceleration is much more severe than linear acceleration, in large part because of the nature of brain tissue (Holbourn, 1945). When the skull is moved in a straight line the brain's *incompressible* nature allows it to move as a whole thereby minimizing distortion. That is to say, the effects of linear force are negligible when compared with rotational forces (Holbourn, 1945). Conversely, when the skull is rotationally accelerated the brain's lack of rigidity prevents it from moving as a whole thus distorting the brain considerably (Holbourn, 1945). However, a study investigating the impact of linear and rotational forces on symptom severity failed to find a clear

relationship (Guskiewicz, Mihalik, et al., 2007). That is to say, the role of linear and rotational force vectors may be clear in terms of brain deformation, but the role that force vectors in clinical outcome remains unclear at present.

Current biomechanical studies of brain deformation have been done using similar gel-filled skull models (Margulies, Thibault, & Gennarelli, 1990; Meaney et al., 1995) which are limited because they cannot account for the effects of heterogeneity, anisotropy, vasculature, meninges, and cerebrospinal fluid on brain deformation. High-speed x-ray on cadavers are also used (Hardy et al., 2001), but cannot account for differences in live subjects as well as spatial limitations. MR techniques in humans (Bayly, et al., 2005; Reese, Feinberg, Dou, & Wedeen, 2002; Sabet, Christoforou, Zatlin, Genin, & Bayly, 2008) on the other hand, provide a rich source of information pertaining to strain fields accompanied by strong spatial and temporal resolution, but are limited by the allowable forces that can be delivered to human subjects. MR studies are consistent in reporting compression in frontal regions and stretching in more posterior regions before subsequent concussion in occipital areas. Furthermore, radial-circumferential shear strains are observed, in kind with those observed in gel models where angular force vectors are applied. Unlike gel models, the MR studies appropriately account for the inherent heterogeneity, natural brain divisions (central fissure and central sulcus), and the brain's suspension composed of the dura mater, falx cerebri, and tentorium membranes. What MR studies cannot account for due to technical limitations as well as ethical guidelines are the shear strains and subsequent axonal injuries.

Understanding the movement of the brain, though important, does not fully describe the nature of concussion. It is equally important to understand the effects of said movement, namely shear strains. Shear strain injuries are deformation of the axon perpendicular to the force vector and are most probable when the skull and brain are accelerated around the midline axis by an angular force (Ommaya & Gennarelli, 1974) though the pathophysiology that leads to traumatic axonal injury (TAI) in TBI is relatively unknown (Ducreux et al., 2005). The current view is that corpus callosum injury is caused by shear-strain rotational forces and/or impact against the free margin of the falx (partition-like fold of the dura that extends into the medial-longitudinal fissure). Ensuing tissue injury is marked by axonal stretching, disruption, and the eventual separation of nerve fibers (Huisman et al., 2004). Animal research (Liu et al., 2006) further suggests that TAI has downstream consequences that affect the integrity of the myelin sheath, the result of which would be significantly reduced neural conduction speed (Barr, 2005). TAI is more commonly seen at gray-white matter junctions (Ducreux, Huynh, et al., 2005; Ducreux, Nasser, Lacroix, Adams, & Lasjaunias, 2005; Huisman, et al., 2004; Le et al., 2005; Lee, Choi, & Chun, 2006; Okumura et al., 2005; Song et al., 2002) because the two tissue types differ in their respective rigidity (Holbourn, 1945) due to the myelin sheaths that surround white matter axons. While the myelin increases the speed of neural conduction in white matter, it also makes the tissue more fibrous (and therefore more rigid) perhaps explaining why TAI is more common in white matter.

Neuroimaging

Investigating how biomechanical forces impact the gross anatomy of the concussed brain has proven difficult. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) can provide information about anatomical and gross structural changes following a concussion. CT is an x-ray dependent technique that constructs a 3-D image of the brain. Clinically, CT scans can reveal brain lesions, contusions, fractures, and intracranial hemorrhaging. They are widely used in the management of closed head injuries, particularly within the first 24 hours post-injury, though it is generally only specified in cases for which there has been LOC (Livingston et al., 2000; Stein & Ross, 1992; Warren & Bailes, 1998) or persistent symptomatology (Rimal, Thapa, Munasinghe, & Errington, 2007).

Research on the utility of CT in sports concussion management is limited as the only studies available focus on boxers. Though there are instances where lesions are detected, the majority of concussed athletes have negative CT findings with weak predictable outcome validity (Jordan et al., 1992; Jordan & Zimmerman, 1988; Ross, Casson, Siegel, & Cole, 1987). The lack of consistent CT findings is quite common in the mTBI literature as well (Gentry, Godersky, Thompson, & Dunn, 1988; Groswasser, Reider-Groswasser, Soroker, & Machtey, 1987; Han et al., 1984; Newton et al., 1992; Zimmerman, Bilaniuk, Hackney, Goldberg, & Grossman, 1986). A large study investigating the prevalence of lesions in mTBI found that approximately only 16% of scans are positive (Iverson, Lovell, Smith, & Franzen, 2000), a rate that is considered quite high based on current clinical findings (Johnston, et al., 2001); moreover, patients

with positive scans share some common characteristics, chief among them being the presence of intracranial abnormalities, LOC, skull fractures, and lower Glasgow Coma Scale (GCS) and Galveston Orientation and Amnesia Test (GOAT) scores. The rates of positive CT findings is even further confounded when one considers that for some studies a GCS score of 13-15 is considered a mTBI (i.e. Iverson, et al., 2000), while for others, a score of only 15 (i.e. Audenaert et al., 2003) is considered a mTBI. Though they are still used in clinical and emergency settings because of their relative cost effectiveness and wide availability (Toga & Mazziotta, 2002), CT scans have fallen out of favour, particularly in research because of their limited ability to detect finite lesions and contusions, especially relative to MRI (Jordan & Zimmerman, 1990; Newton, et al., 1992; Snow, Zimmerman, Gandy, & Deck, 1986).

MRI is a more sensitive technique to investigate anatomical changes given its higher resolution, its capacity to image different planes, and because it provides better distinction between tissue types: gray matter, white matter, and cerebrospinal fluid. Typical injuries detected by MRI resulting from sport concussion include small cortical contusions or subdural hematomas and small white matter hemorrhages (Toga & Mazziotta, 2002) that are generally interpreted to be reflective of TAI (Bazarian, Blyth, & Cimpello, 2006). Though more effective at detecting abnormalities than CT (Jordan and Zimmerman, 1990; Newton et al., 1992), MRI has proven itself inconsistent with only moderate predictive validity in sports concussion (Jordan & Zimmerman, 1990; Newton, et al., 1992) and mTBI in general (Barr, 2005; Bazarian, et al., 2006; Toga & Mazziotta, 2002). Though its resolution is vastly improved over CT, MRI is still not able to detect

lesions in the majority of concussed athletes (Jordan, et al., 1992; Jordan & Zimmerman, 1990), limiting its clinical applicability. Moreover, CT and MRI are unable to provide information about functional alterations in brain function resulting from sports concussion.

Diffusion tensor imaging (DTI) provides information about brain microstructure by quantifying isotropic (typical of gray matter) and anisotropic (typical of white matter) water diffusion. DTI measurements are based on the fact that the network of fibers within the brain is composed of a distinct microstructure that inherently constrains the flow of water molecules. Diffusion tensors numerically model the direction of fastest diffusion in a pattern aligned with fiber orientation. DTI's main utility in neurology is detecting the presence of axonal injury (Toga & Mazziotta, 2002). There is increasing evidence to suggest that TAI is present in TBI and that the extent of the damage is related to the severity of the injury as defined by initial GCS score (Huisman, et al., 2004). Similarly, Kraus et al. (2007) investigated TBI across the severity spectrum using DTI and found the degree of TAI to be related to the severity of the injury with severe TBI patients exhibiting the greatest extent of damage.

Several case studies using DTI on TBI patients from non-athletic populations have consistently noted reduced diffusion in the brain, especially around the splenium (thickened border at the posterior end of the corpus callosum) (Huisman, et al., 2004; Le, et al., 2005; Lee, et al., 2006). The observed axonal shearing in people with concussion has functional consequences. Lee and colleagues (2006) noted that brain areas affected

by axonal injury corresponded to the types of dysfunctions that patients exhibited. Similarly, Huisman and colleagues (2004) compared scores from the GCS with the observed amount of axonal shearing using DTI and found a positive correlation. One of the few studies to focus on mTBI found changes in white matter in the corpus callosum up to five years post injury (Inglese et al., 2005). While it is important to note the persistence of injury after such a long period of time, there are very few studies documenting TAI in the acute post injury phase in mTBI; however, studies suggest that damage can be detected within the first week of injury (Miles et al., 2008; Wilde et al., 2008).

There are several ways to analyze DTI data depending on the research or clinical question. Fractional anisotropy (FA) and mean diffusivity (MD) are the most often used measures of diffusivity (Toga & Mazziotta, 2002). FA is derived from three eigenvalues, the longest (λ_1), middle (λ_2), and shortest (λ_3) distances around a given tensor which correspond to linear (cigar-shaped) and planar (plate-shaped) anisotropic diffusion, and isotropic (spherical) diffusion respectively that define the shape of the tensor as an ellipse. Diffusion anisotropy can be modeled more completely using a set of three matrices where each plane is parameterized in a barycentric space with the three extremes at each corner (Toga & Mazziotta, 2002) making it possible to separate the eigenvalues in order to obtain values of axial diffusivity (AD) taken from the linear plane, and radial diffusivity (RD) derived by averaging the planar and spherical planes. Mean diffusivity is simply the average diffusivity along all three eigenvectors divided by three which gives the overall diffusion of water regardless of direction.

The presence of axonal shearing in people with TBI suggests that the concussed brain will undergo similar changes, though perhaps to a lesser extent when compared to brains that have been severely or moderately injured. Several studies to date have shown FA values to decrease in correlation with the severity of the TBI (Huisman, et al., 2004; Kraus, et al., 2007). Within adults and adolescents having suffered a MTBI, several studies have shown changes in FA in the acute post-injury phase (Miles, et al., 2008; Wilde, et al., 2008). In a pioneering study of concussed athletes Cubon and colleagues (2011) showed a similar decrease of FA in specific brain regions. *We hypothesize that traumatic axonal injury will be detected in concussed athletes and that TAI will be present, though to a lesser extent in the chronic post-injury phase. Specifically, fractional anisotropy and axial diffusivity will be decreased while mean diffusivity measures will show an increase.*

Functional and Hemodynamic Changes

Functional MRI (fMRI) relies on the same principles as MRI, though instead of providing an anatomical picture based on hydrogen protons, it takes advantage of the magnetic properties of haemoglobin. While fMRI can track blood perfusion, the more common paradigm tracks blood oxygenation changes where the blood oxygenation level dependent (BOLD) signal is detected (Toga & Mazziotta, 2002). It is assumed that increased blood flow to a given brain area is related to the cognitive processing inherent to the task and the subsequent increased metabolic demand. Following injury, decreases in blood flow are therefore speculated to represent an impaired functional capacity.

Few studies have specifically targeted sports concussion using fMRI, but each reports dysfunction in concussed athletes (Chen, et al., 2007; Chen, et al., 2004; Chen, et al., 2008; Jantzen, Anderson, Steinberg, & Kelso, 2004; Lovell et al., 2007). Chen and colleagues (2007; 2004; 2008) report functional deficits in working memory in concussed athletes that manifest in reduced activation in dorsolateral prefrontal cortex while Jantzen et al. (2004) noted an atypical BOLD response on a finger tapping sequence in parietal and lateral frontal cortical areas. Furthermore, Chen et al. (2007; 2004; 2008) found that the detectibility of an atypical blood oxygen level dependent (BOLD) signal is directly related to the symptomatology experienced by the athlete. Though useful in understanding persistent symptomatology and its covariates (athletes were tested 1-14 months post concussion), the focus of the studies by Chen and colleagues does not address the acute phase during which deficits, transient though they may be, are most commonly seen in concussed athletes. Lovell and colleagues (2007) scanned concussed athletes in the acute post concussion phase (1 week post concussion) and again approximately one month after injury. Briefly, athletes who displayed hyperactivation on a cognitive task in the acute phase had prolonged recovery times relative to those athletes who demonstrated typical activation in the acute phase. The implication of the findings from Lovell et al. (2007) in concert with those of Chen and colleagues (2007; 2004; 2008) is that atypical activation in the acute phase is related to the presence or absence of symptoms, regardless of time post injury. Unfortunately, to date there has not been an fMRI study that has included other groups suffering from non-concussion related symptoms such as chronic fatigue or migraine thereby making it difficult to implicate the

injury itself over and above the symptomatology. That is to say, it appears that concussions lead to functional changes, but only insofar as those changes are tied to symptomatology. As such, teasing apart the functional changes from the symptomatology is essential in determining if these changes are indeed resultant from concussions or whether they are simply reflective of symptoms regardless of their etiology. Though small in scope, the limited fMRI findings in sports concussion are in agreement with the mTBI literature (McAllister et al., 1999; McAllister et al., 2001; McAllister, Sparling, Flashman, & Saykin, 2001) underscoring the link between symptomatology and corresponding changes in functional brain activation.

Single photon emission tomography (SPECT) provides information about changes in cerebral blood perfusion following a concussion. An intravenously injected radioactive ligand accumulates in different brain areas in proportion to the delivery of nutrients to provide an overall picture of regional cerebral blood flow (rCBF) (Toga & Mazziotta, 2002). With respect to mTBI, changes in rCBF are moderately correlated with clinical outcome (Bonne et al., 2003; Hofman et al., 2001; Umile, Plotkin, & Sandel, 1998), but many of the SPECT studies are limited in that they are carried out in patients with persistent symptomatology at various time points after injury with a wide range in cause of injury. Despite the variability in participant populations, most SPECT studies find focal hypoperfusion in frontal and temporal cortical areas as well as in thalamic-basal ganglia areas (Abdel-Dayem et al., 1998; Abu-Judeh et al., 2000; Abu-Judeh et al., 1999; Bergsneider et al., 1997; Bonne, et al., 2003; Korn, Golan, Melamed, Pascual-Marqui, & Friedman, 2005; Umile, et al., 1998). In a retrospective study Korn and colleagues (Korn,

et al., 2005) found that 85% of Post Concussion Syndrome (PCS) patients in their sample showed a focal reduction in perfusion and an abnormal blood-brain barrier in the over-lying areas. The altered blood-brain barrier was also anatomically correlated with abnormal rhythm generators in the cortex. The results from Sterr (Sterr, Herron, Hayward, & Montaldi, 2006) and Korn (Korn, et al., 2005) suggest a link between blood perfusion and PCS symptomatology that is objectively measurable; however, heterogeneous patient populations and variable testing methods have made it difficult to characterize a link between the neuropsychological and hemodynamic changes in people with PCS.

There is evidence suggesting that concussions change cerebral blood flow (CBF) (Korn, et al., 2005; Lewine et al., 2007; Nilsson & Nordstrom, 1977a; Yuan, Prough, Smith, & Dewitt, 1988). Nilsson and Nordstram (1977a) and Yuan and colleagues (1988) both showed an initial spike in blood pressure in the immediate phase (within 10 minutes) after injury followed by a significant reduction in CBF in the intervening hour with resulting heterogenous changes in CBF patterns in rats. Clinically, an increase in CBF in frontotemporal areas has been documented in patients hours after sustaining injury (Nariai, Suzuki, Ohta, Ohno, & Hirakawa, 2001) as well as the corresponding hypoperfusion in frontotemporal regions seen days and months after the injury (Abdel-Dayem, et al., 1998).

Positron emission tomography (PET) constructs a highly sensitive functional 3-D image of the brain from the emissions of a radio isotope and can directly detect blood

flow as well as glucose and oxygen metabolism in the brain. The majority of PET studies have focused on non sports-related mTBI patients with persistent symptomatology and found that frontotemporal regions are the most consistently impaired regions (Chen, Kareken, Fastenau, Trexler, & Hutchins, 2003; Gross, Kling, Henry, Herndon, & Lavretsky, 1996; Ruff et al., 1994; Umile, Sandel, Alavi, Terry, & Plotkin, 2002; Varney, Pinkston, & Wu, 2001). Unfortunately, all of these studies were conducted with patients well outside of the acute injury phase. PET appears to offer reasonable correlation with neuropsychological deficits, but it is difficult to extrapolate these findings to what is seen in the acute phase in sports concussion. Those people who remain diagnostically symptomatic well after suffering an mTBI represent a very small portion of the population (3-5%) (McCrea, 2008) and as such may not be the best group upon whom to base conclusions. Currently, there have been no studies that have keyed on sports concussion. This general absence of data makes it difficult to assess the efficacy of PET use in mTBI diagnosis and management in general, let alone how it pertains to sports concussion research in particular.

Microfunctional and Neurometabolic Changes

The systemic changes in blood flow which heavily influence mortality and morbidity in TBI (DeWitt & Prough, 2003) may overlay more profound changes that can be observed in ionic shifts that occur after a concussive blow. Concurrent with the initial spike and subsequent decrease in CBF, concussive injuries also cause a hyperexcited brain state via a mechanically elicited neuronal discharge resulting in excitatory activity (Nilsson & Nordstrom, 1977b; Zimmerman & Putnam, 1947) that is not restricted to

mechanoreceptors (Hu & Lewin, 2006; Morris, 1990; Sachs, 1991). Mechanical depolarization as a physiological explanation of concussion was proposed by Denny-Brown and Russell (1940) who postulated that a *hypopolarized* neuronal state followed mechanical stimulation, but it was Walker et al. (1944) who proposed an initial *hyperexcitation*. The hyperexcitation is the end result of a short series of events that begins with the concussive blow creating an area of increased intracranial pressure underneath the skull at the point of impact. This increase in pressure results in high frequency pressure waves that pass through the brain, mechanically depolarizing neural tissue (Walker, et al., 1944). Theoretically, once depolarization has occurred, synaptically-coupled neurons recruit surrounding neurons with a barrage of excitatory discharges in much the same way that an epileptic focus breaks down surround inhibition (Kandel, et al., 2000). The brain eventually regains ionic equilibrium through either neuronal exhaustion where the brain is simply unable to keep up with the tremendous energy demands placed on it due to prolonged hyperexcitation, or through a variety of mechanisms working in concert (Engel & Pedley, 1998).

More precisely, a concussive injury precipitates potassium (K^+) efflux by stretching the axons and opens voltage dependent K^+ channels followed closely by an indiscriminate release of excitatory neurotransmitters (EAAs) (i.e. glutamate) (Hubschmann & Kornhauser, 1983; Julian & Goldman, 1962; Katayama, Becker, Tamura, & Hovda, 1990; Takahashi, Manaka, & Sano, 1981). Energy requiring pumps are then activated to restore ionic homeostasis, which requires roughly two-thirds of the brain's energy resources under normal conditions (Shah & West, 1983; Sunami et al., 1989; Yoshino,

Hovda, Kawamata, Katayama, & Becker, 1991). Meanwhile, excitatory activation becomes wider spread through the involvement of other excitatory receptors including *N*-methyl *D*-aspartate receptors (NMDAr) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors (AMPAr), and kainite receptors leading to even more K^+ efflux (D'Ambrosio, Maris, Grady, Winn, & Janigro, 1999) ergo greater ionic imbalance. The gap between energy supply and demand is further augmented by a decrease in cerebral blood flow (Giza & Hovda, 2001) creating an energy crisis leading to a state of hyperglycolysis (accelerated anaerobic energy production) in an effort to meet the heavy demand for adenosine triphosphate (ATP) (Ackermann & Lear, 1989; Lear & Ackermann, 1989), leaving lactate as a byproduct (Nilsson & Nordstrom, 1977b; Nilsson & Ponten, 1977; Yang, DeWitt, Becker, & Hayes, 1985). Lactate accumulation can lead to neuronal dysfunction through the induction of acidosis, membrane damage, cerebral edema, and altered blood-brain barrier permeability (Gardiner, Smith, Kagstrom, Shohami, & Siesjo, 1982; Ransom & Fern, 1997; Unterberg, Stover, Kress, & Kiening, 2004). The energy scarcity may contribute to post-concussive vulnerability as the depleted resources leave the brain less able to respond to a second injury (Giza & Hovda, 2001) or lead to more long-lasting increased susceptibility to future concussions (Guskiewicz, et al., 2003).

The K^+ efflux, release of EAAs and recruitment of NMDA receptors allow calcium (Ca^{2+}) to enter the cell. Experimentally, the persistent increases in Ca^{2+} influx can last up to 2-4 days (Fineman, Hovda, Smith, Yoshino, & Becker, 1993; Osteen, Moore, Prins, & Hovda, 2001). The prolonged exposure to Ca^{2+} damages mitochondria thus impairing

oxidative metabolism (Verweij et al., 1997; Xiong, Peterson, Muizelaar, & Lee, 1997). The increased calcium levels further complicate neuronal connectivity by disrupting neurofilaments and microtubules (Giza & Hovda, 2001). Additionally, intracellular magnesium (Mg^{2+}) levels are significantly diminished and remain as such for several days post-injury. This contributes further to the energy crisis as Mg^{2+} is essential for generating ATP (energy production). The initiation of protein synthesis and the maintenance of the cellular membrane potential are also dependent in part on Mg^{2+}. Thus the diminished Mg^{2+} levels contribute to the energy crisis by affecting energy production to restore ionic balance and protein synthesis essential to maintaining membrane potential (Giza & Hovda, 2001).

The initial period of hypermetabolism is followed by a longer lasting hypometabolic state where cerebral glucose is diminished for 5-10 days experimentally (Yoshino, et al., 1991) and 2-4 weeks clinically. The lowered cerebral glutamate levels are not correlated with GCS score further underscoring that neurometabolic changes occur whether the concussive injury is accompanied by overt clinical signs or not (Bergsneider et al., 2000). The intracellular increase in Ca^{2+} peaks approximately 48 hours post injury and resolves without obvious morphological damage by 4 days post injury in moderate experimental concussions (Fineman, et al., 1993). More severely concussed animals showed a delayed rise in intracellular Ca^{2+} levels up to 14 days post-injury in remote sites such as the thalamus where cell death was observed (Osteen, et al., 2001). The long term deficit in memory and cognition associated with concussion may be the result of dysfunctional EAA transmission. Post-concussion alterations in

glutamatergic (NMDA) (Miller et al., 1990) adrenergic (Pappius, 1988) and cholinergic (Gorman, Fu, Hovda, Murray, & Traystman, 1996) systems are present in brain areas particularly sensitive to damage such as the hippocampus (D'Ambrosio, Maris, Grady, Winn, & Janigro, 1998; Sanders, Sick, Perez-Pinzon, Dietrich, & Green, 2000; Sick, Perez-Pinzon, & Feng, 1998). Concussive injuries have also been shown to cause changes in choline acetyltransferase in the short term (Gorman, et al., 1996) and a loss of forebrain cholinergic neurons in the long term (Schmidt & Grady, 1995) resulting in deficits in learning and spatial memory in nonhuman animals (Hepler, Olton, Wenk, & Coyle, 1985; Miyamoto, Kato, Narumi, & Nagaoka, 1987).

In addition to the changes noted in EAAs, there is evidence to suggest that γ-Aminobutyric acid (GABA) systems are also implicated in concussion. It has been hypothesized that changes in the GABA system slow down neural networks. Indeed, pharmacological and electrophysiological studies suggest that the late component of the silent period is caused by long-lasting cortical inhibition mediated by GABA-b receptors where the inhibition of GABA re-uptake lengthened the cortical silent period (Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999). The cortical silent period (CSP) is a pause in an otherwise continuous series of electrophysiological events and basically refers to the refractory time of the network. The silent period can be used as a marker of cortical modulation and accompanying changes therein are attributed to learning, disease, or site of damage. It is typically measured using a technique known as transcranial magnetic stimulation that measures central inhibitory/excitatory mechanisms. Studies investigating Huntington's disease (Priori, Berardelli, Inghilleri, Polidori, & Manfredi,

1994) and epilepsy (Hamer et al., 2005; Hattemer et al., 2006; Tataroglu, Ozkiziltan, & Baklan, 2004) found that these patient populations exhibit lengthened CSP. Though Huntington's and epilepsy are very different pathologies supra-typical levels of glutamate and sub-typical levels of GABA are common to both (Barr, 2005; Engel & Pedley, 1998; Teskey et al., 2005). Similar changes in CSP can be observed in patients with ataxia and dementia pugilistica (Restivo et al., 2004). The opposite was found in people with Parkinson's disease where the CSP was found to be shorter relative to healthy controls (Barr, 2005; Priori, Berardelli, Inghilleri, Accornero, & Manfredi, 1994). The net effect of Parkinson's disease is due in part to supra-typical level of GABA. The importance of GABA (inhibitory) and glutamate (excitatory) on functional recovery (Butefisch, 2006) in the brain emphasizes the need to investigate any potential alterations in their relative levels as they may have direct implications on both the acute phase and the persistent phase of brain injury.

The clinical findings (ex. memory dysfunction, concentration problems) are important to the diagnosis of concussion; however, the subclinical findings are just as important in understanding the effects of concussion on the brain. Event-related potential studies found persistent deficits in attention and working memory that were not detected by clinical tests (Gaetz & Bernstein, 2001; Gosselin, Theriault, Leclerc, Montplaisir, & Lassonde, 2006; Lavoie, Dupuis, Johnston, Leclerc, & Lassonde, 2004b; Theriault, et al., 2009; Theriault, et al., 2010). Johnston and colleagues (2001) suggested that the majority of concussed athletes who have documented incapacitating post-concussive symptoms, neuropsychological deficits, and accompanying major lifestyle changes do not have

positive neuroimages. Furthermore, a series of studies by Sojka and Stalnacke (1999; 2006; 2005; 2006; 2004) found that acute levels of biochemical markers of brain tissue damage, S-100B and NSE (neurone-specific enolase), increase in concussed athletes despite negative findings for structural damage. The association between serum chemical changes and disability supports the notion that long-term consequences of a mild brain injury may partly be the result of brain tissue injury.

The work of De Beaumont et al. (2007) may provide the most pressing evidence regarding the persistent and pervasive nature of concussions in athletes as well as further evidence implicating a GABA-glutamate imbalance. Looking at the motor system directly, they were able to determine the effects of multiple concussions in athletes where it was found that the CSP was significantly prolonged after a concussion, and that the occurrence of subsequent concussions augmented these alterations irrespective of the time elapsed since the last incident. This suggests that the intracortical inhibitory interneuron receptors of the motor system may be particularly vulnerable to the effects of sports concussions and that there is a persistent effect (beyond the acute phase of the injury) on the underlying intracortical inhibitory mechanism in asymptomatic young athletes. What De Beaumont et al. have not uncovered is how the metabolism of the concussed brain is altered. Magnetic Resonance Spectroscopy (MRS) affords the opportunity to investigate the metabolic changes in the concussed brain.

MRS images the chemical composition of living tissue and is thus able to detect neuronal damage and changes in the chemical makeup of neurons. It is particularly useful

in corroborating evidence of TAI as detected using DTI because damage related changes to neurons are manifest not only in their physical structure, but also in their composition (Toga & Mazziotta, 2002). Several compounds can be imaged with MRS, chief among them being creatine (Cr), glutamate (Glu), myo-inositol (m-I), and N-acetyl aspartate (NAA). Though other metabolites can be imaged, only the aforementioned neurometabolites will be examined in the current thesis.

MRS can be quantified using two different methods including absolute quantitation (AQ) and relative quantitation (RQ). AQ is a simple and straight forward method where the concentration of a given metabolite is reported. It is generally considered to be the more precise method as it makes no assumptions about the stability of a given neurometabolite, an assumption that is inherent in RQ. However, AQ is much more difficult to process than RQ, and introduces more individual variance (Jansen, Backes, Nicolay, & Kooi, 2006). RQ, on the other hand, is a simpler methodology and has been successfully applied in the diagnosis of several pathologies including epilepsy, cancer, dementia, and multiple sclerosis to name a few. RQ also normalizes the neurometabolic concentrations to an extent as they are expressed as ratios. As mentioned, a key assumption in RQ is the stability of certain metabolites, namely Cr and choline, which are used as reference metabolites. This can complicate the interpretation of spectra as any detectable changes may in fact be due to a change in either the metabolite being measured, a change in the reference metabolite, or both. The current thesis employed the use of RQ with Cr as a reference metabolite which is considered stable in the vast majority of pathologies, including concussion.

Creatine is synthesized endogenously in the liver, kidneys, and pancreas and to a lesser extent in the brain. Exogenously, Cr is obtained in diets containing meat and fish (Andres, Ducray, Schlattner, Wallimann, & Widmer, 2008). It is essential for maintaining the supply of ATP inter- and intracellularly through creatine kinase (CK) and phosphocreatine (PCr) system. Both isoenzymes allow for a large build up of PCr that is a readily available, temporally efficient energy buffer that prevents any major drop-off in ATP concentrations upon cellular activation or stress conditions. Because the brain accounts for roughly 20% of the body's energy consumption, an extremely high rate of ATP turnover is necessary to maintain electrical membrane potentials as well as signaling activities (Andres, et al., 2008). This energy demand is met by the CK-PCr system which provides neurons with an efficient ATP-generating and ATP-consuming processes that function as a sort of energy "shuttle" to bridge sites of energy consumption and energy production. Studies investigating the role of Cr in brain trauma have not revealed any changes in its concentration, but have demonstrated its neuroprotective role. That is to say, higher levels of Cr minimize cell damage (Andres, et al., 2008).

Myo-Inositol (m-I) is synthesized throughout the body, but mostly within the brain where its concentration is also the greatest (Fisher, Novak, & Agranoff, 2002). It serves as a precursor molecule for inositol lipid synthesis, but also as an osmolyte. The concentration of m-I is maintained via three mechanisms responsible for 1) the transport of inositol across the plasma membrane mediated by specific carrier molecules, 2) the synthesis of an isomer, and 3) the efflux of inositol mediated by a volume sensitive osmolyte channel. In functioning cells the osmotic pressure is in a constant state of flux

which must be tightly regulated so that the cell does not sustain damage due to changes in cell volume. Acutely, this is done by the movement of NA^+, K^+, H^+, and Cl^- across the membrane, and chronically by the transport of osmolytes, namely m-I. Physical trauma to the brain can compromise the blood brain barrier resulting in leakage of ions and macromolecules into the cerebral extracellular space which in turn translates into an upregulation of an m-I transporter (sodium m-I transporter) and cellular uptake of m-I.

N-acetylaspartate is present at exceptionally high concentrations in the brain second only to glutamate, and is the largest peak in spectra of healthy brain tissue (Moffett, Ross, Arun, Madhavarao, & Namboodiri, 2007). NAA is synthesized in neuronal mitochondria by Asp-NAT using acetyl CoA and L-aspartate. The synthesis of NAA itself is still not known; however, NAA itself is the precursor to the neurometabolic modulator N-acetylaspartylglutamate (NAAG) (Gehl, Saab, Bzdega, Wroblewska, & Neale, 2004). Aspartoacylase hydrolyzes NAA, localized primarily in oligodendrocytes, producing acetate and aspartate. It is synthesized and exported from neuronal mitochondria (Patel & Clark, 1979) where it is exported to oligodendrocytes as a key contributor in the formation of myelin lipid formation. NAA is also thought to be an osmoregulator as well (Taylor et al., 1994). It had been hypothesized that upon sustaining brain trauma, a loss of NAA was equal to TAI in white matter and neuronal loss in gray matter (Gehl, et al., 2004; Urazaev et al., 2001).

Glutamate, a member of the family of biogenic amines, is the most frequently occurring neurotransmitter in the central nervous system (Barr, 2005; Kandel, et al.,

2000). It is produced from an α-ketoglutarate, and once released after catabolism glutamate is taken up from the synaptic cleft by neurons and glia. Within glia, specifically astrocytes, it is converted to glutamine, a non-neuroexcitatory amino acid, by glutamine synthase which occurs almost exclusively in neuroglia (Broer & Brookes, 2001). Glutamine is then transferred to neurons and express glutaminase activity that catalyzes the hydrolysis of glutamine to glutamate where it is stored until a depolarization occurs (Broer & Brookes, 2001). The role of glutamate is different depending on the type of receptor that it binds with: at ionotropic receptors glutamate is excitatory, whereas at metabotropic receptors it is modulatory (Kandel, et al., 2000). However, glutamate has a role beyond that of neurotransmitter. It is also an important neurometabolite used in the production of nitrogen which is key in the synthesis of proteins and nucleic acid, and essential to the production of other important molecules including GABA.

In the only three sports concussion studies a decrease of NAA is reported, suggesting that there is indeed structural damage in sports concussions despite the negative findings of more traditional imaging techniques (Cimatti, 2006; Vagnozzi et al., 2010; Vagnozzi et al., 2008). Studies outside of the sports literature have also demonstrated abnormalities from individuals with mTBI with otherwise normal-appearing MR scans (Babikian et al., 2006; Govindaraju et al., 2004; Holshouser et al., 2006; Kirov et al., 2007; Shutter, Tong, Lee, & Holshouser, 2006). Significantly depressed concentrations of NAA have been measured in mTBI despite normal findings on conventional MRI (Cecil, Lenkinski, Meaney, McIntosh, & Smith, 1998; Garnett, Blamire, Rajagopalan, Styles, & Cadoux-Hudson, 2000) with the greatest reductions seen

in injury-prone brain tissues which typically include frontal areas and grey-white matter junctions (Holbourn, 1945). In contrast, levels of *myo*-inositol (mI) were shown to be augmented in mTBI (Garnett et al., 2000). This provides yet another indication of cellular injury as higher concentrations of this intracellular compound are associated with glial proliferation (Friedman, Brooks, Jung, Hart, & Yeo, 1998). Though the MRS research is burgeoning and encouraging, more studies for which sports concussion is the focus must be conducted before any conclusions can be made regarding the athlete population.

It is hypothesized that, in the acute phase, the relative concentration of cortical glutamate to creatine will be lower in concussed athletes relative to healthy controls in the acute post-injury phase. We also hypothesize that the relative concentration of N-acetyl aspartate (NAA) to creatine will decrease while the concentration of myo-inositol to creatine will increase in concussed athletes in the acute post-injury phase. It is also hypothesize that m-I will be elevated in the hippocampi, reflective of possible gliosis.

Based on the neurometabolic cascade timeline (Giza & Hovda, 2001), it is further hypothesized that the metabolic changes will be lessened after sixth months, but group differences will still be present between concussed athletes and controls. Specifically, the decrease in NAA will be seen in the dorsal lateral prefrontal cortex and primary motor cortex in the acute post-injury phase, but that neurometabolic changes will have mostly resolved in the chronic post-injury phase. Specifically, NAA and Glu levels will be acutely depressed in both ROIs used in study 3, but will be at or near control levels in the

chronic post-injury phase. Conversely, m-I will be elevated in the acute phase, but will have returned to control levels within the chronic phase.

Article #1

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Acute and Chronic Changes in Diffusivity Measures after Sports Concussion

Running Title: *Postconcussion white matter damage*

Luke C. Henry[1], Julie Tremblay[2], Sebastien Tremblay[1], Agatha Lee[6], Caroline Brun[4], Natasha Lepore[5], Hugo Theoret[1,2], Dave Ellemberg[1,3], Maryse Lassonde[1,2]

1: CERNEC, Université de Montréal, Montréal, Québec, Canada
2: Hôpital Ste. Justine, Montréal, Québec, Canada
3: Département de Kinisiologie, Université de Montréal, Montréal, Québec, Canada
4. University of Pennsylvania, Philadelphia, PA, USA
5. USC, LA, California, USA
6. LONI, UCLA, LA, California, USA

Abstract

Despite negative neuroimaging findings in concussed athletes, studies indicate that the acceleration and deceleration of the brain after concussive impacts result in metabolic and electrophysiological alterations that may be attributable to changes in white matter resulting from biomechanical strain. The present study investigated the effects of sports concussion on white matter using three different Diffusion Tensor Imaging measures (FA, AD, and MD) by comparing a group of 10 non-concussed athletes with a group of 16 concussed athletes of the same age (mean: 22.5 years) and education (mean: 16 years) using a voxel-based approach (VBA) within both the acute and chronic post-injury phases. All concussed athletes were scanned 1-6 days post-concussion and again 6-months later in a 3T Siemens TRIO MRI. Three 2x2-repeated measure ANOVAs were conducted, one for each measure of DTI used in the current study. There was a main effect of group of FA which was increased in dorsal regions of both cortical spinal tracts (CST) and in the corpus callosum in concussed athletes at both time points. There was a main group effect of AD in the right CST where concussed athletes showed elevated values relative to controls at both time points. MD values were decreased in concussed athletes where analyses revealed significant group differences in the CST and corpus callosum at both time points. Although the use of VBA does limit the analyses to large tracts and has clinical limitations with regard to individual analyses, our results nevertheless indicate that sports concussions do result in changes in diffusivity in the corpus callosum and CST of concussed athletes that are not detected using conventional neuroimaging techniques.

Keywords: Diffusion Tensor Imaging, sports concussion, Acute, Chronic

Introduction

According to the Center for Disease Control and the World Health Organization the term "concussion" has officially been replaced by the term mild traumatic brain injury (MTBI), (Gerberding and Binder, 2003; Holm, Cassidy, Carroll, Borg, 2005), but "concussion" is still used ubiquitously in the sports medicine literature where it refers to a type of MTBI typically incurred within the context of sports (Cubon, Putukian, Boyer, Dettwiler, 2011). Until recently, the prevailing attitude in sports culture has been rather dismissive of sports concussions, where they have been considered a minor injury without consequence (Zillmer and Spiers, 2001). This mentality has undergone a major overhaul, particularly in the last 10 years and sports concussions are now taken much more seriously, in part due to the forced retirement of several high profile athletes. As a result, several research and position papers detailing everything from the definition of the injury through to the management of the athlete have been published in an effort to understand sports concussion and the toll that it takes on athletes (Aubry, Cantu, Dvorak, Graf-Baumann, Johnston, Kelly, Lovell, McCrory, Meeuwisse, Schamasch, 2002; Guskiewicz, Bruce, Cantu, Ferrara, Kelly, McCrea, Putukian, McLeod, 2004; Guskiewicz, Bruce, Cantu, Ferrara, Kelly, McCrea, Putukian, Valovich McLeod, 2004; McCrory, Johnston, Meeuwisse, Aubry, Cantu, Dvorak, Graf-Baumann, Kelly, Lovell, Schamasch, 2005; McCrory, Meeuwisse, Johnston, Dvorak, Aubry, Molloy, Cantu, 2009). To date, most research has focused on documenting the clinical and neuropsychological aspects of the injury as well as return to play guidelines. Given that a typical recovery timeline for a sports concussion is roughly 2-10 days in about 90% of

athletes (Dikmen, McLean, Temkin, 1986; Hinton-Bayre and Geffen, 2002; McCrory, Meeuwisse, Johnston, Dvorak, Aubry, Molloy, Cantu, 2009), the longer term physical consequences of these injuries have been largely ignored.

Most conventional imaging techniques rarely reveal structural damage to the sports concussed brain (Ellemberg, Henry, Macciocchi, Guskiewicz, Broglio, 2009; Johnston, Ptito, Chankowsky, Chen, 2001). However, the presence of metabolic disturbances found in young adults (Henry, Tremblay, Boulanger, Ellemberg, Lassonde, 2010; Vagnozzi, Signoretti, Cristofori, Alessandrini, Floris, Isgro, Ria, Marziale, Zoccatelli, Tavazzi, Del Bolgia, Sorge, Broglio, McIntosh, Lazzarino, 2010; Vagnozzi, Signoretti, Tavazzi, Floris, Ludovici, Marziali, Tarascio, Amorini, Di Pietro, Delfini, Lazzarino, 2008) and electrophysiological anomalies found in both young adults and older, former athletes that can persist months (De Beaumont, Brisson, Lassonde, Jolicoeur, 2007; De Beaumont, Lassonde, Leclerc, Theoret, 2007) to several years (De Beaumont, Theoret, Mongeon, Messier, Leclerc, Tremblay, Ellemberg, Lassonde, 2009) after injury suggest that there might yet be a physical injury to the concussed brain, the consequences of which may extend beyond metabolic and electrophysiological alterations.

Peerless and Rewcastle (1967) specified the nature of these physical injuries as being shear strain to white matter or "diffuse axonal injury" (Gentry, 1994), though the term traumatic axonal injury (TAI) is now used more frequently (Buki and Povlishock, 2006; Gennarelli, 1996). TAI is more commonly seen at gray-white matter junctions (Ducreux, Huynh, Fillard, Renoux, Petit-Lacour, Marsot-Dupuch, Lasjaunias, 2005; Ducreux,

Nasser, Lacroix, Adams, Lasjaunias, 2005; Huisman, Schwamm, Schaefer, Koroshetz, Shetty-Alva, Ozsunar, Wu, Sorensen, 2004; Le, Mukherjee, Henry, Berman, Ware, Manley, 2005; Lee, Choi, Chun, 2006; Okumura, Yasokawa, Nakayama, Miwa, Shinoda, Iwama, 2005; Song, Sun, Ramsbottom, Chang, Russell, Cross, 2002) because the two tissue types differ in their respective rigidity (Barr, 2005; Holbourn, 1945) due to the myelin sheaths that surround white matter axons. Shear strain injuries occur when local strains and strain rates exceed a critical threshold where axons are affected (Bain and Meaney, 2000; Gennarelli, Thibault, Tipperman, Tomei, Sergot, Brown, Maxwell, Graham, Adams, Irvine, 1989). They are most probable when the skull and brain are accelerated around the midline axis by a force vector that deforms the brain (Ommaya and Gennarelli, 1974). In landmark studies from the 1940's, Holbourn simulated the movement of the brain using gel models (1943, 1945) where the significance of angular acceleration was first suggested. These studies were supported by animal work that showed very similar movement patterns in nonhuman primates where a clear plastic window was used to view the movement of the brain upon impact (Pudenz and Shelden, 1946).

Diffusion tensor imaging (DTI) provides information about brain microstructure by quantifying isotropic (typical of gray matter) and anisotropic (typical of white matter) water diffusion. DTI measurements are based on the fact that the network of fibers within the brain is composed of a distinct microstructure that inherently constrains the flow of water molecules. Water diffuses primarily along the WM fibers, and is constrained in the direction perpendicular to them. Diffusion tensors numerically model this process via

ellipses whose long axis is aligned with the most likely direction of the fiber in a voxel. One of DTI's main utility in neurology is detecting the presence of axonal injury (Toga and Mazziotta, 2002).

Indeed, there is increasing evidence to suggest that TAI is present in traumatic brain injury (TBI) across the severity spectrum and that the extent of the damage is related to the severity of the injury as defined by initial Glasgow Coma Scale score (Huisman, Schwamm, Schaefer, Koroshetz, Shetty-Alva, Ozsunar, Wu, Sorensen, 2004). Likewise, Kraus et al. (2007) investigated TBI in adults across the severity spectrum using DTI and also found the degree of TAI to be related to the severity of the injury with severe TBI patients exhibiting the greatest extent of damage. The presence of axonal shearing in people with mild, moderate, and severe TBI alike suggests that the brains of concussed athletes will undergo similar changes, though perhaps to a lesser extent than is observed in more severe TBI.

Few studies have focused on TAI within the acute post-injury phase (days to weeks); however, recent work has shown that damage can be detected within the first week of injury (Miles, Grossman, Johnson, Babb, Diller, Inglese, 2008; Wilde, McCauley, Hunter, Bigler, Chu, Wang, Hanten, Troyanskaya, Yallampalli, Li, Chia, Levin, 2008). Several case studies using DTI on TBI patients have consistently noted reduced diffusion in the brain, especially around the corpus callosum in adults soon after injury (Huisman, Schwamm, Schaefer, Koroshetz, Shetty-Alva, Ozsunar, Wu, Sorensen, 2004; Lee, Choi, Chun, 2006). Furthermore, the observed axonal shearing in people with MTBI has

functional consequences. Lee et al. (2006) noted that brain areas affected by axonal injury corresponded to the types of neuropsychological dysfunctions that patients exhibited.

Diffusion alterations are also evident in the chronic post-injury phase (months to years). Several studies investigating MTBI using whole brain DTI analysis techniques in adult samples taken from emergency rooms have found diffuse WM differences in the corpus callosum (Wilde, McCauley, Hunter, Bigler, Chu, Wang, Hanten, Troyanskaya, Yallampalli, Li, Chia, Levin, 2008), frontotemporal (Chu, Wilde, Hunter, McCauley, Bigler, Troyanskaya, Yallampalli, Chia, Levin, 2010), and midbrain regions (Hartikainen, Waljas, Isoviita, Dastidar, Liimatainen, Solbakk, Ogawa, Soimakallio, Ylinen, Ohman, 2010) manifest as changes in fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and/or axial diffusion (AD). A study using tract based statistics to investigate MTBI in adults found that symptom free patients showed no differences in diffusion variables relative to controls, though symptomatic patients demonstrated overall higher mean diffusivity values than both controls and asymptomatic patients in the corpus callosum, the right anterior thalamic radiations and the superior longitudinal fasciculus, the inferior longitudinal fasciculus and the fronto-occipital fasciculus bilaterally (Messé et al, 2010). A study investigating concussed college athletes who had been symptomatic for at least 1 month found no changes in FA, but did find that concussed athletes showed significantly increased MD in the left hemisphere (specifically parts of the inferior/superior longitudinal and frontal-occipital fasciculi, the retrolenticular part of the

internal capsule, and posterior thalamic and acoustic radiations) using a WM skeleton where only the middle of tracts are analyzed (Cubon, Putukian, Boyer, Dettwiler, 2011).

The current study sought to further investigate the presence of TAI in concussed athletes in the acute (2-6 days post-injury) and chronic (6 months post-injury) phases using a whole brain voxel-based method. It is hypothesized that concussed athletes would demonstrate changes, either increases or decreases, in FA and MD as well as AD in concussed athletes relative to control athletes at both time points. We further hypothesized that the corpus callosum would demonstrate particular vulnerability as is consistent with the extant literature (Ducreux, Huynh, Fillard, Renoux, Petit-Lacour, Marsot-Dupuch, Lasjaunias, 2005; Wilde, McCauley, Hunter, Bigler, Chu, Wang, Hanten, Troyanskaya, Yallampalli, Li, Chia, Levin, 2008), but that the corticospinal tract (CST) would also show itself to be vulnerable to the effects of concussion as has been suggested using other techniques such as transcranial magnetic stimulation (De Beaumont, Lassonde, Leclerc, Theoret, 2007; De Beaumont, Theoret, Mongeon, Messier, Leclerc, Tremblay, Ellemberg, Lassonde, 2009) and magnetic resonance spectroscopy (Henry, Tremblay, Boulanger, Ellemberg, Lassonde, 2010).

Methods

Participants

All participants in this study were active male football players for university level intervarsity sports teams and were recruited with help from the team physician and physiotherapists. Athletes were excluded from participation if there was a history of alcohol and/or substance abuse; psychiatric illness; learning disability; neurological

disorders (seizure disorder, central nervous system neoplasm, or brain tumor); and TBI unrelated to contact sports. None of the athletes who participated in this study were taking psychotropic medications at the time of testing. Concussion severity, determined by the team physician, varied from Grade 1 (confusion for less than 15 minutes without amnesia or loss of consciousness) to Grade 3 (loss of consciousness, duration either brief [seconds] or prolonged [minutes]) according to the parameters set out by the American Academy of Neurology Quality Standards Subcommittee and Neurology (1997). The study was composed of one experimental group of 16 athletes who suffered a sports concussion, examined at two different time points and a control group (n=8) composed of athletes who had no history of head injury. The concussed (mean age of 22.08 (*SD*=1.72) years) and the control (mean age of 22.81 (*SD*= 1.53) years) groups were statistically equivalent across age (*p*=0.29). Similarly, concussed athletes had a mean of 15.8 (*SD*=2.16) years of education, while control athletes had a mean of 16.08 (*SD*=1.56) years of education (*p*=0.75). Additionally, at the time of each scan symptom scores were calculated using the post-concussion symptom scale (PCSS) where symptoms are rated on a likert scale from 0 (non-existent) to 6 (very severe). Symptoms on the PCSS include headache, feeling pressure in the head, sensitivity to light, sensitivity to noise, dizziness, vision changes, feeling slowed down, feeling foggy, changes in sleep, fatigue, drowsiness, memory difficulty, concentration difficulty, increased irritability, increased sadness, emotional lability, anxiety, nausea, vomiting, and numbness or tingling. The symptom scores were calculated by adding the likert scale ratings from each symptom as reported by each athlete.

All neuroimaging was performed at the Unité de Neuroimagerie Fonctionelle (UNF) of the Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal, using a Siemens TRIO 3-T whole-body MRI system (Siemens, Erlangen, Germany). This study was approved by the Research and Community Ethics Boards at the UNF and the Université de Montréal in compliance with the code of ethics as stated in the Declaration of Helsinki. All subjects gave informed consent following careful screening for MRI compatibility. None of the concussed athletes contacted for this study refused participation. Concussed athletes were scanned within the first 5 days of injury (mean= 81.92 hours, *SD*= 46.74 hours). The second scan took place six months after the initial scan for the concussed group (mean= 6.375, *SD*= .41) and 18 months after the initial scan for controls (N= 8, mean=18.24, *SD*= 10.29). Only athletes who were able to be scanned at both time points were included in the analyses. While the difference between follow-up scans is quite large the work of Westlye's and colleagues' (2010) study suggests that there are no detectable differences between participants of such a tight age range.

Imaging parameters were: diffusion weighting gradients applied in 64 directions with b values of 0 and 700 s/mm2 and four averages in each direction; repetition time of 12,800 ms, echo time of 101 ms, field of view of 256 x 256 mm2, matrix size of 128 x 128 with partial Fourier reconstructed to 6/8, slice thickness of 2 mm with 0.6 mm gaps, 75 slices. 3D T1-weighted images of corresponding subjects were also acquired with an inversion recovery rapid gradient echo sequence using 3T Trio Siemens MRI scanner. Acquisition parameters were as follows: TI/TR/TE= 1500/2500/3.83 msec; flip angle=15 degrees;

slice thickness = 0.9 mm, with an acquisition matrix of 256x256x256. Total scan time was 28 minutes (4 minutes localizers, 9 minutes MPRage, 15 minutes DTI).

Image Preprocessing and Registration

Extracerebral tissues were removed from the 3D structural MRIs using the iMagic Pro 2.0 (Neuronic S.A., 2007) software followed by manual editing. Edited scans were linearly registered to a high-resolution single-subject brain template image using FLIRT software (Jenkinson and Smith, 2001). A Minimal Deformation Target (MDT) was generated from the 26 subjects (using the method in Kochunov, Lancaster, Thompson, Woods, Mazziotta, Hardies, Fox, 2001) and each 3D structural image was warped to the MDT using a 3D fluid registration algorithm that allows large deformations while guaranteeing diffeomorphisms (Lepore, Brun, Chou, Chiang, Dutton, Hayashi, Luders, Lopez, Aizenstein, Toga, Becker, Thompson, 2008; Lepore, Brun, Chiang, Chou, Dutton, Hayashi, Lopez, Aizenstein, Toga, Becker, Thompson, 2006). Jacobian matrices, representing local volume expansions or contractions relative to the template image, were calculated from the deformation fields for each subject. From the diffusion weighted images, voxel-wise diffusion tensors were generated with MedINRIA (http://www.sop.inria.fr/asclepios/software/MedINRIA). Log-Euclidean tensor de-noising was used to eliminate singular, negative definite, or rank-deficient tensors.

Deformation fields from the nonlinear registration of T1-weighted images were applied to the diffusion tensor images to warp them to a common space. The Finite Strain (FS) method was applied to compute the tensor rotations to the affine transformation matrix *M* (Alexander, Pierpaoli, Basser, Gee, 2001). We then applied a preservation of principal

direction (PPD) algorithm on the higher-order transformation (Alexander, Pierpaoli, Basser, Gee, 2001; Zhang, Yushkevich, Alexander, Gee, 2006).

Voxelwise statistics

The most common univariate anisotropy measure, FA, is defined in terms of the eigenvalues, λ_i, $i = 1,2,3$, of the diffusion tensor as:

$$FA(D) = \frac{\sqrt{3}}{\sqrt{2}} \frac{\sqrt{(\lambda_1 - <\lambda>)^2 + (\lambda_2 - <\lambda>)^2 + (\lambda_3 - <\lambda>)^2}}{\sqrt{\lambda_1^{\,2} + \lambda_2^{\,2} + \lambda_3^{\,2}}},$$

with $<\lambda> = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$. It is widely accepted as an index of white matter integrity and is sensitive to the effects of aging, cognition, trauma, and neurodegenerative disease. MD is a rotationally invariant trace of the diffusion tensor and provides valuable information about the overall diffusion in a voxel or region where MD = $<\lambda> = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$. AD is simply λ_1, the eigenvalue that runs parallel to the axon.

We applied a voxelwise 2x2 repeated measures ANOVA using SPM8 to the derived scalar images (FA, AD, and MD) with a significance threshold of .01 divided by the number of derived scalar images computed for a final significance threshold of .003 in order to be conservative in our estimate of significant differences and to account for the use of multiple comparisons. Because each cluster is composed of several voxels, each with its own F-value, cluster results are reported as ≥ or ≤ .003 along with the maximum F-value in the cluster.

The mean FA values and standard deviation of each cluster shown in Figure 1 were recorded. Similarly, analogous areas from the opposite hemisphere were also included. In concussed athletes, a white matter structure with an FA value increased by more than 2.5 SDs above the mean for that region of interest in the control group was considered damaged. The value of 2.5 SDs was chosen to be consistent with the work of Niogi et al who performed a similar analysis (2008). Such an individualized investigation of the concussed athletes was included to account for the heterogeneity of the injury (see Figure 1 for example FA maps).

Insert Figure 1 about here

Results

Initially, three 2x2 repeated measure ANOVAs were conducted, one for each dependent measure of DTI used in the current study: FA, MD, and AD. All significant results represent voxel clusters with a p-value of .003 or less. Averaged F-values derived from each individual subject were then further calculated.

-Insert Figure 2 about here-

Prominent FA increases were detected in the corpus callosum, as well as throughout the cortical spinal tracts in the concussed group ($F(1, 46) \leq 18.86$, $p < .003$). Though not affected along the entire length, the corpus callosum showed augmented FA values at the genu and posteriorly in the body. Within the right hemisphere, underlying primary motor and sensory cortices continuing ventrally through the cortical spinal tract to the level of the thalamus demonstrated elevated FA values (Figure 2). Contrastingly, the left

hemisphere showed only sparse changes in FA in dorsal regions immediately underlying primary motor and sensory cortices where more ventral regions remained statistically non-significant. There was no main effect of time from the acute phase to the chronic phase ($F(1, 46) \leq 9.7$, $p > .003$), nor was there a significant group X time interaction ($F(1, 46) \leq 9.7$, $p > .003$).

Insert Figure 3 about here

AD showed a more complex pattern of results (Figure 3). There was a main effect of group at the dorsal end of the cortical spinal tract underlying primary motor cortex in the right hemisphere in the same areas showing higher FA values. Concussed athletes showed greater AD values than control athletes ($F(1, 46) \leq 23.77$, $p < .003$). Unlike FA, there were no significant AD differences in the corpus callosum nor were there areas within the left hemisphere that demonstrated significant differences between the two groups. There was no main effect of time and no significant group X time interaction, demonstrating the stability of both groups across time ($F(1, 46) \leq 9.7$, $p > .003$).

Insert Figure 4 about here

MD demonstrated significant differences along the CST and corpus callosum (Figure 4). Though there were no significant group X time interactions ($F(1, 46) \leq 13.23$, $p > .003$), there was a main effect of group seen in the body of the corpus callosum where MD values were lower in concussed athletes relative to their control counterparts ($F(1, 46) \geq 9.8$, $p > .003$). Group effects were also seen within ventral portions of the CST where again concussed athletes showed lower MD than controls ($F(1, 46) \geq 9.8$, $p > .003$).

Similar to what was seen with respect to the FA and AD data, there was no effect of time ($F(1, 46) \leq 9.7$, $p > .003$) which underscores the stability of the control and concussed groups not only across time but also across the diffusion parameters used in the current study.

Insert tables 1 and 2 about here

Results of the individual analyses show that while every concussed athlete showed increased FA in at least one area, some athletes were more affected than others according to the number of clusters affected. Also, more concussed athletes had elevated FA at the second cluster along the CST (nearest the corpus callosum) at both time points. Though nonsignificant at the group level, some athletes appear to also have elevated levels along the right CST. Such different findings at the individual level further underscore that while the biomechanical mechanisms do bear similarities between individuals, there are also differences that are lost in group-based analyses. One-way ANOVAs were conducted to investigate whether the number of regions showing alterations was related with either the reported symptom scores or the total number of concussions reported. The number of regions showing elevated FA levels was not related with the total symptom score ($F=1.072$, $p=.484$) However, the number of regions showing elevated FA values was significantly related with the number of concussions reported ($F=6.95$, $p=.009$) (figure 5). Further post-hoc analyses revealed that this relationship was only significant in those subjects who had a history of three concussions ($p=.005$) but not for those who reported one ($p=.804$) or two ($p=.804$) concussions. The individual analyses for MD did not reveal

any significant differences between the control group and the concussed athletes along the CST. However, within the corpus callosum concussed individuals did show elevated MD. Further, one-way ANOVAs between the total symptom reported scores ($F=1.072$, $p=.297$) and total number of concussions ($F=1.48$, $p=.264$) were not significant.

Include Figure 5 about here

Discussion

We observed significantly higher FA and AD values and lower MD values along the cortical spinal tracts and in the corpus callosum in concussed relative to nonconcussed athletes at both acute and chronic time points. Though there were some differences in the precise locations along the cortical spinal tracts, there was continuity in terms of where in the coronal plane the differences were observed. Specifically, the white matter underlying primary motor and primary sensory cortical areas exhibited the most damage. Similarly, the corpus callosum showed alterations in areas thought to be connected to premotor and motor areas (Hofer and Frahm, 2006). Our results are consistent with other studies that employed DTI in the investigation of MTBI (Chu, Wilde, Hunter, McCauley, Bigler, Troyanskaya, Yallampalli, Chia, Levin, 2010; Mayer, Ling, Mannell, Gasparovic, Phillips, Doezema, Reichard, Yeo, 2010; Wilde, McCauley, Hunter, Bigler, Chu, Wang, Hanten, Troyanskaya, Yallampalli, Li, Chia, Levin, 2008) in that the current study found anisotropic changes. Like previous studies, we found the corpus callosum to be affected by TAI; further the current study identified the cortical spinal tract as being particularly vulnerable.

The current study found consistently increased FA values in concussed athletes in the acute and chronic post-injury phases. Most studies report the inverse, citing disruption to the organizational structure of the tissue, axonal degeneration, and demyelination as pathological hallmarks to TBI (Arfanakis, Haughton, Carew, Rogers, Dempsey, Meyerand, 2002; Kumar, Gupta, Husain, Chaudhry, Srivastava, Saksena, Rathore, 2009; Miles, Grossman, Johnson, Babb, Diller, Inglese, 2008; Newcombe, Williams, Nortje, Bradley, Chatfield, Outtrim, Harding, Coles, Maiya, Gillard, Hutchinson, Pickard, Carpenter, Menon, 2008; Newcombe, Williams, Nortje, Bradley, Harding, Smielewski, Coles, Maiya, Gillard, Hutchinson, Pickard, Carpenter, Menon, 2007; Wilde, Ramos, Yallampalli, Bigler, McCauley, Chu, Wu, Hanten, Scheibel, Li, Vasquez, Hunter, Levin, 2010; Wozniak, Krach, Ward, Mueller, Muetzel, Schnoebelen, Kiragu, Lim, 2007; Wozniak and Lim, 2006). However, all of the aforementioned studies with two exceptions (Miles, Grossman, Johnson, Babb, Diller, Inglese, 2008; Wozniak, Krach, Ward, Mueller, Muetzel, Schnoebelen, Kiragu, Lim, 2007) included mixed TBI samples where the decrease in FA is thought to stem from Wallerian degeneration and subsequent massive cell loss (Arfanakis, Haughton, Carew, Rogers, Dempsey, Meyerand, 2002; Wilde, Ramos, Yallampalli, Bigler, McCauley, Chu, Wu, Hanten, Scheibel, Li, Vasquez, Hunter, Levin, 2010; Wozniak and Lim, 2006). The seemingly contrary increase in FA is thought instead to be reflective of subtle cytotoxic edema and localized inflammatory response due to compressed intracellular space along the cortical spinal tracts and corpus callosum and is more reflective of milder injuries. The subsequent restriction of diffusion then results in increased directionality of the fibers. These results are consistent with

other studies where MTBI was studied exclusively (Chu, Wilde, Hunter, McCauley, Bigler, Troyanskaya, Yallampalli, Chia, Levin, 2010; Mayer, Ling, Mannell, Gasparovic, Phillips, Doezema, Reichard, Yeo, 2010; Wilde, McCauley, Hunter, Bigler, Chu, Wang, Hanten, Troyanskaya, Yallampalli, Li, Chia, Levin, 2008).Within the chronic phase, we also report persistently elevated FA values which may still be characterized by volume loss and myelin degeneration (Bigler, 1999; Meythaler, Peduzzi, Eleftheriou, Novack, 2001). Such an interpretation may also seem counterintuitive as increasing the interstitial space between cells is thought to decrease anisotropy. However, a loss of crossing fibers could result in increased anisotropy (Pfefferbaum and Sullivan, 2003), potentially accounting for the increased FA seen in the chronic phase. Suffice it to say, the increased FA values observed in the acute phase and those observed in the chronic phase may have the same net result, but result from different processes (Hurley, McGowan, Arfanakis, Taber, 2004).

AD quantifies the diffusion along the axon parallel to the predominant fiber orientation. Increases in AD are thought to be reflective of damage to the axon itself, as opposed to radial diffusivity which is thought to measure damage to the myelin (Song, Sun, Ramsbottom, Chang, Russell, Cross, 2002). The acceleration and deceleration forces that characterize concussion cause defects to myelin lamellae and axolemmas, which results in axonal swelling with the release of large amounts of Ca^{2+} (Buki and Povlishock, 2006; Maxwell, Kansagra, Graham, Adams, Gennarelli, 1988), consistent with what is known about the metabolic cascade that follows a concussive blow (Giza and Hovda, 2001). The fact that AD is not reduced may in fact be an encouraging sign as a decrease is

thought to reflect a structural change to the axon (axotomy), whereas an increase may simply mean that while there is swelling, the axonal structure, though affected, is more or less intact (Newcombe, Williams, Nortje, Bradley, Harding, Smielewski, Coles, Maiya, Gillard, Hutchinson, Pickard, Carpenter, Menon, 2007). To that end, the AD and FA values may still normalize over the course of 2-13 months (Chappell, Ulug, Zhang, Heitger, Jordan, Zimmerman, Watts, 2006), well inside the 6-month timeframe employed in the current study.

MD is the average molecular diffusion and is thought to be affected by cellular size and integrity (Pierpaoli, Jezzard, Basser, Barnett, Di Chiro, 1996; Toga and Mazziotta, 2002). Overall, the literature points to cytotoxic edema and inflammation due to homeostatic membrane dysfunctions. There is also some evidence that damaged neurons may potentially repair their damaged membranes, rather than progressing to axotomy (Farkas, Lifshitz, Povlishock, 2006). Other studies investigating ischemic brain injury have speculated that decreased MD values in conjunction with elevated FA values reflect basic alterations in cerebral microstructure (Bhagat, Emery, Shuaib, Sher, Rizvi, Akhtar, Clare, Leatherdale, Beaulieu, 2006; Green, Pena, Price, Warburton, Pickard, Carpenter, Gillard, 2002). The metabolic changes observed in concussion (Giza and Hovda, 2001; Henry, Tremblay, Boulanger, Ellemberg, Lassonde, 2010) support the notion that both membrane dysfunctions and altered microstructure are driving the lowered MD values in the current study. This corresponds well with the current study's findings where apparent cellular edema normalizes in the chronic post-injury phase as cellular edema is thought to

be an acute phenomenon (Chu, Wilde, Hunter, McCauley, Bigler, Troyanskaya, Yallampalli, Chia, Levin, 2010; Wilde, McCauley, Hunter, Bigler, Chu, Wang, Hanten, Troyanskaya, Yallampalli, Li, Chia, Levin, 2008).

The results of the individual analyses support the group findings. The clusters along the cortical spinal tract were greater than 2.5 standard deviations above the mean of the control group in the majority of the concussed athletes with the exception of the dorsal-most cluster (left CST cluster 1). Perhaps most clinically relevant, the number of affected regions correlated significantly with the total number of concussions. Such a finding, though certainly preliminary further adds to the ever growing evidence regarding the deleterious effects of multiple concussions (Bruce and Echemendia, 2009; De Beaumont, Brisson, Lassonde, Jolicoeur, 2007; Gaetz, Goodman, Weinberg, 2000; Guskiewicz, McCrea, Marshall, Cantu, Randolph, Barr, Onate, Kelly, 2003; Theriault, De Beaumont, Tremblay, Lassonde, Jolicoeur, 2010).

Magnetic resonance spectroscopy (MRS) captures unique information about white matter pathology through the detection and quantitation of several brain metabolites *in vivo*. We, along with others, have previously reported metabolic alterations in primary motor cortex in the acute phase (Henry, Tremblay, Boulanger, Ellemberg, Lassonde, 2010; Vagnozzi, Signoretti, Tavazzi, Floris, Ludovici, Marziali, Tarascio, Amorini, Di Pietro, Delfini, Lazzarino, 2008) as well as in the chronic phase (Henry, Tremblay, Leclerc, Khiat, Boulanger, Ellemberg, Lassonde, submitted). The metabolic results support the DTI results with remarkable consistency as the MR regions of interest were set in the cortical

spinal tracts underlying motor cortex, precisely where the diffusion changes are manifesting FA and AD abnormalities. The acutely and chronically diminished concentrations of *N*-acetylasparte (NAA) (Henry, Tremblay, Leclerc, Khiat, Boulanger, Ellemberg, Lassonde, submitted) and increased phosphocreatine (Gasparovic, Yeo, Mannell, Ling, Elgie, Phillips, Doezema, Mayer, 2009) concentrations are related to disrupted consumption and production of ATP in the injured neuron to regain homeostatic equilibrium and repair (Giza and Hovda, 2001; Henry, Tremblay, Boulanger, Ellemberg, Lassonde, 2010; Vagnozzi, Signoretti, Tavazzi, Floris, Ludovici, Marziali, Tarascio, Amorini, Di Pietro, Delfini, Lazzarino, 2008). It is possible that the restorative metabolic process results in increased intracellular water retention (Mayer, Ling, Mannell, Gasparovic, Phillips, Doezema, Reichard, Yeo, 2010). Such a hypothesis is confirmed by looking at cellular mechanisms of osmotic regulation at different time points after injury. While ions are called upon to regulate osmotic pressure in the acute phase (Lang, Busch, Ritter, Volkl, Waldegger, Gulbins, Haussinger, 1998; Strange, 1992), other metabolites that do not alter the electrophysiological balance of the cell like myo-Inositol are called upon to regulate the osmotic pressure in the long term (Lang, Busch, Ritter, Volkl, Waldegger, Gulbins, Haussinger, 1998). Such a metabolic alteration would fit with the current study's DTI findings where MD is decreased due to cellular edema in the acute phase and through metabolic alteration in the chronic phase. That is to say, mechanisms of osmotic regulation after sustaining a concussion, though different between the acute and chronic phases may underlie the initial and continued alteration in MD.Transcranial magnetic stimulation (TMS) studies have demonstrated chronic

subclinical motor system dysfunctions specific to intracortical inhibition that was exacerbated in young athletes who had suffered more severe and/or multiple injuries (De Beaumont, Lassonde, Leclerc, Theoret, 2007). More alarmingly, similar anomalies were found in retired athletes, decades removed from their last concussion (De Beaumont, Theoret, Mongeon, Messier, Leclerc, Tremblay, Ellemberg, Lassonde, 2009). In another study, de Beaumont and colleagues found the same intracortical inhibition problems in concussed athletes in addition to neurophysiological and behavioral markers of subclinical motor dysfunction (De Beaumont, Mongeon, Tremblay, Messier, Prince, Leclerc, Lassonde, Theoret, accepted). The exact link between the current study's DTI findings and the motor system dysfunctions as documented using TMS is not clear but the fact that both point to the motor cortex and the underlying cortical spinal tract indicate that this brain area as being particularly vulnerable.

The current study is not without limitations. Because mTBI is such a diffuse and heterogeneous injury, a voxel based analysis (VBA) approach may lack the sensitivity to identify alterations because only those injured brain areas that are common to the experimental group will be detected. Likewise, from a clinical standpoint, it has not been established that VBA has enough statistical power for legitimate single-subject analysis. From a technical standpoint, VBA requires spatial normalization, which can introduce error if inaccurate leading to false positive results due to poor alignment (Shen, Szameitat, Sterr, 2007). Also, because VBA requires spatial smoothing to allow each subject's brain to fit into a common space, there is further loss of sensitivity leaving only changes in large tracts detectable. However, employing a VBA approach provides

certain advantages. For example, VBA is fully automated, data-driven, and ideal for group-based analyses. Further, VBA does not require a priori hypotheses about where changes in the brain might be occurring, and it can be used in an exploratory fashion whereby the whole brain can be examined which is important in mTBI owing to the heterogeneity of the injury.

Overall, the results of the current study suggest that there is WM damage after a sports concussion in the acute and chronic post-injury phases in the form of increased FA and AD values and decreased MD values primarily along the cortical spinal tracts and to a lesser extent in the corpus callosum. These results are consistent with other studies that have investigated WM changes after MTBI (Chu, Wilde, Hunter, McCauley, Bigler, Troyanskaya, Yallampalli, Chia, Levin, 2010; Mayer, Ling, Mannell, Gasparovic, Phillips, Doezema, Reichard, Yeo, 2010; Wilde, McCauley, Hunter, Bigler, Chu, Wang, Hanten, Troyanskaya, Yallampalli, Li, Chia, Levin, 2008), but are in the opposite direction of TBI studies showing decreased FA and increased MD (Arfanakis, Haughton, Carew, Rogers, Dempsey, Meyerand, 2002; Kumar, Gupta, Husain, Chaudhry, Srivastava, Saksena, Rathore, 2009; Miles, Grossman, Johnson, Babb, Diller, Inglese, 2008; Newcombe, Williams, Nortje, Bradley, Chatfield, Outtrim, Harding, Coles, Maiya, Gillard, Hutchinson, Pickard, Carpenter, Menon, 2008; Newcombe, Williams, Nortje, Bradley, Harding, Smielewski, Coles, Maiya, Gillard, Hutchinson, Pickard, Carpenter, Menon, 2007; Wilde, Ramos, Yallampalli, Bigler, McCauley, Chu, Wu, Hanten, Scheibel, Li, Vasquez, Hunter, Levin, 2010; Wozniak, Krach, Ward, Mueller, Muetzel, Schnoebelen, Kiragu, Lim, 2007; Wozniak and Lim, 2006), which may be due to the use

of more severely injured participants, different imaging techniques, and different time intervals in these studies. Regardless of the nature of the findings, the emerging pattern of findings demonstrates WM injury after MTBI, including sports concussions as demonstrated in the current study. The precise nature of the ensuing pathology must be further documented to better understand the time course of WM damage and the possibility of recovery after a concussion. Future research should also concentrate on delineating the nature of diffusion changes resulting from moderate and severe TBI versus more mild injuries as there appears to be different processes at work that are not explained by the degree of injury severity.

Conflict of Interest

The authors report no conflict of interest.

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Figure Legends

Figure 1: Example FA maps of one control athlete (1A) and one concussed athlete (1B). These maps show FA after linear and nonlinear registration to a common template. Examples of MD maps from a control athlete (1C) and a concussed athlete (1D) are also shown.

Figure 2: Differences in fractional anisotropy between control and concussed athletes. 2A: The mean FA value from significant clusters demonstrating increased FA values in the concussed group at each time point relative to control group at each time point. The ** denotes a significant group effect ($F(1, 46) \leq 18.86$, $p < .003$). 2B: Significant clusters demonstrating increase FA values in concussed athletes relative to controls moving posteriorly through the corpus callosum. 1C: Significant clusters demonstrating increased FA values in concussed athletes relative to controls moving posteriorly through the cortical spinal tract.

Figure 3: Differences in axial diffusivity between control and concussed athletes. 3A: The mean AD value from significant clusters demonstrating increased AD values in the concussed group at each time point relative to control group at each time point. The ** denotes a significant group effect ($F(1, 46) \leq 23.77$, $p < .003$). 3B: Significant clusters demonstrating increased AD values in concussed athletes relative to controls moving posteriorly through the cortical spinal tract.

Figure 4: Differences in mean diffusivity between control and concussed athletes. 4A: The mean MD value from significant clusters demonstrating decreased MD values in the concussed group at each time point relative to control group at each time point. The ** denotes a significant group effect ($F(1, 46) \geq 9.8$, $p > .003$). 4B: Significant clusters demonstrating decreased MD values in concussed athletes relative to controls in the corpus callosum (far left) moving posteriorly through the cortical spinal tract (middle and right).

Table 1: Concussed athletes compared to the control mean FA in brain regions showing significant differences at the group level and their opposite hemisphere homologues. The structure was noted as damaged if the FA was 2.5 SDs greater than the average FA of 8 healthy control subjects in either hemisphere. The total number of subjects showing FA below which a structure was considered to have a traumatic axonal injury lesion is listed in the final row. The final column shows the number of structures in which each subject showed altered FA values at either time point (T1= acute phase, T2= chronic phase). The last two columns depict the total number of symptoms reported at the time of the first scan and the number of total concussions sustained according to self-report respectively.

Table 2: Concussed athletes compared to the control MD mean in brain regions showing significant differences at the group level. No opposite hemisphere homologues were added as the regions are either midline (corpus callosum) or spanned into each hemisphere (right and left CST). The structure was noted as damaged if the FA was 2.5 SDs less than the average MD of 8 healthy control subjects in either hemisphere. The

total number of subjects showing MD above which a structure was considered to have a traumatic axonal injury lesion is listed in the final row. The final column shows the number of structures in which each subject showed altered MD values at either time point (T1= acute phase, T2= chronic phase). Only areas within the corpus callosum detected MD differences at the individual level.

Figure 1: Example FA and MD maps

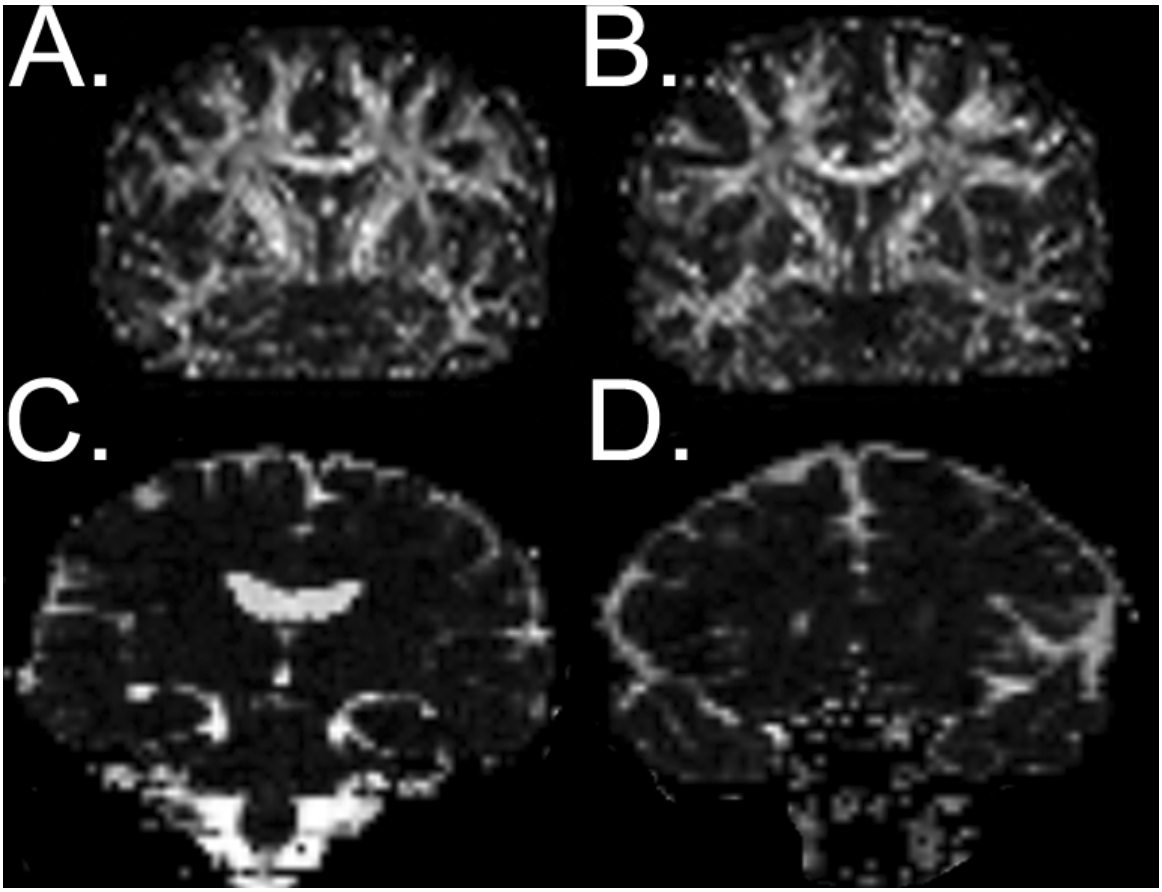


Figure 2: FA Alterations

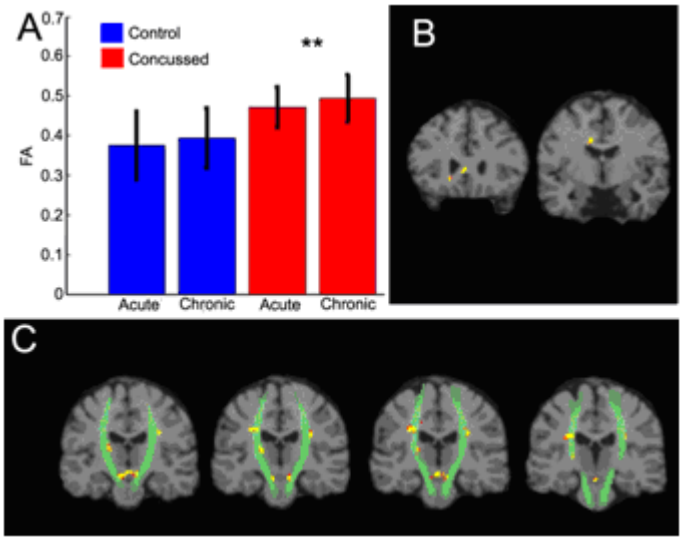


Figure 3: AD Alterations

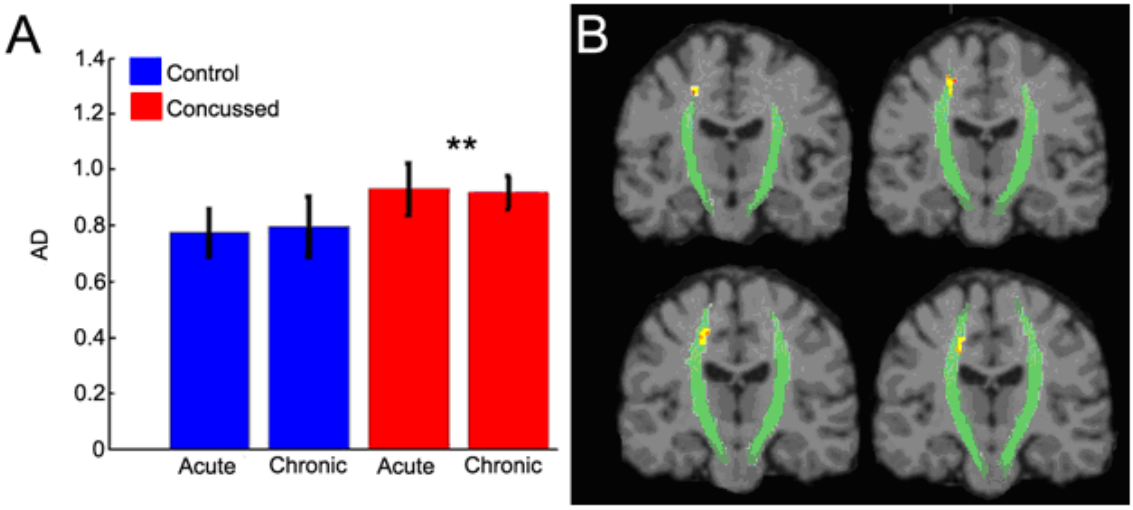


Figure 4: MD Alterations

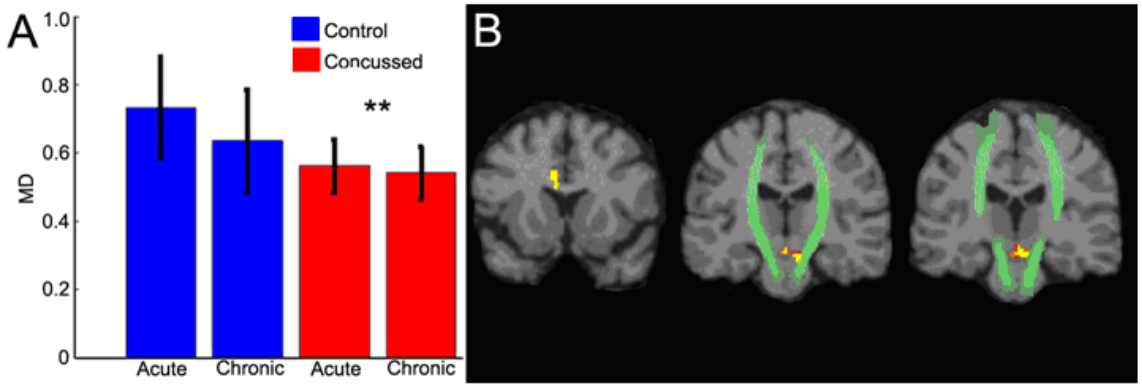


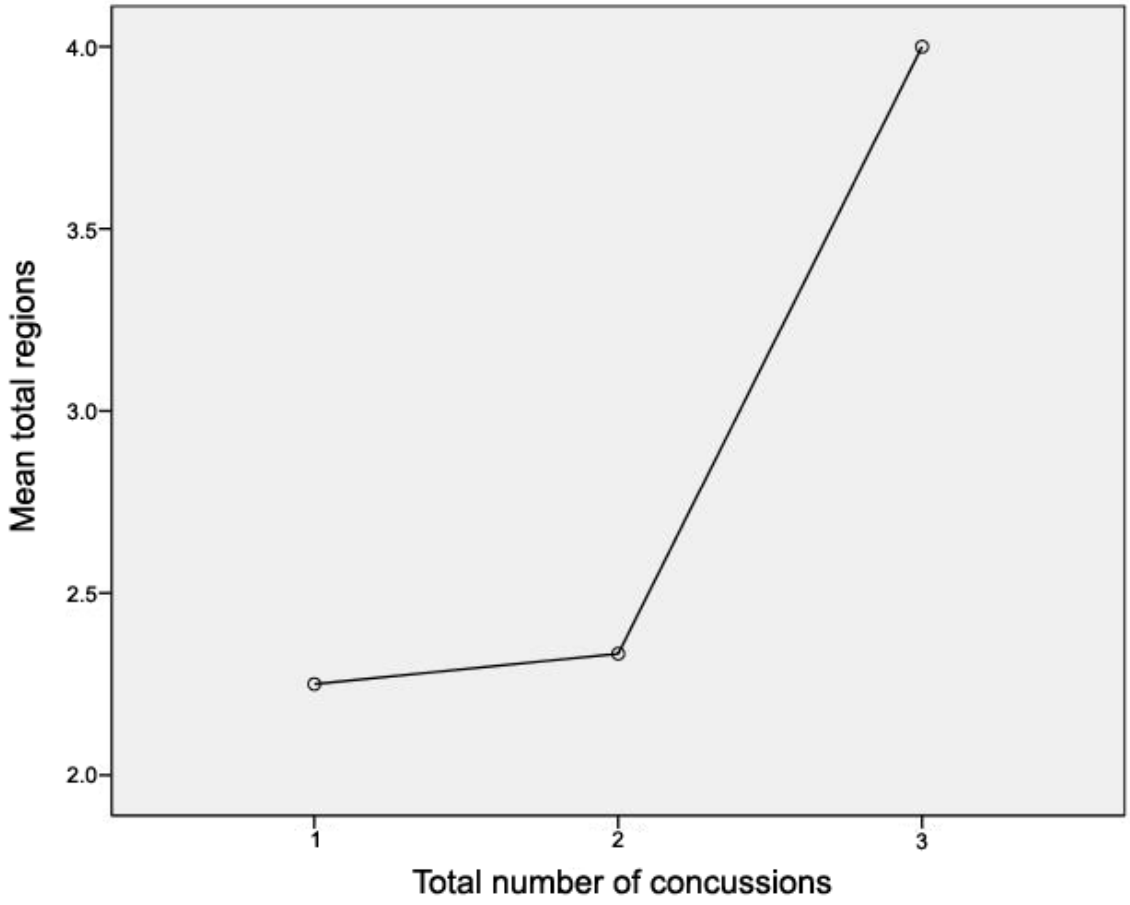
Table 1: Locations of traumatic axonal injury based on FA values in each concussed athlete

	Left CST Cluster 1		Right CST Cluster 1 mirror		Left CST Cluster 2		Right CST Cluster 2 mirror		Left CST Cluster 3		Right CST Cluster 3 mirror		Left CST Cluster 4				
Subject	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	**Total Regions**	**Total Symptoms**	**No. of concussions**
1	X				X										**2**	**41**	**2**
2					X	X			X				X	X	**3**	**7**	**1**
3					X	X			X	X	X				**3**	**8**	**2**
4					X	X			X				X	X	**3**	**13**	**1**
5			X						X		X				**3**	**17**	**2**
6						X					X				**2**	**13**	**1**
7					X	X							X	X	**2**	**13**	**1**
8					X	X							X		**2**	**7**	**1**
9					X	X							X		**2**	**8**	**2**
10	X												X		**2**	**7**	**2**
11					X	X			X	X	X		X		**4**	**0**	**3**
12									X		X		X		**3**	**2**	**1**
13	X				X	X			X	X			X	X	**4**	**1**	**3**
14					X	X									**1**	**5**	**1**
15						X							X		**2**	**0**	**2**
16									X				X		**2**	**9**	**1**
Total	**3**	**0**	**1**	**0**	**10**	**11**	**0**	**0**	**8**	**3**	**5**	**0**	**11**	**4**			

Table 2: Locations of traumatic axonal injury based on MD values in each concussed athlete

	Left CST Cluster		Corpus Callosum	
Subject	T1	T2	T1	T2
1				
2				
3				
4				
5			X	
6				
7				
8				X
9			X	X
10				X
11				X
12			X	
13				X
14				
15				X
16				X
Total			**3**	**7**

Figure 5: Altered regions and number of concussions



Article #2

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Neurometabolic changes in the acute phase following sports concussions correlate with symptom severity

Henry, Luke C.[1*], Tremblay, Sébastien[1*], Boulanger, Yvan[3], Ellemberg, Dave[1,2], and Lassonde, Maryse[1]

[1]Centre de Recherche en Neuropsychologie et Cognition, Department of Psychology, University of Montreal, Montréal, Québec, Canada
[2]Centre de Recherche en Neuropsychologie et Cognition, Department of Kinesiology, University of Montreal, Montréal, Québec, Canada
[3]Department of Radiology, University of Montreal and Hôpital Saint-Luc, Montréal, Québec, Canada
[*] These authors contributed equally to this work.

Abstract

Sports concussion is a major problem affecting thousands of people in North America every year. Despite negative neuroimaging findings many athletes display neurophysiological alterations and post-concussion symptoms such as headaches and sensitivity to light and noise. It is suspected that neurometabolic changes may underlie these changes. The present study thus investigated the effects of sports concussion on brain metabolism using ^{1}H-MR Spectroscopy by comparing a group of 12 non-concussed athletes with a group of 12 concussed athletes of the same age (mean: 22.5) and education (mean: 16 yrs). All athletes were scanned 1-6 days post-concussion in a 3T Siemens MRI as well as administered a symptom scale to evaluate post-concussion symptomatology. Participants also completed a neuropsychological test battery to assess verbal memory, visual memory, information processing speed, and reaction time where no group differences were detected relative to controls. Concussed athletes showed a higher level of symptoms than non-concussed athletes and they also showed a significant decrease in glutamate in primary motor cortex as well as significant decrease in N-acetyl aspartate in prefrontal and primary motor cortices. No changes were observed in the hippocampus. Furthermore, the metabolic changes in M1 correlated with self-reported symptom severity despite equivalent neuropsychological performance. These results confirm cortical neurometabolic changes in the acute postconcussion phase and demonstrate for the first time a correlation between subjective self-reported symptoms and objective physical changes which may be related to increased vulnerability in the concussed brain.

Keywords: MRI spectroscopy, sports concussion, symptomatology, metabolism

Introduction

Despite concussions being labeled an epidemic (Holm et al., 2005), with a conservatively estimated annual incidence of more than 300,000 in the United Sates alone (Thurman et al., 1998), and injury descriptions dating back more than 3000 years to ancient Egypt (Verjaal & van 'T Hooft, 1975), very little is understood about how a concussion changes the brain on either a structural or neurochemical level. There is no standard definition of concussion, but it is generally considered to be a closed head injury due to a direct blow to, or shaking of the head from an impulsive force resulting in a transient alteration in mental status and brain processes that may include loss of consciousness, memory dysfunction (amnesia), impairment of reflex activity, and/or disorientation (Binder, 1986; Echemendia, 2006; Quality Standards Subcommittee and Neurology, 1997). Typical injuries resolve spontaneously within 7-10 days, a timeframe understood to be the acute phase (Collins et al., 1999; Iverson, 2005; Lovell et al., 2004; McCrea et al., 2003), in correspondence with the neurometabolic cascade as demonstrated in rats (Giza & Hovda, 2001). The seriousness of concussions has long been dismissed in the sports world, but because of the forced retirement of several high-profile athletes who suffered multiple concussions, the gravity of the effects of sports concussions is finally coming to the forefront (Gaetz & Weinberg, 2000; McCrory et al., 2005).

For all their associated symptoms (headache, light sensitivity, sleep disturbance, etc.) (see McCrea et al., 2008 for a complete list of symptoms) sports concussions have yet to reveal any sort of consistent injury pattern detectable using neuroimaging (Johnston et al. 2001; McAllister et al., 2001 ; Mendez et al., 2005). CT and MRI have yielded limited

and inconsistent results. Of the few CT studies available, all conducted on boxers, the vast majority of scans came back negative for any sort of lesions or damage with only a few of the most severe cases showing cerebral atrophy Jordan, B.D., et al., 1992; Jordan, & Zimmerman, 1988; Ross et al., 1987). MRI data follows a similar pattern of results with very few lesions being detected despite its superior precision to CT (Jordan, B.D. and Zimmerman, R.D. (1990, Newton, M.R., et al. (1992).

Functional imaging techniques have faired somewhat better. Using fMRI, Chen and colleagues found different activation patterns on a working memory task for symptomatic concussed athletes compared to uninjured athletes; further, they found that atypical activation patterns are highly related to symptomatology months after the injury (2007, 2004, 2008). Lovell et al. (2007) and Jantzen et al. (2004) confirmed these findings in athletes imaged in the acute (within 7-10 days) post-concussion phase. PET and SPECT have yet to be used to study sports concussions specifically, but their use in mild traumatic brain injury (mTBI) has garnered mixed results with frontotemporal regions exhibiting the most consistent impairment in patients with persistent symptomatology (Abu-Judeh et al., 2000 ; Abu-Judeh et al., 1999; Bonne et al., 2003; Chen et al., 2003, Korn, et al., 2005; Umile et al., 2002; Varney et al., 2001).

The inconsistency of neuroimaging findings is in stark contrast to the electrophysiological alterations that have been detected in concussed athletes using event-related potentials (ERP) and transcranial magnetic stimulation (TMS) paradigms. In symptomatic and asymptomatic athletes alike, concussions produced auditory and visual

processing alterations well after the acute post-injury phase that may be reflective of brain injury (Dupuis et al., 2000; Gaetz & Weinberg, 2000; Gosselin et al., 2006). Gaetz and colleagues (2000) and De Beaumont and colleagues (2007a) respectively demonstrated the cumulative effects of concussions on latency and amplitude changes in the P300 wave in concussed athletes months after the last incident. Another study using TMS found altered intracortical inhibition in concussed athletes that was positively correlated to the number of concussions sustained, suggesting long-term and cumulative effects in the motor system (De Beaumont et al., 2007b).

How can the concussed brain demonstrate altered electrophysiology (ERP and TMS) and detectable, though variable changes in functional response (fMRI, PET, and SPECT) in the face of a seemingly uninjured and asymptomatic brain according to anatomical imaging (CT and MRI)? Almost all other examples of altered functions are accompanied by either a known lesion/abnormality or chronic pathological condition, including epilepsy (Goyal et al., 2008; Krendl et al, 2008; Mazerolle et al., 2007; Moran et al., 2008), Alzheimer's disease (Chapman et al., 2007; Heckemann et al., 2008; Levy-Cooperman et al., 2008; Olichney et al., 2008), and Parkinson's disease (Bokura et al., 2005; Hallevi et al., 2008; Li et al., 2005; Nandhagopal et al., 2008). It is likely that there may be micro-structural and/or metabolic changes associated with concussions that underlie the persistent alterations in electrophysiology. The subtlety of these changes has evaded traditional imaging techniques leaving concussion poorly understood while the underlying neural changes, be they anatomical or metabolic have yet to be defined, particularly in the acute phase.

Magnetic resonance spectroscopy (MRS) allows the detection and quantitation of several brain metabolites *in vivo* including, though not limited to creatine/phosphocreatine (Cr), a general energy marker, choline-containing compounds (Cho), a marker of neuronal damage and membrane turnover, *myo*-inositol (mI), a glial marker, glutamate (Glu), the principal excitatory neurotransmitter, and N-acetyl aspartate (NAA), a marker of neuronal integrity. MRS is thus able to detect neuronal damage and changes in the chemical make up of the brain. It is particularly useful in corroborating evidence of diffuse axonal injury because damage related changes to neurons are manifest not only in their physical structure, but also in their composition (Toga & Mazziotta, 2002). MRS has been used to look at the neurometabolic changes after moderate and severe TBI (Babikian et al., 2006; Garnett et al., 2000; Groswasser et al., 1987; Holshouser et al., 2006). Studies from outside of the sports literature have also demonstrated significantly depressed NAA concentrations in mTBI patients months to years after injury despite normal findings on conventional MRI (Cecil et al., 1998; Cohen et al., 2007; Garnett et al., 2000 ; Govindaraju et al., 2004; Kirov et al., 2007; Signoretti, S., et al., 2008).

There are two sports concussion studies using MRS in the acute phase, both of which report a decrease of NAA, though with some variability in their findings (Cimatti, 2006; Vagnozzi, et al., 2008). Cimatti's study looked at six different cases (four boxers) and compared baseline scan data to post-trauma scan data. The results of the study, although interesting, are variable where changes in NAA are reported in some but not all cases. The results are also rendered difficult to interpret by a lack of specific details regarding the participant demographics. Indeed, with only six participants in the study, and the fact

that previous head injuries are not accounted for it is difficult to draw conclusions about either the state of the post-concussion brain or the utility of MRS in assessing concussion.

Vagnozzi and colleagues (2008) included the initial post injury scan and two follow-up scans that allowed for the time-course of metabolic recovery to be charted in 14 concussed athletes of both genders ranging in age from 21 to 35, 10 of whom were injured in the course of a combat sport (boxing and kickboxing). Their main finding is centered on a decrease in the concentration of NAA in a single region of interest in each hemisphere of the frontal lobes. They report that concussions open a temporal window of metabolic imbalance that returns to normal levels within 30 days, but can be further exacerbated by subsequent injury within the restoration time frame.

The results of these two studies suggest that there may be structural damage in sports concussions despite the negative findings with more traditional imaging techniques (Cimatti, 2006; Vagnozzi et al., 2008). MRS is thus able to detect damage related changes to the physical structure and the composition of neurons following a concussion; however, with only two studies investigating sports concussion with MRS on a grand total of 20 subjects, we are still precluded from making any definitive statements as to the application of MRS in sports concussion either for diagnostic or research purposes. Furthermore, Cimatti's (2006) and Vagnozzi's and colleagues' (2008) studies still leave unanswered questions. The MRS protocol used in these two studies favors a less specific, but more robust spectrum in a single region of interest (Toga & Mazziotta, 2002). Also, the control group used by Vagnozzi et al is not composed of athletes and the study

population contains both men and women with a sizeable age range and no mention of education level. To overcome these limitations, the current study used an all-male university athlete study population with a very tight age gap in recognition of the fact that concussions exert differential effects on men and women (Farace & Alves, 2000; Granito, 2002; Roof et al., 1993). The current study also included more regions of interest to investigate the potential for differential effects in different brain regions and a more elaborate, though lest robust spectral sequence in order to investigate different metabolites.

The current study looked at the acute effects of sports concussion (within 7 days) on the chemical composition in primary motor cortex (M1), dorsolateral prefrontal cortex (DLPFC), and hippocampus, as well as neuropsychological test performance in collegiate athletes. The short post-injury time frame was chosen in light of the fact that the vast majority of athletes return to play within the first week, some within two days post-injury (Echemendia, 2006). The primary motor cortex was chosen as a region of interest based on the alterations in intracortical inhibition seen in concussed athletes (De Beaumont et al., 2007b). Likewise, the DLPFC was chosen based on both ERP studies (Dupuis et al., 2000; Gaetz & Weinberg, 2000; Gosselin et al., 2006) and fMRI studies (Chen et al., 2007; Chen et al., 2004; Chen et al., 2008; Jantzen et al., 2004; Lovell et al., 2007). The hippocampus was chosen because of its susceptibility to damage (Barr, 2005; Kandel et al., 2000) and its involvement in the generation of the P300 wave in ERP (Picton, 1992; Polich, 2004). It is hypothesized that all regions of interest will show diminished levels of NAA, increased levels of mI, and reduced levels of glutamate. We also speculate that

concussed athletes will perform more poorly on neuropsychological tests; furthermore, we expect significantly different neuropsychological test performance to correlate with neurochemical changes in the region of interest (ROI) thought to underlie the abilities substantiated by the test in question.

Methods

Participants

All 24 participants in this study were active players for university level intervarsity sports teams and were recruited with help from the team physician and physiotherapists. The following exclusion criteria were applied to select the athletes who took part in this study: a history of alcohol and/or substance abuse; psychiatric illness; learning disability; neurological disorders (seizure disorder, central nervous system neoplasm, or brain tumour); and TBI unrelated to contact sports. None of the athletes who participated in this study were taking psychotropic medications at the time of testing. The study was composed of two experimental groups. The control group consisted of 12 non-concussed athletes who had no history of neurological insult. The experimental group consisted of 12 athletes who suffered a sports concussion within the 6 days prior to being scanned (mean= 81.92 hours, *SD*= 46.74 hours). Concussion severity was assessed by the team physician. It varied from Grade 1 (confusion for less than 15 minutes without amnesia or loss of consciousness) to Grade 3 (loss of consciousness, duration either brief [seconds] or prolonged [minutes]) according to the parameters set out by the American Academy of Neurology Quality Standards Subcommittee and Neurology (1997). Additionally, all head injuries were classified as mild, with Glasgow Coma Scale scores ranging between

13 and 15 at the time of injury. Control and concussed athletes were equivalent with regard to age (t= .90, p= 0.19) and level of education (t= .18, p= 0.74; see Table 1). A standardized concussion-history form was administered to obtain detailed information about the number of previous concussions (if any), approximate date(s) of each concussion, descriptions of the accident(s), nature and duration of relevant postconcussion symptoms (confusion and/or disorientation, retrograde and/or anterograde amnesia, and loss of consciousness). Reported symptom scores were significantly different (p= 0.003), with the concussed athletes showing a relatively high level of symptom severity on a postconussion symptom scale from the NHL battery (Lovell & Collins, 1998) (see Table 1). Symptoms were further divided into equal subcategories by type including cognitive (feeling slow, feeling foggy, difficulty concentrating, memory problems), mood (sadness, irritability, anxiety, increased emotionality), sleep/arousal (fatigue, difficulty falling asleep, sleep changes, loss of energy), somatic (nausea/vomiting, balance, pins and needles, dizziness), and cranial (headache, pressure, sensitivity to light, sensitivity to noise) symptom clusters to identify commonly reported symptoms (Table2).

Insert Table 1 about here

Neuropsychological Testing

Neuropsychological tests from the National Hockey League neuropsychological testing program were used to assess multiple aspects of cognitive functioning (Echemendia & Julian, 2001). This battery includes classic neuropsychological tests (Lavoie et al., 2004; Lovell & Collins 1998) selected to evaluate retrograde and anterograde amnesia (orientation questions), attention processes (Pennsylvania State University cancellation task), visual scanning and mental flexibility (Color Trails A and B and Symbol Digit Modality Test), visual memory (Brief Test of Visual Memory and incidental memory recall of Symbol Digit Modality Test), verbal memory (Hopkins Verbal Learning Test), and speech fluency (verbal fluency, phonemic). Standardized and uniform neuropsychological testing procedures were carried out by a trained neuropsychology student for all participants.

Neuroimaging

MR Imaging

All studies were performed at the Unité de Neuroimagerie Fonctionelle (UNF) of the Centre de Recherche, Institut Universitaire de Gériatrie de Montréal, using a Siemens 3-T whole-body MRI system (Siemens, Erlangen, Germany). This study was approved by the Research and Community Ethics Boards at the UNF and the Université de Montréal and done in compliance with the code of ethics as stated in the Declaration of Helsinki. All subjects gave informed consent following careful screening for MRI compatibility.

Insert fig. 1 about here

MR Spectroscopy

We positioned our regions of interest using a rigorous anatomical localization protocol. Subjects were placed in the scanner and underwent a localizer scan prescribed parallel to the hippocampus (anterior commissure-posterior commissure). Voxels were then prescribed for the hippocampus (20mm x 40mm x 16mm), dorsolateral prefrontal cortex (DLPFC) (16mm x 16mm x 16mm), and primary motor cortex (M1) (16mm x 20mm x 32mm) of the left and right hemispheres (see Fig. 1). All voxels were placed on an AC-PC-oriented oblique axial slice corresponding to the region of interest first on a sagittal view, and then confirmed using coronal and axial views to ensure adequate distance from ventricles, fatty tissue, and bone. Single-voxel 1H-MRS spectroscopic measurements were performed using a PRESS (Point RESolved Spectroscopy) sequence (TE (echo time)=30 ms, TR (repetition time)=1500 ms, 256 acquisitions, 1200 Hz bandwidth, 1024 points, duration 6:30 minutes) on a 12-channel head coil. We opted for a moderate TR and shorter TE to balance between T1- and T2-associated signal losses and scan time to ensure that all six regions of interest could be captured within reasonable scan duration to ensure the behavioural quiescence of our participants in the scanner. Outer-volume suppression bands contiguous with the PRESS-selected volume were automatically placed in all three dimensions based on the voxel size of each ROI.

Metabolite quantitation was done using Linear Combination (LC) model (Provencher, 1993), an operator-independent spectral analysis software that estimates metabolite concentrations and their ratios relative to Cr using a set of basis reference spectra

acquired from individual metabolites on our MR instrument. Values were retained when uncertainties estimated as Cramer-Rao lower bounds (%SD) were less than 20%. The LCModel operator was blind to group membership. Figure 2 displays a typical LCModel output spectrum from a control participant. Values of NAA/Cr, Cho/Cr, mI/Cr and Glu/Cr were analyzed.

Insert Fig 2 about here

Statistics

Statistical analyses were done using SPSS (PC version 15.0). Coefficients of variance (CV) were calculated for each metabolite. All subsequent statistical analyses were performed only for metabolite ratios that had overall CV values < 20 % (i.e., NAA/Cr, Glu/Cr and mI/Cr). The values from the left and right hemispheres were averaged for all three regions of interest in the current study. The rationale for averaging the regions of interest across hemispheres is twofold: First, the literature suggests no lateralization effects in the regions used in the current study (Zimmerman et al., 2008; King et al., 2008; Szentkuti et al., 2004; Geurts et al., 2004). Second, it is not known if the effects of a concussion are greater at the site of impact or whether any resulting changes are distributed diffusely, regardless of impact site. As such, because it impossible to determine the exact location of impact without the use of helmets equipped with sensors, it was decided to combine the spectra from both hemispheres within each region of

interest. This was confirmed in our control group across regions of interest and metabolite ratios ((hippocampi: Glu/Cr $t(20) = 1.06$, $p = 0.15$, mI/Cr $t(22) = -.59$, $p = 0.72$, NAA/Cr $t(22) = .86$, p= 0.20) (DLPFC: Glu/Cr $t(22) = -.18$, $p=0.57$, mI/Cr $t(22) = 0.0$, $p = 0.50$, NAA/Cr $t(22) = -1.45$, $p = 0.92$)(M1 Glu/Cr $t(22) = -2.18$, $p = .98$, mI $t(22) = .05$, $p = .48$, NAA $t(22) = .26$, $p = .40$)). Though the Glu/Cr and NAA/Cr levels are near significance in the hippocampi, this is not considered to be suggestive of a lateralization effect, but rather reflective of three control participants who are driving the nonsignificant differences. It should be noted that one participant was excluded for the Glu/Cr ratio in the hippocampus as he had an abnormally high value on the left side (> 3.29 SD). The different metabolites in any given voxel are unrelated in principle and are not correlated (Braun et al. 2002). As such, the metabolite ratios of the two groups were compared using Student's independent t-tests.

Student's t-tests were carried out for each neuropsychology test. Also, in order to look at the neuropsychological performance of the control athletes and the concussed athletes, all test scores were converted in to Z-scores to derive an overall neuropsychology battery composite score. Pearson correlations were performed on significant neurometabolic findings and significant clinical findings with a Bonferroni correction for multiple comparisons. The effect sizes of all inter-group comparisons are reported as Cohen's *d*.

Results

Neuropsychological Testing

Performance was equivalent between control and concussed athletes across all neuropsychological tests used to assess the various cognitive domains (Table 3). Due in

part to a lack of statistical power, no between group differences reached significance across all neuropsychological tests used to assess the various cognitive domains (Table 3).

Include Table 3 about here

Magnetic Resonance Spectroscopy

Overall spectroscopy values across groups were quite stable with very little variance owing in large part to the relative quantification technique. There were no significant differences between control and concussed subjects in the hippocampi. Glu/Cr was statistically equivalent ($t(22) = .55$ $p = .59$) as were mI/Cr ($t(22) = -1.176$ $p = .25$) and NAA/Cr ratios ($t(22) = .45$ $p = .66$) (Fig. 3A). Results from the DLPFC (Fig. 3B) revealed a mixed pattern of changes. While there were no differences in Glu/Cr ($t(22) = .40$ $p = .70$) and mI/Cr ($t(22) = .28$, $p = .78$), there was a significant difference in NAA/Cr ($t(22) = 2.36$ $p = .03$, $d=.99$) suggesting at least a partial metabolic disruption within the acute phase. M1 revealed an even stronger pattern of metabolic alterations (Fig. 3C). Glu/Cr levels were lowered significantly ($t(22) = 3.47$ $p = .004$, $d=.83$). mI/Cr levels remained unaffected in M1 ($t(22) = .18$ $p = 0.86$), but similar to the change seen in DLPFC, NAA/Cr ratios were significantly affected with concussed athletes showing reduced levels ($t(22) = 2.912$ $p = 0.008$, $d=1.16$) (Fig. 3C).

Include Fig. 3 about here

Correlations between significant metabolite changes and symptom severity were carried out as symptom severity was the only clinical variable distinguishing concussed and non-concussed athletes. Correlations between NAA/Cr and self-reported symptom scores were not significant in DLPFC (r= -0.132, p= 0.559). However, NAA/Cr in M1 was significantly negatively correlated with self-reported symptom scores (r= -0.554, p= 0.008) (Fig. 4A). Similarly, Glu/Cr in M1 also showed a significant negative correlation with symptom severity (r= -0.637, p= 0.001) (Fig. 4B). Two athletes were excluded from the correlation analysis, one control and one concussed. The control athlete was excluded for an unusually high symptom score that was related to somatic pain and cognitive stress related to an impending knee surgery. The concussed athlete was also excluded for an abnormally high symptom score that had him more than three standard deviations from the group norm.

Further *post-hoc* correlations were performed on neurometabolites and symptom clusters including cognitive (feeling slow, feeling foggy, difficulty concentrating, and memory problems), mood (anxiety, sadness, irritability, and increased emotionality), sleep/arousal (fatigue, difficulty falling asleep, sleep changes, loss of energy), somatic (nausea/vomiting, balance, pins and needles, dizziness), and cranial (headache, pressure in the head, sensitivity to light, sensitivity to noise) categories (see Table 2) using a Bonferroni correction for multiple comparisons. The *post-hoc* symptom-neurometabolite correlations in M1 did not reveal significant correlations for NAA/Cr and cognitive symptoms (r= -0.3, p= 0.18), mood symptoms (r= -0.24, p= 0.28), sleep/arousal symptoms (r= -0.36, p= 0.10), or somatic symptoms (r= -0.43, p= 0.05). The correlation

between NAA/Cr and cranial symptoms in M1 was equal to the cut-off value after multiple comparisons (r= -0.53, p= 0.01). The same pattern of results was found for Glu/Cr and cognitive symptoms (r= -0.491, p= 0.02), mood symptoms (r= -0.179, p= 0.43), sleep/arousal symptoms (r= -0.047, p= 0.84), and somatic symptoms (r= -0.37, p= 0.09), with only the Glu/Cr-cranial symptoms correlation in M1 being significant after correcting for multiple comparisons (r= -0.86, p= 0.000).

Insert Fig. 4A and B about here

Discussion

The current study investigated neuropsychological and neurometabolic differences between 12 non-concussed athletes and 12 concussed athletes of similar age and education. Neuropsychological testing did not reveal any significant differences between control and concussed participants. Despite normal neuropsychological test results and anatomical MRI, concussed athletes reported post-concussion symptoms and had altered neurometabolic profiles. The neurometabolic alterations, as measured using ^{1}H-MR spectroscopy were not seen in all ROI. There were no changes detected in the hippocampus within the acute post-injury time frame. However, there were significant alterations in cortical areas. Depressed levels of NAA were seen in DLPFC and M1. Furthermore, glutamate levels were depressed in M1, though a similar depression was not seen in DLPFC. These results are important in light of the typical recovery window (7-10 days) and return-to-play protocols commonly used by athletic teams for sports concussion. The current study demonstrates that even though an athlete may perform at

typical neuropsychological test levels, this does not mean that the brain has returned to its pre-injury state. The MRS results of the current study correlate with the symptoms reported by the concussed athletes. Vagnozzi et al. (2008) concluded that the closure of metabolic brain imbalance (as measured by MRS) did not correlate with the closure of clinical symptoms. By contrast, the current study reports a correlation between the MRS findings and the self-reported symptoms.

Indeed, our results did not reveal any significant between-groups differences in neuropsychological test performance. The absence of differences is common to many sports concussion studies (De Beaumont et al., 2006), especially using paper-pencil testing (Broglio et al. 2007; Grindel et al., 2001), a testament to the elusiveness of the pathology as well as to the insensitivity of the majority of available test batteries. Another reason why we may not have found neuropsychological deficits is the mild nature of the injuries in concert with the education levels of the athletes (Grindel, S.H., Lovell, M.R., Collins, M.W., 2001). This is in sharp contrast with the subjective report of post-concussive symptoms, which were found to correlate with metabolite changes in cortical areas. Without doubt, the major problem with respect to the current study and the neuropsychological test results is the sample sizes. Indeed, those studies where neuropsychological differences are found generally have sample sizes three or four times larger than that used in the current study (Echemendia & Julien, 2001; Gosselin et al., 2006; Collins et al., 2003; Lau et al., 2009). With a more sensitive battery and a larger sample size, neuropsychological differences are more likely to emerge in a pattern consistent with what is reported in studies where neuropsychological testing is the focus

(Echemendia & Julien, 2001; Gosselin et al., 2006; Collins et al., 2003; Lau et al., 2009). While the small sample size means that our neuropsychological test results should be taken with a grain of salt, it also underscores the strength of the effect of the neuroimaging data.

Given the susceptibility of the hippocampus to damage due to its histological properties (Barr, M. 2005; Kandel et al., 2000) and its role as a P300 wave modulator (Fushimi, M. et al., 2005), we expected to see changes in the hippocampus; however, from a biomechanical standpoint it is not surprising that we did not find any changes in this ROI in the acute phase. The anatomical location of the hippocampus is further removed from any initial impact delivered to the head at the time of injury relative to the other two ROI. By virtue of its histology and location, pathological changes reach the hippocampus at a different rate than the cortex (Elkin & Morrison, 2007). That is to say, the brain is not uniform and different regions respond differently to the same mechanical forces. This is corroborated by the nature and extent of hippocampal injury in moderate to severe TBI. According to recent animal research using the fluid percussion method, pathological changes in the hippocampus correlate with the severity of the injury (Golarai et al., 2001; Hellmich et al., 2005) where injury to the hippocampus increases with the force of the impact. It is therefore less likely for damage to be detectable in the hippocampus with less severe injuries. The hippocampus is still capable of being injured from a mTBI, but the relative mildness of a sports concussion makes it less likely than an injury to neocortex. However, it must also be noted that other studies (Yoshino et al., 1991, 1992) have found metabolic changes in the hippocampus subsequent to fluid percussion injury.

Yoshino and colleagues (1991) found that cells in areas not mechanically damaged had dramatically altered functioning in terms of their glucose metabolism that was found bilaterally, though more pronounced ipsilateral to the injury. Of note, these changes were more pronounced in the neocortex and the hippocampus. Yoshino et al. (1992) further discovered that these metabolic changes were directly related to glutamatergic projections within the hippocampus. We did not find a similar depression of glutamatergic metabolism within the hippocampus. This may be due to differences between sports concussion and the fluid percussion model. It may also be due to biomechanical differences related to head-cervical anatomy between bipedal and quadruped animals, or further still may be due to limitations inherent to the imaging technique used in the current study. The nonsignificant increase in mI may be indicative of gliosis, a common feature of neural scarring, a process that can take years to appear in TBI patients (Diaz-Arrastia, R., et al. 2000; Golarai et al., 2001; Swartz, B.E., et al., 2006).

Changes to DLPFC function are perhaps the best documented in the sports concussion literature with changes reported in neuropsychology (Broshek & Freeman, 2005; Gosselin et al., 2006), electrophysiology (De Beaumont et al., 2007a; Gosselin et. al, 2006), and functionality (Chen et al., 2004; Lovell et al., 2007). Consistent with the changes seen in DLPFC and the existing literature (Cimatti, 2006; Vagnozzi et al., 2008) our results showed a decrease in NAA/Cr in M1. The decrease in NAA levels in M1, as in DLPFC, indicates a change in the metabolism of neurons. Understanding the biosynthesis of NAA further supports the notion that the cost of NAA is a decrease in ATP production (see Moffett et al. 2007, for a complete review). When the brain is in

homeostatic balance this cost is not high and proper levels of NAA are maintained. However, when the brain is injured, in this case by a concussive blow, that metabolic balance is shifted. Concurrent with the initial spike and subsequent decrease in cerebral blood flow (Nilsson & Nordstrom, 1977a; Yuan et al., 1988), concussive injuries also cause a hyperexcited brain state via a mechanically elicited neuronal discharge resulting in excitatory activity (Nilsson & Nordstrom, 1977b; Zimmerman & Putnam, 1947) that is not restricted to mechanoreceptors (Hu & Lewin, 2006; Morris, 1990; Sachs, 1991). This hyperexcited state puts a high metabolic demand on restoring the resting state, a key aspect of which is the production of ATP resulting in a decrease of NAA. Vagnozzi et al. (2008) indirectly support this by charting the recovery of NAA levels which return to typical levels at 30 days post-injury.

Though limited to M1, we are the first to report a decrease in Glu/Cr in sports concussion. The Glu/Cr decrease is corroborated by the prolongation of the cortical silent period (CSP) in M1 reported by de Beaumont and colleagues (2007b). The CSP is a pause in an otherwise continuous series of electrophysiological events. This suggests that the receptors of intracortical inhibitory interneurons of the motor system may be particularly vulnerable to the effects of sports concussions. Though diminished Glu/Cr levels do not speak to changes in intracortical inhibition, they do further suggest that M1 as a ROI is affected by concussive blows despite normal neuropsychological performance. The augmentation of the inhibitory action speculated to underlie the prolongation of the CSP may be further bolstered by the diminished levels of glutamate, chiefly responsible for excitatory activity in the brain.

It is not clearly understood why Glu/Cr levels are so drastically affected in M1 while they remain statistically equivalent in DLPFC. Histological differences between M1 and DLPFC may account for some of the difference. Betz cells are giant pyramidal cells found only in layer V of M1 (Barr, 2005) and are thought to be excitatory. That is to say, while both M1 and DLPFC possess layer V pyramidal cells, only M1 is equipped with the giant Betz cells adapted to facilitate excitatory transmission to descending pathways. It is possible that the pushing of the top of the brain against the cranial vault that occurs during rotational forces in a concussion (Bayly, et al., 2005) may affect the biosynthesis of glutamine in astrocytes or its hydrolyzation into glutamate in excitatory neurons. The downstream affect may be manifest in diminished levels of glutamate as seen in M1. In turn, the absence of Betz cells in DLPFC may be involved in its exemption from glutamatergic alterations.

The breadth of the metabolic changes in M1 (decreases in NAA/Cr and Glu/Cr) within the concussed athletes might be explained by the biomechanics of head injury (Bayly et al., 2005; Denny-Brown & Russell, 1940; Holbourn, 1945). The respective impacts of rotational and linear forces in producing a concussion (Bayly et al., 2005; Denny-Brown & Russell, 1940; Greenwald, 2008; Holbourn, 1945; Mihalik, et al., 2007) suggest that M1 is consistently vulnerable to the white matter injury of shear strain. The differential effects on Glu/Cr in M1 versus DLPFC are further corroborated by changes detected using diffusion tensor imaging where patients who had suffered a mTBI demonstrated reduced fractional anisotropy in the corticospinal tract indicating diffuse axonal injury where no such injury pattern was found in frontal regions (Kraus et al., 2007).

Interestingly, the *post-hoc* correlations by symptom type (cognitive, sleep/arousal, mood, somatic, and cranial) revealed a significant correlation of both NAA/Cr and Glu/Cr with cranial symptoms including headache, pressure in the head, sensitivity to light, and sensitivity to noise. Within this symptom cluster, feeling pressure in the head, sensitivity to light, and sensitivity to noise can all be considered symptoms of headache (IHS, 1997). Headaches themselves are common as a post-traumatic symptom (Gibbs, 1994; Matthews, 1972; Williams & Nukada, 1994a, b). Indeed, posttraumatic cranial symptoms may be related to the pathophysiology of concussion (Alves et al., 1986) such as diffuse axonal injury or excitotoxic amino acid release and subsequent neural injury (Giza & Hovda, 2001). Furthermore, altered cerebral haemodynamics and slowed cerebral circulation have been described in the post-concussion syndrome (Gilkey, et al., 1997) and are likely due to vasomotor instability (Alves et al., 1986) resulting in a decrease in regional cerebral blood flow (Gilkey et al., 1997).

Significant correlations between changes in NAA/Cr and symptoms as well as Glu/Cr and symptoms in M1 are clinically important. Indeed, the diagnostic and prognostic importance of postconcussion symptoms underscores the need to establish a less subjective metric to measure recovery without the confounds of either lying about symptom resolution to return before it is safe to do so on one extreme to embellishing symptoms for litigious or compensatory reasons on the other extreme. Neurometabolic changes in M1 may provide such a means. The results of the current study demonstrate a link between self-reported symptoms, even in the relatively minor injury of sports concussion, and neurometabolic alterations. Though these correlations do not prove

causation between metabolic depression and experienced symptoms, they do lend credibility to the relevance of self-reported symptoms. It is important to note that the neurometabolic changes reported in the current study and the subsequent correlations with reported post concussion symptoms was found in a small sample size. However, the effect sizes are robust and indicate a consistent metabolic alteration that is coherent with the accepted pathophysiology of concussion (Giza & Hovda, 2001). Changes in neurometabolic balance also open the door for questions about the use of pharmaceuticals in the management and perhaps even prevention or attenuation of concussions. Changes in neurometabolism represent the first step in characterizing the physical consequences of suffering a blow to the head, a positive step in understanding the pathophysiology of concussion.

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Figure Legends

Figure 1: Regions of interest (ROI) for MRS data acquisition depicted in the sagittal, coronal, and axial planes in the hippocampus (top; 20 mm x 40 mm x 16 mm), dorsolateral prefrontal cortex (DLPFC) (middle; 16 mm x 16 mm x 16 mm), and primary motor cortex (M1) (bottom; 16 mm x 20 mm x 32 mm). Spectra were recorded in both the left and right hemispheres.

Figure 2: Proton spectrum of a control subject in M1 showing the peaks corresponding to the metabolites of interest, creatine (Cr), *myo*-inositol (mI), glutamate (Glu), and *N*-acetylaspartate (NAA). Concentrations are derived from the area under the peaks.

Figure 3: Bar graphs of the mean Glu/Cr, mI/Cr and NAA/Cr metabolite ratios for control (black bars, n = 12) and concussed (gray bars, n = 12) athletes in A, hippocampi, B, dorsolateral prefrontal cortex (DLPFC) and C, primary motor cortex (M1). Values are the mean of 24 voxel spectra (12 left hemisphere, 12 right hemisphere) per group. Standard errors of the means are represented by vertical bars. An asterisk indicates a statistically significant difference relative to controls (p= 0.03 for NAA/Cr in B; p = 0.005 for Glu/Cr in C; p = 0.01 for NAA/Cr in C).

Figure 4: Correlations between NAA/Cr and cranial symptom cluster scores in M1 (A.) and Glu/Cr. and cranial symptom cluster scores in M1 (B.). These correlations were statistically significant after a Bonferroni correction for multiple comparisons (r=-.53, p= .01 and r=-.86, p= .000 respectively).

Table 1: Demographic Data

Group	Age (years)	Education (years)	# of Concussions	Symptom score
Control	23 ± 0.71	16.1 ± 0.70	0	3.33 ± 2.71
Concussed	22.1 ± 0.77	15.8 ± 0.97	1.67 ± 0.4	13.96 ± 4.22

Table 2: Symptom Cluster Results

Symptom Cluster	**Group**	**Mean (± SEM)**	***t*-value**	***Cohen's d***	***p***
Cognition	Control	.64 ± .41	-2.91	1.24	.011
	Concussed	2.41 ± .81			
Mood	Control	.36 ± .30	-1.67	.74	.12
	Concussed	1.27 ± .75			
Sleep/Arousal	Control	1.18 ± .91	-2.77	1.18	.012
	Concussed	3.4 ± .77			
Somatic	Control	0.00 ± 0	-2.78	1.19	.019
	Concussed	1.09 ± .58			
Cranial	Control	.09 ± .13	-3.37	1.44	.007
	Concussed	3.32 ± 1.42			

Table 3: Neuropsychology Results

Tests	**Condition**	**Group**	**Mean ± SEM**	***t*-value**	***Cohen's d***	***p***
Hopkins	Immediate recall	Control	26.9 ± 1.7	.025	.28	0.51
		Concussed	27.82 ± 1.07			
Hopkins	Delayed Recall	Control	87.82% ± 7.37	-.26	.24	0.40
		Concussed	82.2 ± 5.78			
SDMT	Total Correct	Control	61.45 ± 2.83	.39	.04	0.65
		Concussed	63.82 ± 5.35			
BVMT-R	% Retention	Control	99% ± 1.17	-1.15	.74	0.13
		Concussed	95% ± 3.12			
Verbal Fluency	Total Correct	Control	34.00 ± 2.85	.50	.17	0.69
		Concussed	35.83 ± 4.42			
PSU	Total Correct	Control	43.11 ± 3.26	-.56	.27	0.29
		Concussed	46.5 ± 2.22			
Color Trails A	Completion Time	Control	25.27 ± 2.37	.59	.01	0.72
		Concussed	24.09 ± 3.72			
Color Trails B	Completion Time	Control	58 ± 4.96	1.32	.04	0.90
		Concussed	57.09 ± 7.98			
Composite Z-Score	Neuropsychology Battery	Control	.132 ± .559	-.21	.09	.84
		Concussed	.191 ± .755			

Figure 1: Regions of Interest

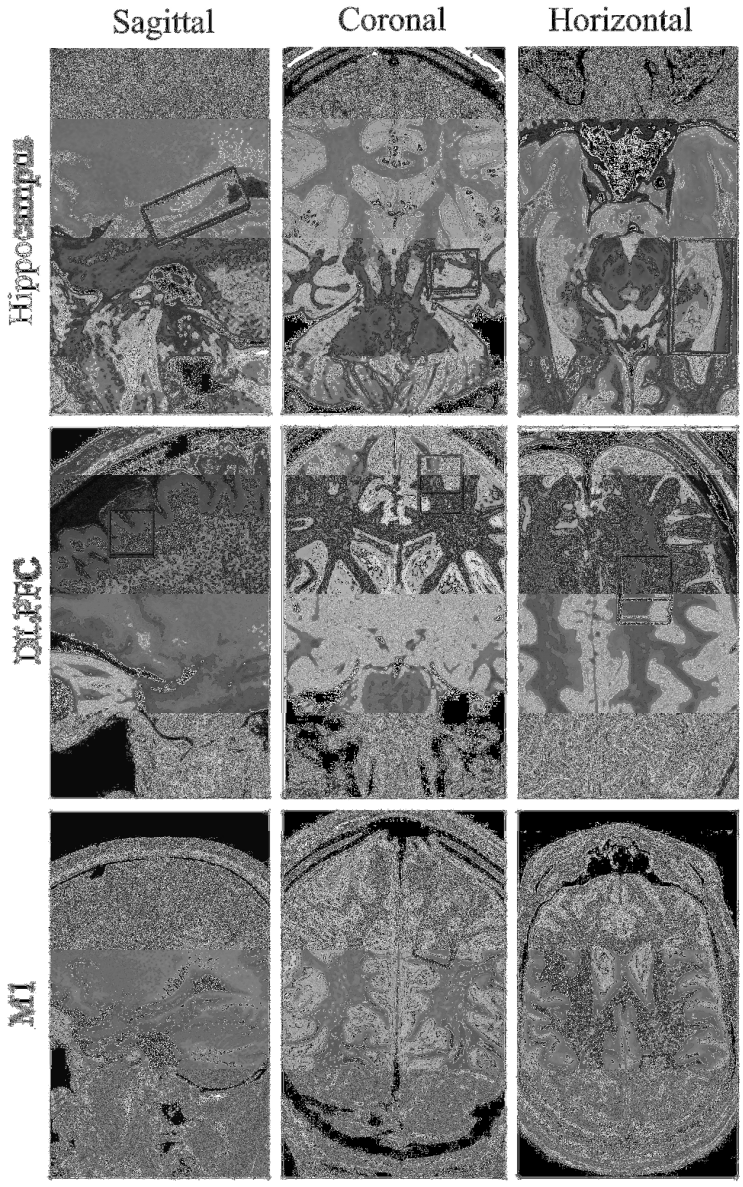


Figure 2: Typical spectrum from M1

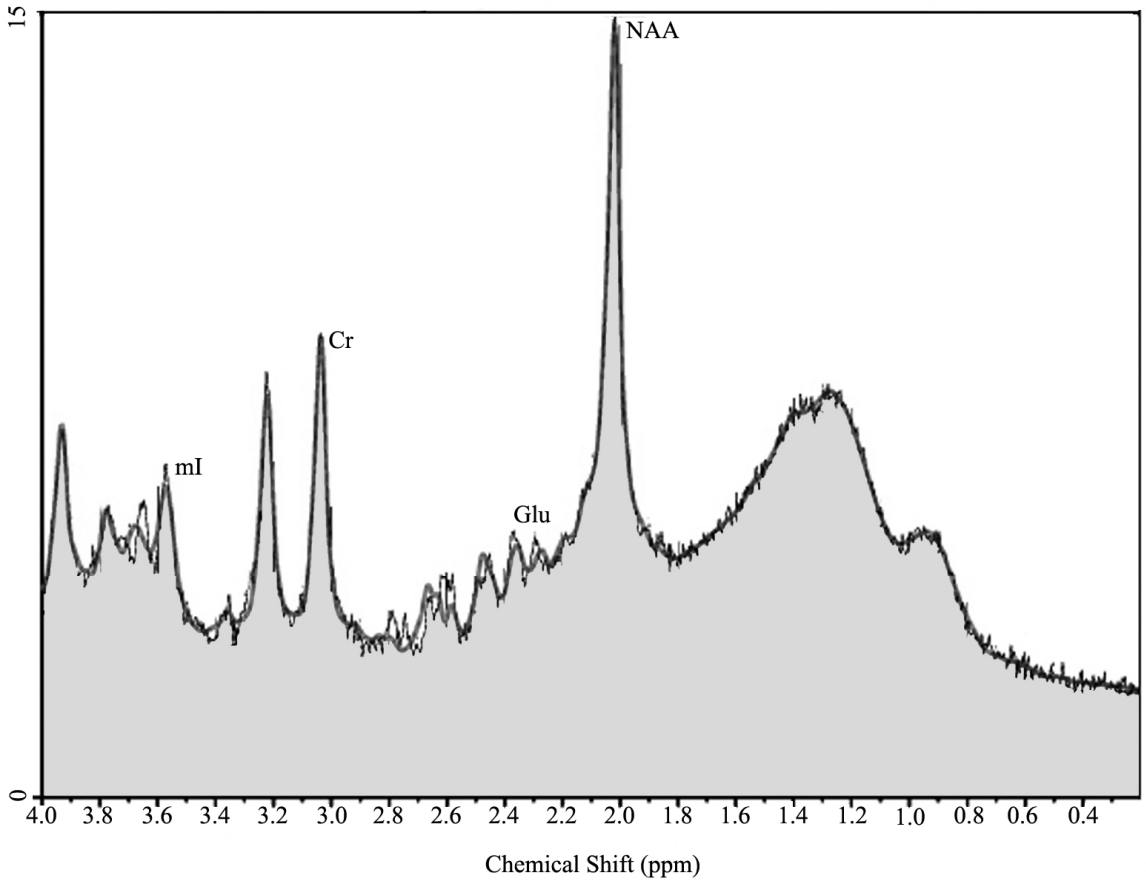


Figure 3: Averaged metabolite ratios in each of the 3 regions of interest

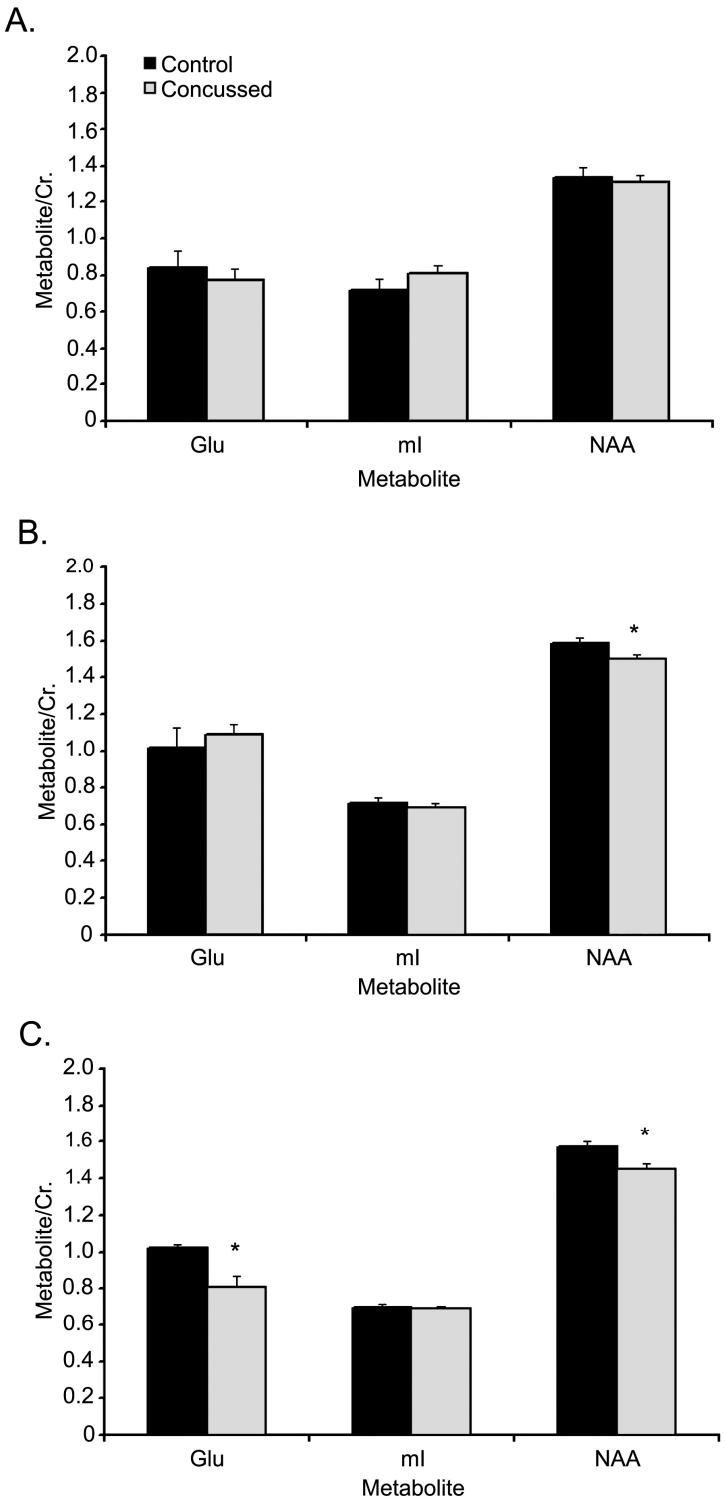
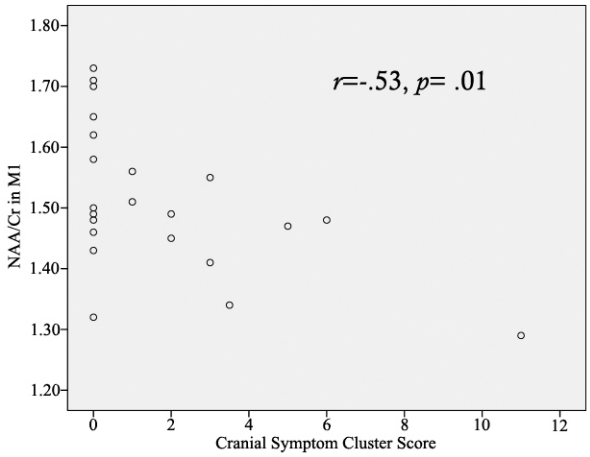


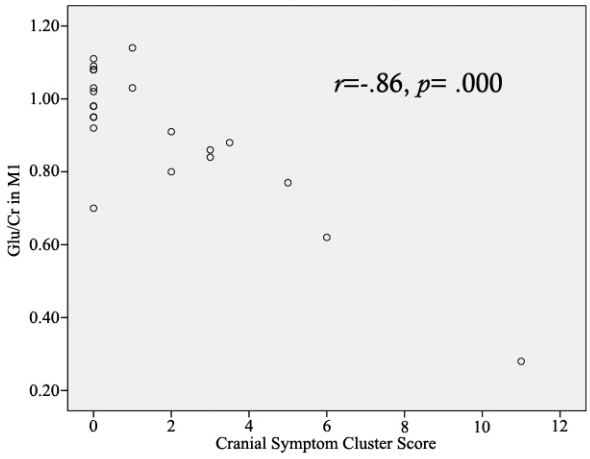
Figure 4: Cranial Symptom Cluster Correlations

Figure 4

A.


r=-.53, p= .01
NAA/Cr in M1
1.80
1.70
1.60
1.50
1.40
1.30
1.20
0
2
4
6
8
10
12
Cranial Symptom Cluster Score

B.


r=-.86, p= .000
Glu/Cr in M1
1.20
1.00
0.80
0.60
0.40
0.20
0
2
4
6
8
10
12
Cranial Symptom Cluster Score

Article #3

Article accepted:

Henry LC, Tremblay S, Leclerc S, Khiat A, Boulanger Y, Ellemberg D and Lassonde M. (accepted). Metabolic changes in concussed American football players during the acute and chronic post-injury phases. BMC Neurology.

Metabolic changes in concussed American football players during the acute and chronic post-injury phases

Luke C. Henry[1], Sébastien Tremblay[1], Suzanne Leclerc[2], Abdesselam Khiat[3], Yvan Boulanger[3], Dave Ellemberg[1,2], Maryse Lassonde[1]

[1]Centre de Recherche en Neuropsychologie et Cognition, Department of Psychology, University of Montreal, Montréal, Québec, Canada

[2]Centre de Recherche en Neuropsychologie et Cognition, Department of Kinesiology, University of Montreal, Montréal, Québec, Canada

[3]Department of Radiology, University of Montreal and Hôpital Saint-Luc, Montréal, Québec, Canada

Abstract

Background: Despite negative neuroimaging findings many athletes display neurophysiological alterations and post-concussion symptoms that may be attributable to neurometabolic alterations. **Methods:** The present study investigated the effects of sports concussion on brain metabolism using ^{1}H-MR Spectroscopy by comparing a group of 10 non-concussed athletes with a group of 10 concussed athletes of the same age (mean: 22.5 years) and education (mean: 16 years) within both the acute and chronic post-injury phases. All athletes were scanned 1-6 days post-concussion and again 6-months later in a 3T Siemens MRI. **Results:** Concussed athletes demonstrated neurometabolic impairment in prefrontal and motor (M1) cortices in the acute phase where NAA:Cr levels remained depressed relative to controls. There was some recovery observed in the chronic phase where Glu:Cr levels returned to those of control athletes; however, there was a pathological increase of m-I:Cr levels in M1 that was only present in the chronic phase. **Conclusions:** These results confirm cortical neurometabolic changes in the acute post-concussion phase as well as recovery and continued metabolic abnormalities in the chronic phase. The results indicate that complex pathophysiological processes differ depending on the post-injury phase and the neurometabolite in question.

Keywords: MRI spectroscopy, sports concussion, recovery, metabolism

Background

The perception of sports concussions has undergone a gradual overhaul throughout the past decade where an injury that was once considered to be inconsequential has come to be understood within the neuropsychological and medical communities to be an injury with quantifiable changes to the brain that are both transient [1-3] and persistent [4-7]. According to the current literature, transient changes are by far more abundant as most of these occur within the acute phase where athletes exhibit neurocognitive changes [8-10] in addition to neurophysiological alterations [11-15]. Persistent changes have also been documented [4-7, 16-19], though some doubt their clinical legitimacy, citing litigation and other secondary gains as confounds [20-23].

There is a disproportionate amount of research focusing on the acute post-injury phase owing largely to the fact that this is where the most overt effects of a sports concussion can be detected. The acute post-injury phase has no strict cut-off but is generally understood to be within three months though 80-90% of patients exhibit full recovery within the first 10 days [24, 25]. Thus the chronic phase is understood to be anywhere from three months and outward post-injury in accordance with the DSM-IV-TR definition of Post-concussion Syndrome [26].

Indeed, most of the quantifiable changes associated with sports concussion are either subclinical or recovered in the acute phase [25, 27]. Typically, post-concussive symptoms all but disappear within 2-3 weeks of concussion [24, 28, 29] with only a small percentage of cases exhibiting post-concussion effects passed the acute phase [7, 30-32].

Neuropsychological findings are similar, with typical neurocognitive recovery taking place on the order of days to weeks, well within the 3-month window [1, 10, 15, 33-35] though it can take longer in some cases, particularly if the injury is not properly managed [13, 36, 37].

Brain imaging studies conducted across the post-concussion timeline reveal variable results contingent upon the imaging technique used [38 for review, see 39]. Morphological changes are difficult to characterize using technologies like CT [40, 41] and MRI [41, 42] regardless of proximity to injury. Even in emergency room cases where patients are scanned within 72 hours of injury, MRI did not reveal any consistent pattern of injury across patients with injuries categorized as mild [43]. However, more recent studies have found subtle changes in the white matter of mild traumatic brain injury in non sports-related patients in the acute phase [44] and well after the acute phase [45] that are otherwise undetectable with conventional imaging techniques. Given their lack of precision in detecting signs of injury in concussion, the utility of CT and MRI in characterizing sports concussion is quite limited except in the most severe cases where symptomatology is either abnormally prolonged or severe [46].

Other imaging changes have yielded strong patterns of results in the acute (fMRI) and chronic post injury phases (transcranial magnetic stimulation-TMS, event related potentials-ERPs). Functional changes have been characterized, sharing an apparent link to symptomatology [2, 3, 5, 47, 48]. Of note, all of these studies were conducted within the acute phase when athletes were still expressing elevated symptoms. That is to say,

functional alterations are linked to symptomatology and these changes dissipate concurrently with self-reported symptoms and neurocognitive recovery. Because of the strong effect of functional recovery shown there are no fMRI studies investigating sports concussion in the chronic phase. Electrophysiological techniques such as TMS [4, 16, 17] and ERP paradigms [18, 49-51] have demonstrated alterations at various time points post injury with some remarkably enduring effects well past the acute phase, even decades after the last injury.

With the paucity of data implicating any morphological changes and the lion's share of studies reporting functional changes, sports concussions are largely understood to be a functional injury [52]. This has been demonstrated more directly by three studies that used 1H-magnetic resonance spectroscopy (MRS) to characterize the post-concussion metabolic spectra [53-55]. Consistent to these studies is a diminished level of *N*-acetylaspartate (NAA) which is thought to be indicative of reversible neuronal and/or mitochondrial dysfunction [56]. In accordance with the decrease in energy (ATP) production [57], NAA levels fall in the acute post injury phase [53, 54], but can recover in injuries that do not involve substantial permanent tissue destruction [56]. Indeed, Vagnozzi and colleagues [54] demonstrated metabolic recovery of NAA levels after 30 days in singly concussed athletes. All of this strongly suggests that there is metabolic recovery after a sports concussion, at least as it concerns NAA, but can the same be said of all neurometabolites?

Previous research has suggested that monitoring NAA levels is sufficient to conclude “full cerebral metabolic recovery” [54] after injury. Such a statement is based on two assumptions. The first is that NAA is the only neurometabolite to be affected after a concussion. Our previous work [53] demonstrated a decrease in glutamate levels in primary motor cortex in the acute post injury stage. This decrease in glutamate is consistent with the hypoglycolic state that is known to occur after closed head injury [57, 58]. Indeed, while there is an immediate post-impact spike in glutamate and glycolysis, the co-occuring drop in cerebral blood flow leads to an extended energy crisis owing to the lack of available calcium resulting in impaired oxidative metabolism [57]. This drop in glutamate has been shown to correlate with injury severity in humans, and has been shown to persist for 2-4 weeks [58]. Other studies have demonstrated metabolic alterations in choline following mTBI [59-62]. Given that metabolites other than NAA have been shown to be affected due to concussion, neurometabolic recovery cannot be presumed based on NAA recovery alone. The second assumption presumes that even if other neurometabolites are affected by a concussion, all neurometabolites recover at the same rate. The current study aims to investigate this notion by comparing spectra obtained within one week (2-5 days) post concussion versus spectra obtained six months after the injury in the same athletes. Two regions of interest were employed. The first region of interest, the dorsal-lateral prefrontal cortex, was chosen based on both electrophysiological [49, 51, 63] and fMRI studies [2, 3, 5, 47, 48] that implicate this region in the effects of sports concussion. The second region, primary motor cortex, was chosen as a region of interest based on the alterations in intracortical motor inhibition

seen in concussed athletes [4, 17]. Each region of interest was imaged in the left and right hemispheres for a total of four spectra per subject. Within each region of interest, three neurometabolites will be analyzed using relative quantification methods: NAA, glutamate, and myo-Inositol. N-acetylaspartate is present at exceptionally high concentrations in the brain second only to glutamate, and is the largest peak in spectra of healthy brain tissue [56] and is thought to be a key contributor in the formation of myelin lipid formation as well as an osmoregulator [64]. Glutamate, a member of the family of biogenic amines, is the most frequently occurring neurotransmitter in the central nervous system [65, 66]. The role of glutamate is different depending on the type of receptor that it binds with: at ionotropic receptors glutamate is excitatory, whereas at metabotropic receptors it is modulatory [65]. However, glutamate has a role beyond that of neurotransmitter. It is also an important neurometabolite used in the production of nitrogen which is key in the synthesis of proteins and nucleic acid which is essential to the production of other important molecules including GABA. Myo-Inositol is synthesized throughout the body, but mostly within the brain where its concentration is also the greatest [67]. It serves as a precursor molecule for inositol lipid synthesis, but also as an osmolyte. In line with previous research we hypothesized a recovery of NAA [54] and glutamate levels [58, 68] in all regions of interest. However, we further hypothesized that myo-inositol levels would be increased in the chronic phase relative to the acute phase in concussed athletes in all regions of interest based on what has been demonstrated in other TBI studies [69, 70].

Methods

Participants

All participants in this study are the same participants used in Article #2. The athletes were active players for university level intervarsity sports teams and were recruited with help from the team physician and physiotherapists. The following exclusion criteria were applied to select the athletes who took part in this study: a history of alcohol and/or substance abuse; psychiatric illness; learning disability; neurological disorders (seizure disorder, central nervous system neoplasm, or brain tumour); and TBI unrelated to contact sports. None of the athletes who participated in this study was taking psychotropic medications at the time of testing. The study was composed of one experimental group examined at two different time points and a control group (N=10) composed of athletes who had no history of head injury, sports related or otherwise, also scanned at two different time points. The experimental group consisted of 10 athletes who suffered a sports concussion. They were first scanned within the 5 days of injury (mean= 81.92 hours, *SD*= 46.74 hours). The second scan took place six months after the initial scan for the concussed group (mean= 6.375, *SD*= .41) and 18 months after the initial scan for controls (N= 10, mean=18.24, *SD*= 10.29). Though there is a vast difference in time between scans, the follow-ups for control athletes should not demonstrate significant change, neurometabolites remaining relatively stable in the uninjured brain [71]. Symptoms scores were taken from both groups at each time point using the Post Concussion Symptom Scale (PCSS).

All head injuries were classified as mild, with Glasgow Coma Scale scores ranging between 13 and 15 at the time of injury. A standardized concussion-history form was administered to obtain detailed information about the number of previous concussions (if any), approximate date(s) of each concussion, descriptions of the injury(ies), nature and duration of relevant postconcussion symptoms (confusion and/or disorientation, retrograde and/or anterograde amnesia, and loss of consciousness). Concussed athletes followed the return to play protocol that was adopted after the second consensus statement on concussions in sport [72] and re-endorsed after the third international consensus statement on sports concussion [52]. In brief, the athletes followed a graded return to play beginning with complete rest, followed by light physical activity. From there, athletes progressed to sport-specific exercise and then non-contact drills before returning to game play. As is standard for return to play, athletes only advanced to the next stage of physical activity if they remained symptom free at the previous one.

Neuroimaging

MR Imaging

All studies were performed at the Unité de Neuroimagerie Fonctionelle (UNF) of the Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal, using a Siemens 3-T whole-body MRI system (Siemens, Erlangen, Germany). This study was approved by the Research and Community Ethics Boards at the UNF and the Université de Montréal and done in compliance with the code of ethics as stated in the Declaration

of Helsinki. All subjects gave informed consent following careful screening for MRI compatibility.

MR Spectroscopy

We positioned our regions of interest using a rigorous anatomical localization protocol. Subjects were placed in the scanner and underwent a localizer scan prescribed parallel to the hippocampus (anterior commissure-posterior commissure). Voxels were then prescribed for the dorsolateral prefrontal cortex (DLPFC) (16mm x 16mm x 16mm), and primary motor cortex (M1) (16mm x 20mm x 32mm) of the left and right hemispheres (see Fig. 1). All voxels were placed on an AC-PC-oriented oblique axial slice corresponding to the region of interest first on a sagittal view, and then confirmed using coronal and axial views to ensure adequate distance from ventricles, fatty tissue, and bone. Single-voxel 1H-MRS spectroscopic measurements were performed using a PRESS (Point RESolved Spectroscopy) sequence (TE (echo time)= 30 ms, TR (repetition time)= 1500 ms, 256 acquisitions, 1200 Hz bandwidth, 1024 points, duration 6:30 minutes) on a 12-channel head coil. To ensure that all four regions of interest could be captured within reasonable scan duration and to ensure the behavioural quiescence of our participants in the scanner we opted for a moderate TR and shorter TE to balance between T1- and T2-associated signal losses and scan time. Outer-volume suppression bands contiguous with the PRESS-selected volume were automatically placed in all three dimensions based on the voxel size of each ROI.

Include Fig 1 about here

Linear Combination (LC) model (Provencher, 1993), an operator-independent spectral analysis software that estimates metabolite concentrations and their ratios relative to creatine/phosphocreatine (Cr) using a set of basis reference spectra acquired from individual metabolites on our MR instrument was used for metabolite quantitation. NAA/Cr, Glu/Cr and mI/Cr were only analyzed if the estimated uncertainties calculated as Cramer-Rao lower bounds (%SD) were less than 20%. The LCModel operator was blind to group membership.

Statistics

Statistical analyses were done using SPSS (PC version 16.0). Coefficients of variance (CV) were calculated for each metabolite for metabolite ratios that had overall CV values < 20 % (i.e., NAA/Cr, Glu/Cr and mI/Cr). The values from the left and right hemispheres were averaged for both regions of interest in the current study. The rationale for averaging the regions of interest across hemispheres is twofold: First, the literature suggests no lateralization effects in the regions used in the current study (Zimmerman et al., 2008; King et al., 2008; Szentkuti et al., 2004; Geurts et al., 2004). Second, it is not known if the effects of a concussion are greater at the site of impact or whether any resulting changes are distributed diffusely, regardless of impact site. Because we could not otherwise be certain as to which side of the brain received the impact in the concussed athletes, we opted to combine the spectra from both hemispheres within each region of interest. The different metabolites in any given voxel are unrelated in principle and are not correlated (Braun et al. 2002). As such, the metabolite ratios of the two groups were compared using 2-way group X time repeated measures ANOVA for each

metabolite in each ROI. Tests of the simple effects were carried out on metabolites in regions that differed between concussed and control athletes. Also, tests of the simple effects were also carried out on metabolites in ROIs that had been found to differ significantly between control and concussed athletes in our previous work carried out in the acute phase only [53].

Include Fig. 2 about here

Results

Total symptom scores from the PCSS revealed a significant interaction ($F(1,18) = 23.23$; $p = .000$), where there was a significant effect of time ($F(1,18) = 24.73$; $p = .000$), and a main effect of group ($F(1,18) = 5.94$; $p = .025$), where concussed athletes were significantly more symptomatic in the acute post-injury phase ($p = .003$), but were statistically equivalent to controls in the chronic post-injury phase ($p = .43$) (results not shown).

Analyses of Glu:Cr in the DLPFC revealed no significant interaction ($F(1,18) = 0.11$; $p = .75$), no main effect of Time ($F(1,18) = 1.23$; $p = .28$) and no main effect of Group ($F(1,18) = 2.28$; $p = .15$) (Figure 2A). Similar results were found for m-I:Cr where there was no interaction ($F(1,18) = 0.33$; $p = .58$), no main effect of Time ($F(1,18) = 0.02$; $p = .96$), and no main effect of Group ($F(1,18) = 0.72$; $p = .41$) (Figure 2B). Though there was no significant interaction of NAA:Cr in DLPFC ($F(1,18) = 0.69$; $p = .42$) and no effect of Time ($F(1,18) = 0.24$; $p = .63$), there was a main effect of Group ($F(1,18) =$

6.87; p= .017). As seen in Figure 2C, the concussed group differed from the control group at both time points.

Include Fig. 3 about here

Within M1, Glu:Cr concentrations (Figure 3A) showed a significant time by group interaction (F (1,18) = 9.21; p= .007). There was also a significant main effect of Time (F (1,16) = 6.07; p= .024); while there was not a significant main effect of Group, there was a trend (F (1,18) = 3.48; p= .08). A simple effect analysis revealed a significant difference between control and concussed athletes in the acute phase (p= .009) but not in the chronic phase (p=.64). Further within group simple effects comparisons revealed no significant differences between time points for the control group (F (1,9) = 0.004; p= .95) while the concussed group did show a significant difference over time (F (1,9) = 11.01; p= .009). By contrast, m-I:Cr concentrations showed an interaction trend toward significance (F (1,18) = 2.84; p= .1), a trend in main effect of Time (F (1,18) = 2.79; p= .10) and no significant differences between Groups (F (1,18) = 2.75; p= .115). Despite the nonsignificance, as the group by time interaction showed p-values with a trend toward significance, further simple effects analyses were conducted (see Fig. 3B). Analyses of the between-group effects revealed no significant differences in the acute phase (p=.93) but a significant difference in the chronic phase (p=.037). Within group differences between the acute and chronic phases did not reveal any significant differences in the control subjects (F (1,9) = 0.013; p= .91) but did reveal significant changes across time in the concussed subjects (F (1,9) = 5.23; p= .048). A pattern of results similar to what was

shown in DLPFC was also shown in M1 concentration of NAA:Cr (see Fig. 3C). There was no significant interaction (F (1,18) = 1.90; *p*= .19), nor was there a significant main effects of time (F (1,18) = 2.41; *p*= .14); however, the groups tended to differ at both time points (F (1,18) = 3.10; *p*= .095).

Discussion

The current study investigated neurometabolic differences between 10 non-concussed athletes and 10 concussed athletes of similar age and education in the acute and chronic post-injury phases. In the DLPFC, NAA:Cr levels remained lower in the concussed group across time. All other comparisons in DLPFC revealed no significant differences or trends. Within the motor cortex there were variable changes depending upon the metabolic ratio in question. Concussed athletes demonstrated a recovery of Glu:Cr levels across time (Figure 3A). Levels of m-I/Cr were equal between control and concussed athletes in the acute post-injury phase but there was a significant difference in the chronic phase suggesting metabolic disruptions that emerged over time as opposed to being immediately reactionary to the injury. NAA/Cr levels in M1 tended to distinguish control and concussed athletes at both time points, suggesting a similar pattern as was seen in the DLPFC.

The profiles of Glu:Cr and m-I:Cr in the DLPFC demonstrate stability within and between groups. This is consistent with the findings of Shutter et al. [73] who found that Glu:Cr levels were not predictive of outcome in patients with good outcomes either immediately post-injury or eight months post-injury relative to controls. It is difficult to draw parallels with other findings involving either increases or decreases in Glu:Cr levels

as other studies have taken spectra from different brain regions and in more severely injured populations [74, 75]. Though we have previously demonstrated changes in Glu:Cr in primary motor cortex, we did not see similar alterations in DLPFC suggesting that there are biomechanical influences that are present in primary motor cortex that are not present in prefrontal areas [53, 76]. Similarly, m-I:Cr levels were also very stable both across time and between groups.

The nature of the NAA:Cr findings was unexpected given the current literature implicating decreased levels of NAA:Cr [53, 54, 62, 77]. Indeed, past research investigating the time course of NAA alterations report mixed results as one moves chronologically further away from the point of injury. Vagnozzi and colleagues [54] report recovery within 30 days, except for those athletes who received a second concussive blow during the acute phase. We reported a similar finding in a group of 12 athletes who were scanned days after sustaining a concussion [53]. Conversely, Cohen and colleagues [77] found that whole brain NAA levels remained depressed in patient groups that were days to over a year post injury. Other studies have found diminished NAA levels in similar brain regions ranging from days [70] to one month [62] to months [78] to a year [79] post-injury. Though our results are consistent with the latter group of studies showing continued depression of NAA:Cr levels, they are contrary to those of Vagnozzi and colleagues [54]. The exact nature of why there is continued depression is not immediately evident, but a few explanations for these metabolic alterations are plausible. Firstly, the current study's sample is composed of student athletes. Though the athletes followed the return to play protocol as specified in the consensus statements [52,

72], they continued to take classes and in most cases resume practice within one week after the injury during the season in addition to continued physically demanding training in the months after the season when the follow-up data were obtained. This continued cognitive and physical effort may protract a full recovery [12, 52, 80] even when return to play protocols are properly followed such that a return to preconcussion levels does take place, but outside of the window used in the current study. Another possibility that may explain the continued metabolic depression is perhaps unique to contact sports like football and hockey. Even though no athletes reported a second concussion in a single season, sustaining subconcussive blows during practices and games may have also delayed metabolic recovery, even without resulting in a second injury as some studies suggest there are consequences, even if short lived, to sustaining multiple subconcussive blows [81, 82]. Finally, it is also possible that sustaining a concussion persistently lowers NAA:Cr levels. There is ample evidence to suggest this is the case after a mTBI [70, 77, 79]. Future studies charting the time course of metabolic injury and recovery need to be conducted in order to determine whether there is recovery, in whom there is recovery, and when the recovery occurs. Even though the current study investigates sports concussion, which are not necessarily equivalent to mTBI, the comparison is still worth making until more data specific to sports concussion becomes available.

Results from primary motor cortex paint a more complex picture of the metabolic state of the brain after a concussion. While depressed in the acute phase, Glu:Cr levels in concussed athletes rebound to those of control athletes in the chronic phase, elegantly demonstrating metabolic recovery. There is no precedent for Glu:Cr recovery in the

mildly brain injured population, let alone in the sports literature. However, given the seemingly short lived metabolic disturbance of glutamate levels as illustrated in the neurometabolic cascade [57], we predicted just such a recovery. What remains to be further explored is when exactly between the injury and the 6-month post injury time point as measured in the current study does Glu:Cr concentration achieve physiologically typical levels and whether this metabolic resolution corresponds to symptom recovery. The reasons for affected Glu:Cr levels in M1 but not in DLPFC are not immediately apparent. However, examination of the literature investigating the biomechanics of mTBI intimate that the rotational forces associated with concussion suggest that M1 is consistently vulnerable to shear strain [19, 76, 83-85].

M-I:Cr in M1 also showed a complex pattern of results. While there are no differences in the acute phase, there appears to be a pathological increase in m-I:Cr in the chronic phase. Other studies investigating either mixed TBI groups [70] or severe TBI [69, 74] though in different brain regions, have noted increased concentrations of m-I months and years after injury. The current data are consistent in this respect, but why these differences are not seen in the acute post injury phase is not immediately apparent. One possible explanation may be that there are two different mechanisms that help to regulate osmotic pressure in neurons and glia. Within the acute post-trauma phase this is primarily regulated by the rapid transport of Na^+, K^+, H^+, and Cl^- across the plasma membrane [86, 87]. Indeed, such an account is supported by the neurometabolic cascade as described by Giza and Hovda [57] where axonal swelling is indicative of hypernatremia [88]. To offset the ensuing water loss, the brain accumulates m-I to avoid

a rapid over correction which could have devastating consequences to the brain [89]. Such a fast acting mechanism would preclude any observable changes in m-I in the acute phase which is in line with the current study's results. However, long term changes in cellular tonicity are offset by the transport of non-perturbing osmolytes that do not alter the electrophysiological state of the cell, namely m-I [87]. The increase in m-I might also be indicative of gliosis [see 90 for review]. Myo-Inositol increase in association with decreased NAA:Cr ratios has been associated with gliosis in other populations [90], while other work suggestions that gliosis is not necessarily related to altered neurometabolism [91]. Other studies investigating TBI also report increased m-I:Cr levels in both severe [69, 74] and mild injuries [70]. In addition to TBI, other pathologies that have been associated to increased levels of m-I include drug addiction and stroke [see 67 for a complete review]. Though many questions remain as to the functional significance of such an increase in m-I, we are the first to report such an effect in the sports concussion population. Further confirmation is needed to confirm the robustness of this finding in a larger sample as well as the temporal nature of the changes.

The breadth of the metabolic changes in M1 (increases in m-I/Cr and Glu/Cr) within the concussed athletes in the chronic post-injury phase as well as the significant decrease of Glu:Cr in the acute phase may be due to the biomechanics of how the brain moves within the skull when a rotation force is applied [76, 85, 92]. The respective impacts of rotational and linear forces in producing a concussion [76, 85, 92-94] suggest that M1 is consistently vulnerable to the white matter injury of shear strain. The differential effects on Glu:Cr and m-I:Cr in M1 versus DLPFC are further corroborated

by changes detected using diffusion tensor imaging where patients who had suffered a mTBI demonstrated reduced fractional anisotropy in the corticospinal tract indicating diffuse axonal injury where no such injury pattern was found in frontal regions [19].

NAA levels in M1 showed a statistical trend between concussed and control athletes. Indeed, the overwhelming evidence implicates diminished levels of NAA after a brain injury, whether it be mild [53, 54, 62, 70, 77-79, 95] or severe [60, 70, 74, 75, 78, 96, 97]. Though the current results were not statistically significant in M1, this is consistent with what the current study demonstrates in the DLPFC. Decreased levels of NAA may be reflective of diffuse axonal injury in white matter and neuronal loss in gray matter [98], but this is a less probable interpretation given the heterogeneous nature of the neuropathological response to trauma. Declines in NAA levels are linked to decreases in ATP where the greater the initial decrease, the lesser the observed recovery. Indeed, recovery is observed in all injuries that do not include the substantial permanent destruction of brain tissue. That is to say, neurological recovery may be observed in conjunction with varying degrees of metabolic recovery where the latter need not be complete in order to observe clinical recovery in the former [56]. The persistent reduction in NAA:Cr levels observed in the current study may therefore be the consequence of a continued reduction of ATP due to the disruption of neuronal mitochondria due to the influx of Ca^{2+} and lactate, which is consistent with the observed post-injury cellular pathology [57]; furthermore, clinical signs seem to bare little relation to the neurometabolic anomalies observed in patients suggesting a highly variable relationship between injury severity and metabolic changes passed the immediate (minutes) post-

injury phase [57]. It is thus conceivable, despite the findings of Vagnozzi et al (2008) that concussed athletes do have continued metabolic disruptions despite being clinically recovered in terms of their PCSS scores. That is to say, even though concussive injuries do not typically result in observable brain trauma (i.e. MRI, CT scan), and concussed individuals typically recover from a symptom standpoint within weeks after the injury, there is a sustained and persistent effect on cellular metabolism. The continued neurometabolic alterations observed in the current study may be reflective of other pathological processes such as gliosis or cell loss. Cell loss would seem to be less probable given the time frame of 6 months post injury used in the current study, though changes in volumetry have been shown as a consequence to mTBI while other studies have measured brain volume at one year post-injury [77, 99]. Indeed, a study investigating mTBI 6 months post injury showed atrophy only in participants who had positive MR findings [100] while another some three months post injury also failed to find differences in participants who had suffered a MTBI [101]. Gliosis, as mentioned above is another possibility, but given that the current study observes an increase of m-I only in M1, it does not explain the persistence of metabolic disturbance observed in the DLPFC.

Conclusions

The current study shows a complex and varying pattern of recovery and persistent metabolic depression in different cortical areas and in different metabolites. The return of Glu:Cr levels in the concussed athletes to those of controls from the acute to the chronic phase clearly demonstrates recovery, at least insofar as glutamate is concerned. The lack

of group-time interactions of NAA:Cr concentration, both in M1 and DLPFC was somewhat surprising. It may be reflective of the fact that recovery from concussion is best achieved through cognitive and physical rest as described above [52, 80]. It may also be reflective of a persistent pathological state. Indeed, there are several neuropathologies that demonstrate continued depression of NAA levels including, though not limited to, stroke, TBI of all severities, multiple sclerosis, brain tumours, Alzheimer disease, and neuro-AIDS and other infections [56]. Clearly these represent different pathologies operating on different mechanisms. However, it is also indicative of the global role of NAA as a marker of neurometabolic health, irrespective of the underlying pathology.

Though our results demonstrate recovery in one instance (Glu:Cr in M1), they also show continued metabolic disturbance in another (NAA:Cr in DLPFC and M1), and altogether new neurometabolic alteration in yet another (m-I:Cr in M1). While at first this may seem self-contradictory, that need not necessarily be the case. Currently, all we know about concussive neurometabolic changes is that several different neuronal processes are affected [57]. What is far less understood is how these processes are related and which of these processes are necessarily concurrent to one another. Subsequently, the recovery of these respective processes may indeed follow differential recovery curves as is already noted on a micro-level [57]. Future studies should include larger samples and more time intervals to chart the metabolic recovery and stability of not just Glu, NAA, and m-I, but also of GABA and choline containing compounds with the understanding that not all metabolites will follow the same recovery curve, nor will all brain areas.

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Figure Legends

Figure 1: Typical spectrum and regions of interest

A. Proton spectrum of a control subject in M1 showing the peaks corresponding to the metabolites of interest, creatine (Cr), *myo*-inositol (mI), glutamate (Glu), and *N*-acetylaspartate (NAA). Concentrations are derived from the area under the peaks. **B**. Regions of interest (ROI) for MRS data acquisition depicted in the sagittal, coronal, and axial planes in the dorsolateral prefrontal cortex (DLPFC) (middle; 16 mm x 16 mm x 16 mm), and **C.** primary motor cortex (M1) (bottom; 16 mm x 20 mm x 32 mm). Spectra were recorded in both the left and right hemispheres.

Figure 2: Spectra in DLPFC

A. Line graph of the mean Glu/Cr ratios, **B** represents the means of m-I:Cr ratios and **C.** represents NAA:Cr ratios for control (black lines, n = 10) and concussed (gray bars, n = 10) athletes in the dorsolateral prefrontal cortex (DLPFC) at the acute and chronic post injury time points. Values are the mean of 24 voxel spectra (10 left hemisphere, 10 right hemisphere) per group. Standard errors of the means are represented by vertical bars. G represents a group effect and an asterisk indicates a statistically significant difference of $p \leq 0.05$.

Figure 3: Spectra in M1

A Line graph of the mean Glu/Cr ratios, **B** represents the means of m-I:Cr ratios and **C.** represents NAA:Cr ratios for control (black lines, n = 10) and concussed (gray bars, n = 10) athletes in the dorsolateral prefrontal cortex (DLPFC) at the acute and chronic post injury time points. Values are the mean of 24 voxel spectra (10 left hemisphere, 10 right hemisphere) per group. Standard errors of the means are represented by vertical bars. I represents an interaction of Group and Time, T represents an effect of time, and G represents a group effect. Statistically, *t* represents a trend where $p \leq .10$ and an asterisk indicates a statistically significant difference of $p \leq 0.05$ and a double asterisks indicates $p \leq .01$.

Figure 1: Typical spectra and regions of interest

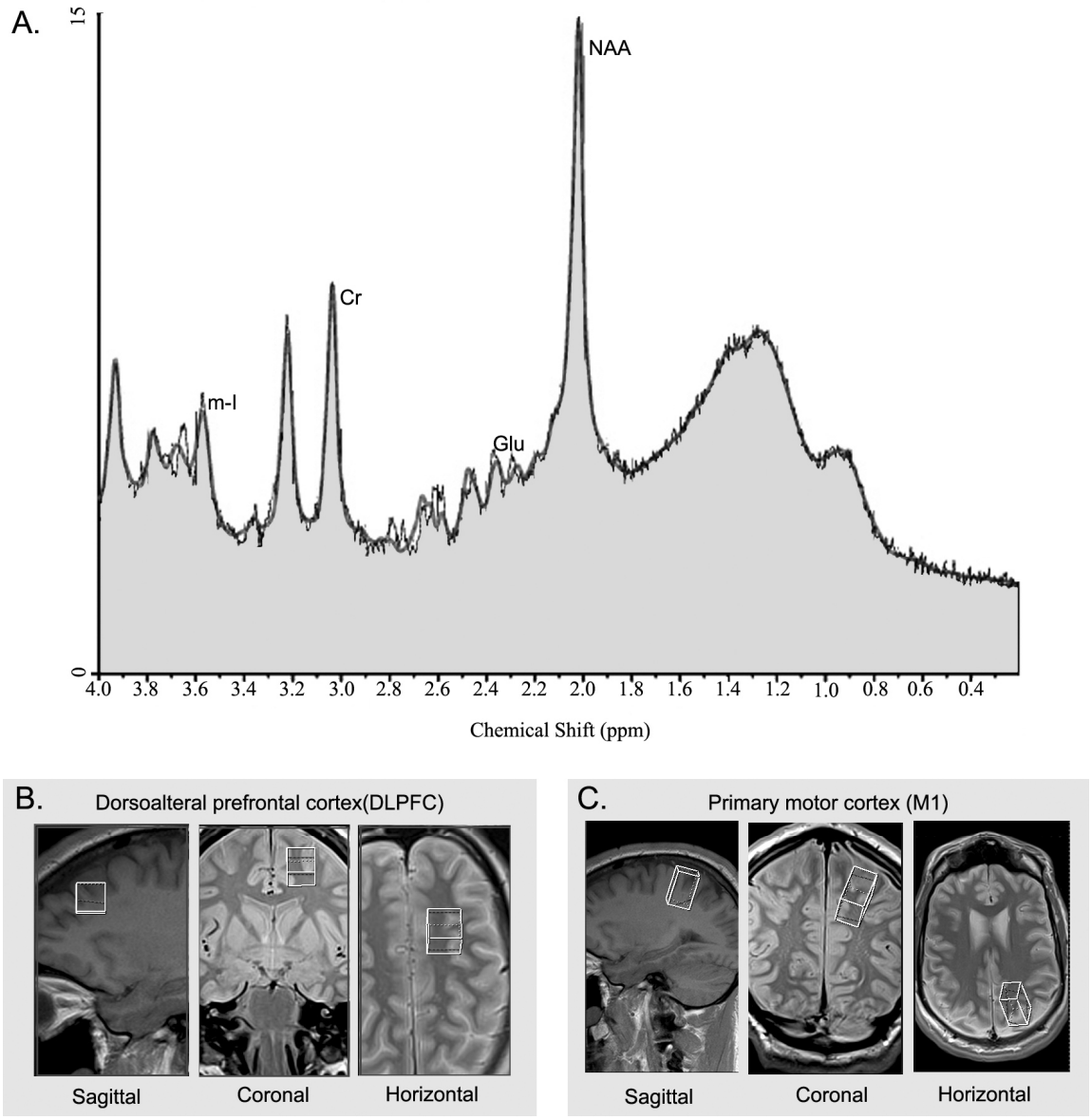


Figure 2: Metabolic changes in the DLPFC

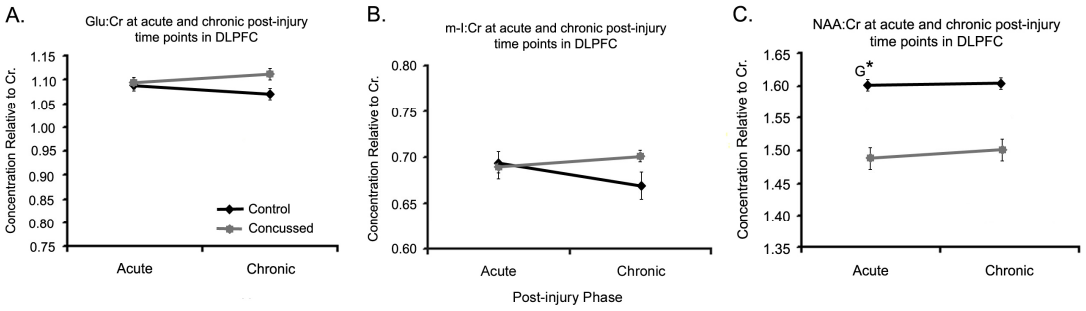
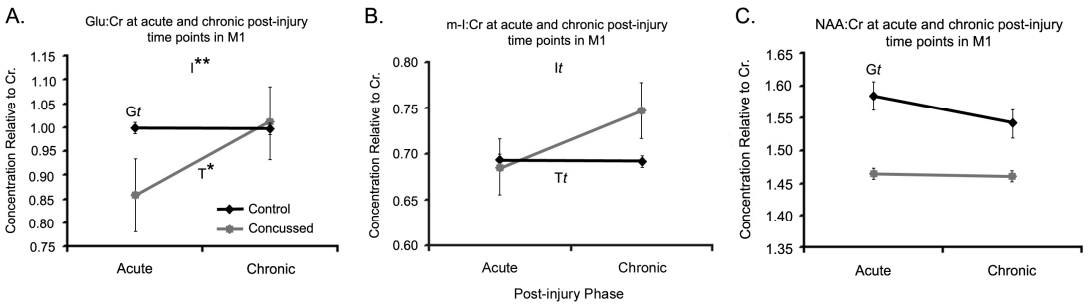


Figure 3: Metabolic changes in M1



General Discussion

General Discussion

Summary and Implications

The aim of the present thesis was to examine the Microstructural and neurometabolic consequences of sports concussions in the immediate (acute) post-injury phase as well as in the later (chronic) post-injury phase in a group of male athletes relative to their peers. To date, most concussion research has focused on the acute post-injury phase. Given that most symptoms resolve themselves within a relatively rapid timeframe (10-14 days for total symptom resolution), it makes logical sense to direct the bulk of our efforts toward understanding the underpinnings of concussive injuries within the timeframe when the effects are most salient. Even as the extant literature has greatly deepened the general understanding of symptomatology and injury management (Cantu, 1992; Grindel, Lovell, & Collins, 2001; Guskiewicz et al., 2004; Lovell, Collins, & Bradley, 2004; McCrory, et al., 2009) there has been very little human research done to understand the consequences of concussion above and beyond the neurocognitive dysfunctions and symptomatology. Indeed, sports concussions were understood to represent a purely functional injury (Aubry, et al., 2002). Functional imaging studies supported such a notion as it had been demonstrated that functional anomalies resolved as symptoms resolved (Chen, et al., 2004; Chen, et al., 2008; Fazio, et al., 2007). Conspicuous by its absence in a functional-centric view of sports concussions is the means by which function might be altered due to an injury that is not accompanied by any apparent physical injury. Such a view point has only been bolstered by the fact that

the overwhelming majority of studies that have employed traditional brain imaging techniques to investigate sports concussion have failed to detect any anatomical alterations (see Ellemberg, et al., 2009; Johnston, et al., 2001 for review).

Yet even as concussions are widely regarded by many in both the research and medical communities as being a transient injury requiring only acute management, the case for concussions having consequences beyond the duration of symptoms, regardless of how long their resolution takes, is rapidly building. It is now known that sports concussions result in altered electrophysiological profiles in concussed athletes (De Beaumont, Brisson, et al., 2007; Dupuis, Johnston, Lavoie, Lepore, & Lassonde, 2000; Gosselin, et al., 2006; Lavoie, Dupuis, Johnston, Leclerc, & Lassonde, 2004a; Theriault, et al., 2009; Theriault, et al., 2010), but in the absence of any clinical signs, meaning that concussions have consequences beyond a simple transient functional dysregulation. Similarly, TMS has been successfully used to document changes in the concussed brain, present in both active (De Beaumont, Lassonde, et al., 2007) and retired (De Beaumont, et al., 2009) athletes. How then can electrophysiological anomalies due to sports concussion persist if the injury has no long term physical consequences? To help understand this one must turn to different means of imaging the brain capable of elucidating different aspects of anatomy and metabolism.

To address the underlying mechanisms of sports concussion, the current thesis sought to expound on the growing, though still fledgling, research directed at understanding the structural and metabolic changes that ensue following a sports concussion (Chappell et al., 2006; Cubon, et al., 2011). DTI was used to investigate

potential post-injury anatomical changes in white matter given its particular vulnerability to shear strain forces. MR spectroscopy was employed as a means of investigating the mechanisms underlying the functional and electrophysiological changes observed after a concussion. The first study investigated the consequences of concussions on white matter in the brain, again comparing the acute and chronic post-injury phases (Study 1). It was hypothesized that diffuse axonal injury would be detected in concussed athletes and that evidence of TAI would diminish, though still be present in the chronic post-injury phase. Precisely, fractional anisotropy and axial diffusivity were predicted to decrease while mean diffusivity measures were hypothesized to increase. Our results, in terms of the directionality of the DTI measures, were in fact precisely the opposite of what was hypothesized: FA and AD both increased in concussed athletes while MD decreased. Though not entirely inconsistent with the literature (Chu et al., 2010; Mayer et al., 2010; Wilde, et al., 2008), these findings were unexpected. We interpreted these findings to reflect edema at both the cellular and local levels in conjunction with membrane dysfunction and altered microstructure. Such an interpretation actually bodes well for the outcome of the young concussed brain that has sustained only one or two injuries. Indeed, decreased FA and AD with increased MD values are associated with more terminal outcomes including greater cell death, volume loss, and axotomy (Farkas, Lifshitz, & Povlishock, 2006). Almost more importantly than what was found is where it was found. Our results showed that the CST seems to be particularly vulnerable to the effects of sports concussions. Biomechanically, this makes sense (Bayly, et al., 2005; Sabet, et al., 2008), but the majority of TBI research suggests, and quite logically so, that the corpus

callosum is particularly vulnerable (Wilde, et al., 2008). Whether this has something to do with how the head moves in a car accident versus a direct body impact or perhaps is related to greater rotation forces being exerted around the midline due to the added weight of the helmet is not known at this point. What is known is that there is an alarming and ever growing link between concussions, chronic traumatic encephalopathy (CTE), and amyotrophic lateral sclerosis (ALS) (McKee, et al., 2009; McKee et al., 2010). Particularly provocative about this triad in light of the first study is that ALS preferentially attacks the upper motor neuron, namely the CSP. Granted, concussions do not equate to ALS any more than they equate to Alzheimer, but the evidence suggesting that they can be a major contributing factor in the development of a neurodegenerative disease, be it ALS or Alzheimer is becoming apparent (Lindsay et al., 2002; McKee, et al., 2009; McKee, et al., 2010).

The second and third studies of the current thesis examined the neuropsychological, symptomatic, and metabolic sequelae of sports concussions in the acute post-injury phase (Study 2) and compared the acute and chronic post-injury phase neurometabolic responses (Study 3). It was hypothesized that the relative concentration of cortical glutamate to creatine would be lower in concussed athletes relative to healthy controls in the acute post-injury phase. We further hypothesized that the relative concentration of NAA:Cr would decrease while the concentration of myo-inositol to creatine would increase in concussed athletes in the acute post-injury phase. Based on the neurometabolic cascade timeline (Giza & Hovda, 2001), it was also hypothesized that the metabolic changes would be lessened after sixth months, but group differences would

still be present between the concussed athletes and controls. The results of the second study, where only the acute phase was analyzed, showed that within the DLPFC, NAA:Cr levels were significantly diminished. Similarly, NAA:Cr levels in M1 were also depressed, as were Glu:Cr levels. These findings are consistent with the literature (Vagnozzi, et al., 2010; Vagnozzi, et al., 2008) where a decrease in NAA is almost ubiquitous across pathologies (see Moffett, et al., 2007 for a comprehensive review). Most intriguingly is that the results differed depending on the region of interest, and that a change in one neurometabolite in a given area does not necessarily equate to changes in other neurometabolites. Such heterogeneity of effect suggests that the consequences of a concussion cannot be considered to affect all brain areas equally and in the same way.

The third study examined the neurometabolic consequences in the acute and chronic post-injury phases. Though not identical, the results were very similar to what was seen in Study 2. NAA levels were depressed in both post-injury phases suggesting a persistent effect in the DLPFC. Similarly, NAA levels within M1 showed a group trend, where levels were depressed at both time points. Interestingly, Glu:Cr levels recovered, again suggesting that different neurometabolites are affected in different ways in different brain areas. The recovery of Glu:Cr levels may prove to be a positive prognosticator for good recovery. Also within M1, m-I:Cr levels demonstrated a trend effect of time suggesting that levels increase over time. An increase in m-I is thought to be reflective of slower acting osmoregulation processes that would not be present within the acute phase (Fisher, et al., 2002). It is also thought to be reflective of gliosis and therefore more

permanent damage (Fisher, et al., 2002). This may indeed be the case in concussed athletes, though it is far too early to make such a determination.

Taken altogether, the results of the three studies indicate that sports concussions result in structural and metabolic alterations; furthermore, these alterations last beyond any clinical manifestations and persist in the face of functional recovery. In practical terms, the fact that after a concussed individual has "recovered" for all intents and purposes means that the structural and metabolic perturbations have no bearing on day-to-day activities. This does not mean that changes in the diffusivity properties of WM and in the efficiency of cellular energy consumption are spurious to the concussed athlete. In actuality the true consequences of these changes are not yet known. What is known is that the effects of a concussion or even two are not clinically detectable and many athletes who sustain three or more injuries, especially when managed correctly, do not demonstrate any clinical signs or symptoms (Asplund, McKeag, & Olsen, 2004; Barth, et al., 1989; Broglio, Macciocchi, & Ferrara, 2007; Chen, et al., 2007; Collins, et al., 2003; Fazio, et al., 2007; Guskiewicz, Ross, & Marshall, 2001; Iverson, 2005; Iverson, Brooks, Collins, et al., 2006; Lovell, et al., 2004). However, it may be that the effects of concussions on white matter integrity and the efficacy of cellular energy consumption take time to manifest. It may also be the case that for the vast majority of athletes, concussions do not result in deleterious effects in and of themselves, and only bear long term consequences in conjunction with other factors specific to the injuries (number, severity, age at which the injuries occurred), and genetics (Lindsay, et al., 2002). What seems apparent at this point is that multiple concussions can result in serious

consequences (McKee, et al., 2009). Presently, there is a seeming inundation of former high level professional athletes who suffered careers worth of unmanaged concussions, returning to play without missing a single sequence in some cases. Obviously this is not true of all former athletes who suffered multiple concussions, but the fact that it is true for a noticeable, if not sizeable minority demands further attention. How the changes in WM and neurometabolites may contribute differentially in those athletes who do not develop CTE versus those that do is up for debate and speculation at present.

Limitations

The studies that comprise the current thesis are not without their limitations. Some of the issues related to the current work are technical, while others stem from more practical issues. While none of these issues undermine the totality of the work, they do temper the degree to which the current results are valid in one sense and generalizable in another.

The most obvious limitation of the current work is a common issue in neuroimaging studies- the sample size. Though obviously every effort was made to recruit the highest number of subjects possible, the small number of subjects does place a limit on the extent to which the current findings may be generalized to all sports concussions specifically, and to mild traumatic brain injuries generally. While the number of subjects was adequate to make conservative, reasonable conclusions, prudence should be exercised in making any broad reaching statements. Furthermore, the fact that all of the concussed athletes were football players may also limit the applicability to other athletic populations. Though the differences are likely minimal with respect to sports like

hockey, the comparability to combat sports is as yet undetermined and any attempts to create links there between should be done with some degree of caution.

The current study used university level athletes as subjects. While this allowed us a great deal of control over demographic factors such as age and education level, other limitations emerge as a result. Given that the studies in the current thesis were not the only research studies that many of these athletes took part in, there was a degree of familiarity with the neuropsychological tests that were used by the subjects in the current studies that were also common to other studies. Though it is unclear the degree to which this familiarity influenced test performance, the lack of group differences, particularly during the acute phase, suggest that there may have been some carry over effects. Granted, this does not take away from the main findings of the current thesis, but it may have limited the findings by dampening effects that might have otherwise emerged. Similarly, the vast majority of the subjects recruited in the current thesis are football players, which again is positive in terms of group-to-group comparison; however, the fact that nearly all of the athletes would have most assuredly sustained several subconcussive blows does color the results to an extent. In some ways, it emboldens the current results as the metabolic and anatomic effects of concussions emerge over and above the effects of subconcussive blows demonstrating a reliable effect. However, it precludes a clean investigation of the effects of sports concussion relative to a brain that has experienced few if any subconcussive blows.

The highly technical nature of the imaging techniques used in the current thesis allowed for the collection of novel data, but of course are not without their limitations either. MRS is extremely sensitive at detecting the metabolic profile of the region of interest. However, it is also difficult to acquire clean samples in certain brain areas. The mere location of the hippocampus coupled with the size of the shim box used in the current study to isolate the hippocampus made it difficult to consistently obtain clean spectra in this ROI. By sizing the box to be slightly shorter and narrower this problem could have been minimized. Also, the current study employed relative quantitation, using creatine as a reference metabolite for all other metabolites. This is an oft-used technique and is widely accepted in the literature; however it would have been useful to also have the absolute values which can only be obtained on Siemens MRI systems by specifying so before scanning. Though not a critical lacuna, having the absolute values as a means of further comparing the metabolic differences would have added another dimension to the current thesis.

Finally, DTI is still a relatively young technique. It is clearly an overall advantage to be able to use cutting edge technology, but one of the drawbacks can be the conflicting interpretation of the outcome measures. For example, some studies found FA values to be increased in injured brains while others report a decrease. Though sound and reasonable interpretations can be made to state the case for either finding, the apparent lack of consistency is at times difficult to reconcile. It may very well be that in a certain population one, the other, or both can be correct, while in another population the same parameters mean different things. What those conditions are is currently unclear.

Future Directions

Many questions have emerged from the present results. The current thesis investigated the effects of concussion on white matter integrity and brain metabolism using DTI and MRS respectively in an all male athlete sample. While there are clear gender differences between males and females that make mixed sample studies somewhat problematic, future investigations of a similar nature in female athletes should be conducted. Furthermore, a comparison of female to male athletes may also be warranted to better understand potential differences in injury presentation, anatomical and metabolic alterations, and recovery timeline. The methods employed by the current thesis could also be well applied to athletic populations longitudinally. Different from the current studies, future studies should image concussed athletes at several time points between the initial scan (2-5 days post) and the chronic post-injury phase (6 months post) such that scans are also done at 14 days, 30 days, and two months post-injury to better chart the metabolic response to concussion.

Originally an aspect I had hoped to investigate as a part of the current thesis that had to be dropped due to the number of participants was a comparison of singly concussed athletes versus multiply concussed athletes using both DTI and MRS. Such a study would provide an important piece of the puzzle in determining how the brain responds to an initial injury and how that response changes in the face of the effects of accrued impacts. Also, particularly if athletes who sustain a career's worth of injuries are studied, invaluable information as to how CTE develops in some athletes while others remain unaffected may emerge. Clearly the aspects surrounding CTE are incredibly

complex, and though only a minority of athletes succumb to this horrific outcome, any attempt to understand and more importantly to minimize such a fate should be taken. Though not the only factor, understanding how the anatomical changes, mild and subclinical as they are in young athletes, can contribute to neurodegenerative processes, such as mild cognitive impairment, amyotrophic lateral sclerosis and Alzheimer Disease to name a few, should not be overlooked. Indeed, the findings of the current studies highlight the importance of and need for such future investigations.

Within the mTBI literature there is a clear delineation between all other kinds of mTBI and sports concussion. On one hand, this seems logical. Many of us would hesitate to compare the impact of an automobile collision with an open-ice body check based on biomechanics, velocity, and the obvious situational differences of a vehicle-on-vehicle versus body-on-body impact. The idea that there is a difference between the two is strongly reflected in the nomenclature found in the current literature where concussions are used to refer to sports-related injuries almost exclusively, while mTBI is the term of choice for almost all other non-penetrating mild head injuries. However, by most standard clinical tools such as GCS and neuropsychological tests there is no stand-out difference between the two injuries. Whether it is correct to regard the sports concussions and mTBIs sustained by other means as separate injuries remains to be seen. Future studies should employ methods like MRS and DTI to document and understand the differences between mTBI and sports concussion, if any.

The heterogeneity of the current thesis's findings as well as the heterogeneity inherent to concussions further brings to mind questions about the relative importance as to how the injuries themselves were sustained. It may be possible that coup injuries result in different structural and metabolic changes than contrecoup injuries, thereby creating differential effects in the two hemispheres. Future research should strive to include the point of impact and degree of rotational force as a means of further parsing the effects of sports concussions. Having sensory-equipped helmets as a part of standard equipment for all football players would not only eliminate this particular conundrum from a research perspective, but may also be of tremendous help to clinicians when seeking to apply both general principles to all their patients, but also individualized treatment based on an athlete's particular physiology and injury.

Already briefly mentioned, MCI, ALS, and Alzheimer's disease are suspected to be more likely outcomes in individuals who have suffered repeated sports concussions. There are other factors that figure heavily into the equation, the largest of which is genetics; however, the role of sports concussion has to be considered as a significant risk factor in the later development of a neurodegenerative disease. While there have already been studies investigating the anatomical and metabolic aspects of neurodegeneration, there has yet to be a comparison between former athletes who have sustained concussions and those older adults already diagnosed with a neurodegenerative disease. Following former concussed athletes later in life to differentiate those who develop MCI or any other similar disease from those who do not will bolster our understanding of the importance of sports concussions as a risk factor; further, such a study would also deepen

our knowledge of injury or metabolic processes that underlie the mechanism of sports concussion as a risk factor for neurodegenerative disease.

Even before an individual who has sustained several concussions reaches a life stage advanced enough for late-life neurodegeneration to set in, another more insidious problem may emerge in a small, though important segment of former athletes: CTE. CTE can only be diagnosed upon autopsy, but evidence of it can be detected beforehand. It is perhaps best understood when the entire constellation of symptoms is present in the retired athlete. Retired boxers and football players allow the clinical and research community a unique opportunity to examine such a state. Signs and symptoms of CTE can involve deficits that span the range of cognitive, behavioral/affective, and motor impairments. The cognitive deficits are most likely to affect the domains of attention, memory, and executive function (Mendez, 1995). The behavioral/affective aspects are more difficult to separate from potentially pre-existing traits, but are thought to encompass disinhibition, irritability, periods of euphoria combined with periods of hypomania, paranoia, impaired insight, and violent outbursts (Mendez, 1995). Such a behavioral profile is consistent with the cognitive degradation of executive functions which are thought to include emotion regulation and inhibition (Barr, 2005; Kandel, et al., 2000). Finally, motor impairments including bradykinesia, ataxia, spasticity, impaired coordination, and at the extreme end Parkinsonism (Mendez, 1995). Employing tools such as DTI and MRS in individuals suspected of having CTE may provide us with valuable information as to how the deteriorating brain is affected and which areas are most severely injured.

Conclusion

It is now known that concussions are not a mere transient event, and are certainly nothing to be shrugged off and brushed aside. Sports concussions represent a physical trauma to the brain that has immediate consequences and persistent effects. In the short term the concussed individual experiences a host of symptoms such as mental fogginess, headache, confusion, and retrograde and anterograde amnesia to name a few. In the long term, though subclinical, concussed athletes continue to demonstrate electrophysiological anomalies (De Beaumont, Brisson, et al., 2007; De Beaumont, Lassonde, et al., 2007; De Beaumont, et al., 2009; Dupuis, et al., 2000; Gosselin, et al., 2006; Lavoie, et al., 2004a; Theriault, et al., 2009; Theriault, et al., 2010) as well as white matter and metabolic alterations as shown by the current thesis. With all of this in mind, it is worth reconsidering how we think about these injuries. Rather than thinking about concussions as single events, the paradigm needs to shift where concussions are thought of as processes. While the injury itself is the event that starts the process, the constellation of data that has emerged, including the current thesis, suggest that the effects of concussions continue long after the acute symptomatic phase. Even those concussed individuals who report no ill effects are known to be more vulnerable to concussions subsequent to future impacts and that the effects of concussions are not discrete, but rather bootstrap such that their effects are cumulative, worsening in severity and duration (McCrory, et al., 2009).

The first study of the current thesis examined the anatomical consequences of sports concussions. Athletes were imaged 2-5 days and six months post-injury using a rigorous DTI protocol and compared to a control group of their peers. The results indicate

that there is indeed altered white matter integrity in both the acute and chronic post-injury phases. The areas that demonstrated the strongest and most stable alterations were along the cortical spinal tracts and areas of the corpus callosum. Biomechanically, these areas are the most vulnerable to rotational force vectors, though the corpus callosum is typically considered to be more vulnerable than the cortical spinal tract given its size and location. While the results of Study 1 do show alterations in the corpus callosum, the cortical spinal tracts were more greatly affected in terms of the overall area showing altered DTI measures. Though there is no immediate explanation for this finding, it might be speculated that the added bulk of football helmets preferentially weight the head to move more violently around the midline axis which would create a larger force vector around the cortical spinal tracts. While helmets undoubtedly decrease the impact of linear force vectors it may well be the case that they add rotational velocity. Such a biomechanical investigation has yet to be undertaken, but may prove crucial in designing head gear that better protects against the rotational forces of concussive blows.

The second and third studies of the current thesis were designed to investigate the metabolic alterations associated with concussive blows. Study 2 examined athletes within the acute post-injury phase only. The results showed lowered levels of NAA:Cr in both prefrontal and primary motor cortices. Additionally, lowed Glu:Cr levels were also found in primary motor cortex only. These results are consistent with Study 1 in that the primary motor cortex seems to be the brain area that is most greatly affected. The metabolic alterations were also correlated to self-reported symptoms. This correlation is likely reflective of two independent processes that are simultaneously occurring. Such an

explanation is supported by the results of Study 3 where both the acute and chronic post-injury phases were examined. Though the metabolic changes were not as strongly differentiated in this study due to sample size, the statistics are by and large consistent with those of Study 2. Noteworthy, some metabolic alterations return to typical levels (Glu:Cr in M1) while others remain depressed (NAA:Cr in DLPFC and NAA:Cr in M1) and others still emerge only in the chronic phase (m-I:Cr in M1). The conclusion here is that different metabolites recover at different rates in different brain areas depending on their location. The metabolic profile of concussion has yet to be fully elucidated. More studies examining this aspect of concussion will serve to better characterize how concussions alter the brain's energy use, where it is most altered, and for how long it is altered.

The book on sports concussions is only beginning to be written. The current thesis aimed to contribute novel data as to how the brain changes microstructurally and metabolically subsequent to sustaining a sports concussion. Though far from conclusive, the current results demonstrate that concussions are more than a mere transient phenomenon lasting 10-14 days. Rather, concussions may be the prime mover in a cascade of events that alters the brain electrophysiologically, structurally, metabolically, and functionally throughout the course of an athlete's playing career in the short term and his or her life in the long term.

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