Towards a neurophysiological approach of managing sport-originated brain injury

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Contents

List of figures

List of tables

List of appendices

List of abbreviations

ACC	accelerometer
AUC	area under the curve
BCAA	branch chained amino acids
BCTT	Buffalo concussion treadmill test
BDNF	brain-derived neurotrophic factor
BG-SA	Brain Gauge somatosensory assessments
CNN	convolutional neural network
DHA	docosahexaenoic acid
DUR	duration discrimination
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
GSI	global severity index
KD	ketogenic diet
mTBI	mild traumatic brain injury
NP	neurophysiological
NPV	negative predictive value
NUT	nutritional intervention
PPV	positive predictive value
PSC	predominant symptom cluster
PSDI	positive symptom distress index
PST	positive symptom total
PUFA	polyunsaturated fatty acids
SCAT-5	Sport Concussion Assessment Tool Version 5
SOBI	sport-originated brain injury
SRC	sport-related concussion
SR-mTBI	sport-related mild traumatic brain injury
SSS	symptom severity score
TBI	traumatic brain injury
TOJ	temporal order judgement
TOJc	temporal order judgement with confounding stimulus

Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements) nor material which to a substantial extent has been accepted for the award of any other degree or diploma of a university or other institution of higher learning.

Chapters 2 to 8 of this thesis represent separate papers that have either been published or have been/will be submitted to peer-reviewed journals for consideration for publication. My contribution and the contributed by the various co-authors to each of these papers are outlined at the beginning of each chapter. All co-authors have approved the inclusion of the joint work in this doctoral thesis.

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Candidate contributions to co-authored papers

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We, the undersigned, hereby agree to the percentages of participation to the chapters identified above

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Personal view

Until the age of 20, my entire life revolved around my engagement in sport. I cannot remember a season going by without spending countless hours participating in skateboarding, skiing, ice hockey, lacrosse, American football, or rugby. I loved spending hours upon hours on my own firing pucks at my hockey net or practicing place kicks on my front lawn where I pretended two trees were goal posts. My self-image revolved around being an athlete, until October 2012 when I suffered two traumatic brain injuries in the same week playing rugby for my university.

The first injury was a late and high tackle that took me clean off my feet, whipped my head back, and caused me to crash hard to the ground headfirst. I was winded by the collision and a little disoriented, but never lost consciousness. I had taken hard hits and had "my bell rung" many times in the past when I was always able to tough it out and keep playing. This time was no different, so I got up and finished off the game. Later that evening I had some headaches but concerns about if I had just injured my brain never crossed my mind. During the next few days, I had some more headaches along with feeling more tired than usual and difficulty concentrating, but this was during midterm exams, so I dismissed these issues due to long days of studying. The following Saturday I played in another game, where I was forced to make a tackle on a much larger player and during the tackle their elbow collided violently with my forehead. This time I knew something wasn't right immediately. I was seeing stars, felt extremely disoriented and nauseous, and had difficulty maintaining my balance. But it was the last game of the season, I was the team captain, and we were out of substitutions; so for the second week in a row I finished the game with no concern about the state of my brain. The rest of that day I felt unusual, but again brushed it off as having "my bell rung", which was no big deal. Fast forward a month later when rugby was over, and midterms were finished but something still wasn't right. Every night I would sleep 2-3 hours longer than normal but wake up exhausted, I couldn't concentrate in class and my memory was severely diminished, I was unusually irritable, my coordination was off, and I struggled to tolerate exposure to day-to-day levels of noise and light. I finally accepted that I needed to seek medical help when I tried, and failed, to complete a basic fitness test due to a crippling headache that seemed to worsen during exercise. I saw a physician at the university medical clinic, and after going through my symptoms and history, was informed that I had "post-concussion syndrome" due to the hits I had taken a few weeks prior. Since exercise seemed to worsen my symptoms, I was told to avoid all exercise, physical activity, and sport until I was completely symptom free at rest which could take anywhere from a couple weeks to many months. The physician referred me to a neuropsychologist but admitted that it would likely take 6-12 months to get an appointment, and then ended the appointment by wishing me good luck and that there was no need for a follow-up. While I was initially skeptical, I trusted the medical advice I received because my aspirations at the time were to gain admission to medical school to train as a surgeon. Little did I know that this advice would send me in a

downward spiral that would lead to persistent symptoms, depression, and questioning whether my mind was creating my own suffering.

After approximately 12 months of debilitating symptoms and complete avoidance of sport and physical activity, I finally had my appointment with the neuropsychologist. Before this appointment I had a renewed sense of hope that I would receive the specialist advice I needed to turn things around. After 6 hours of intense assessment the clinician concluded that overall, I performed well above average on nearly all metrics except processing speed, and that without a pre-injury baseline it wouldn't be possible to interpret my findings further. When I asked about treatment recommendations none were provided. Finally, I asked if I just had to treat my situation as my new reality and give up the pursuit of my career aspirations. I was told that things might never be the same as before, but the only thing that would stop me from accomplishing my goals was my willingness to adapt and overcome. While I was frustrated that I didn't receive a detailed treatment plan, armed with this advice I decided I'd go to the university library and start looking for answers myself.

I quickly realised that my frustration with the clinicians I had seen to that point was misplaced. The reason they didn't know what to tell me was because there was little to no evidence in the literature for them to draw from. Most guidelines and recommendations at that time said that 80-90% of patients spontaneously get better within two weeks, and that cognitive and physical rest until symptoms abated was the advised course of action for patients with prolonged issues. I was beginning to lose all hope until I found the work of Dr John Leddy from the University of Buffalo. Leddy's work described an exercise is medicine method to improve symptomology through progressive loading below the symptom exacerbation threshold. This research described an approach to rehabilitating persistent symptom after mild traumatic brain injury (mTBI) similar to the strategies I had learned for musculoskeletal injuries and confirmed my initial skepticism of the rest, rest, rest approach. I spent a great deal of time reading about the links between exercise, neuroplasticity, and brain health in the fields of stroke and dementia. I began to apply these principles to myself to feel "normal" again. I had to relearn how to learn, I had to rebuild my body and fitness, and I had to overhaul my mental health. My progress was anything but linear, many times I pushed too hard and felt like I was taking one step forward and two back. Nearly two years of trial and error after my injuries I felt close to my old self.

I have always wanted to help people and make a difference and my personal experiences with brain injury put me in a unique position to contribute to the field. This realisation led to the decision to pursue a career in research and academia rather than medicine and motivated me to endeavour towards addressing some of the gaps in the literature in a manner that provides clinicians with the information they need to improve the outcomes of their patients. My experience led to the completion of a Master's degree evaluating the utility of sub-symptom threshold aerobic and balance exercise for people with persistent symptoms after

mTBI. Knowledge gained throughout my Master's led to working closely with local physicians and receiving referrals to apply my research to help people struggling after mTBI. My personal experience allowed me to build rapport with patients easily because I intimately understood what they were dealing with. All these experiences culminated in receiving a full scholarship to move across the world and pursue this PhD here in New Zealand. Over eight years after my injuries I still wouldn't say I'm 100% recovered. I still deal with frequent severe headaches, fog, and issues with memory that never troubled me before October 2012. But I have learned how to manage these issues and given the opportunity to go back in time, I wouldn't change a thing because I wouldn't be chasing my dream nor the person I am today. The amount of work that needs to be done in this field might be daunting to some, but it fills me with excitement to dedicate my career to advancing the clinical management of brain injury in any way I can.

Abstract

Of the estimated 27 million traumatic brain injuries (TBIs) annually, 95% are considered mild TBIs (mTBIs), that are not life threatening but lead to disturbances in normal brain function. While these injuries are described as “mild” a considerable proportion of patients suffer mTBI-related symptom burden for weeks, months, and in some cases years post-injury. The number of patients suffering from persistent mTBI-related complications, and the cost for healthcare systems, indicates a need to advance the clinical management of these injuries. Approximately one fifth of TBIs result from a sport/physical activity related mechanism of injury, with 98% of these sport-originated brain injuries (SOBI) considered to be mTBI.

SOBI is a problem for clinicians who manage athletes with these injuries. The advances in our understanding of the neurophysiological consequences of TBI provide targets for novel assessments and interventions to improve clinical outcomes. Considerable gaps exist in current clinical management pathways that contribute to poor recovery outcomes and prolonged burden. These gaps range from needing to understand which clinical factors contribute to variability in recovery outcomes, identifying objective measurements that can feasibly assist clinicians who work with mTBI patients, and innovative methods to proactively reduce the damaging neurophysiological consequences of brain injury.

Many mTBI researchers focus their effort in a specific subdiscipline to gain intimate knowledge of a specific aspect of mTBI. However, holistic research efforts are needed to link the findings of these endeavours to translate this knowledge to the benefit of mTBI patients. This PhD aimed to evaluate how a diverse collection of evolving neurophysiological approaches to assessing and managing mTBI might translate from bench to bedside for SOBI. The two main research questions were “what factors influence time to recovery following SOBI?“ and “what is the translational potential of neurophysiological approaches to advance clinical management of SOBI?”. The purpose of structuring the thesis into two sections was to develop a diverse technical skillset and multidisciplinary understanding of what is required to improve translation of promising findings from highly controlled studies into real-world clinical environments. The methodological approach to this thesis was to conduct embedded quantitative research within a functioning clinical SOBI service. The design, analysis, and interpretation of studies that were not clinically embedded prioritised ecological validity of findings. Overall, this thesis presents a case study, systematic review, cross-sectional study, experimental study, and multiple cohort studies (both retrospective and prospective).

There were consequences caused by suboptimal clinical assessment protocols as reported in our case study of an individual with a history of SOBI experiencing prolonged symptoms. Using retrospective analysis of SOBI clinic data, predictors of SOBI recovery trajectories were identified. Insufficiencies in clinical pathways were the greatest modifiable factor that influenced recovery outcomes post-SOBI. Identification of subgroups of SOBI patients based on their predominant symptom cluster was a key factor related to

recovery. Patients with vestibulo-ocular symptomology were particularly vulnerable to negative outcomes. Therefore, education and training of clinicians so they can recognise and treat these symptom profiles more effectively is needed. Figures claiming the majority of SOBI patients will be recovered by 14-days post-injury are out of date and require updating given our study showed that closer to 50% of patients experienced symptoms beyond this timeframe.

Our systematic review of experimental literature evaluating the effect of nutritional interventions to improve neurophysiological outcomes of TBI identified that anti-oxidant, branched chain amino acid, and ω-3 polyunsaturated fatty acid supplementation appear to be the most promising candidates to proactively lessen brain damage and burden caused by TBI. Since the biofidelity of the animal studies was low and no studies have evaluated nutritional interventions on neurophysiological outcomes in humans, a feasibility study would be necessary before dedicating time and resources to a clinical trial.

The translational potential and clinical utility of neurophysiological based assessments to provide objective data to assist clinical decision making and enable the evaluation of a nutritional intervention to improve SOBI recovery outcomes were evaluated. Clinical utility of somatosensory assessments to assist clinicians working with SOBI patients when making diagnostic decisions, predicting recovery trajectories, and determining if neurophysiological recovery coincided with clinical recovery was assessed. Machine learning was used to develop an algorithm using wearable sensor data acquired during an incremental stress test to classify mTBI symptom subclusters. Brain-derived neurotrophic factor (BDNF), a biomarker of neuroplasticity, was measured non-invasively within saliva under clinically realistic conditions to begin to understand the relationships between BDNF and symptom burden.

Several methodological issues that are likely to prevent the translational of neurophysiological approaches to the benefit of current clinical practice in the future were identified. Neither salivary-BDNF or somatosensory assessments appear to offer utility to improve clinical assessment of SOBI, nor to evaluate the potential benefit of a nutritional intervention. The potential value of using wearable sensors and machine learning algorithms to collect and analyse objective data during an already established clinical test was the most promising finding but requires a great amount of development. Overall, the evidence did not suggest that nutritional interventions or neurophysiological assessments will be coming to the aid of clinicians and their SOBI patients soon.

Future studies need to prioritise study designs that emphasise biofidelity and ecological validity so that findings are more likely to translate to clinical practice. In the interim, responsibility falls on healthcare organisations, educational institutes, and sporting bodies to improve the education, standards, and pathways moving forward to reduce the burden caused by persistent complications following SOBI.

Variable vernacular

Before presenting relevant background information justifying the research questions evaluated in the chapters of this thesis a discussion about inconsistencies in terminology within the field of TBI is necessary. A casual search of the literature reveals discordance in the use of the terms concussion and mTBI; with some papers using the terms interchangeably, while others describe the two injuries as separate entities. The Latin roots for concussion are *concutere* (to shake violently) and *concussus* (to strike together). Excluding penetrating brain injuries like those caused by gunshot and stab wounds, violent shaking of the brain and the striking of the brain against the skull are consistent with the mechanisms of injury for all severities of TBI, not just mild TBI [1]. In my experience as an athlete and working with athletes to associate the term concussion with "head knocks" or "bell ringers". The association between these terminologies with concussion is clinically unhelpful as it does not accurately communicate to the affected individual that they have suffered an injury to their brain which should be taken seriously.

In 2015 a published review titled "Concussion is confusing us all" provides compelling arguments that concussion and post-concussion syndrome are unhelpful terms that should be retired, citing that they lack any diagnostic precision and encourage lazy diagnostic approaches [2]. In 2018 the Centers for Disease Control and Prevention recommended single use of mTBI over concussion and minor head injury because "they have different connotations for families, researchers, and health care professionals, allowing for misinterpretation" ([3], p. E2). The term concussion (and related terminology including post-concussion syndrome and subconcussions) are still widely used despite these recommendations. Working clinically with patients who have suffered mTBI is challenging and the need to navigate the different terminology used adds to the complexity (i.e., what does a clinician mean by a given term). There is a clear need for global adoption of a singular set of clear definitions.

Over the course of completing this PhD my personal stance on this issue has evolved from viewing mTBI and concussion as interchangeable terms to firmly agreeing with the call to retire concussion-centred terminology in research and clinical practice. Mild, moderate, and severe TBI are useful labels to describe the degree of structural damage and risk of permanent disability/death; but it seems reasonable to speculate that the large number of mTBI patients who experience prolonged complications would be unlikely to describe their injury as "mild". Moreover, describing an injury to the brain as "mild" may cause athletes (who are known to underreport and minimise their injuries [4-8]) to misinterpret the seriousness of these injuries while also potentially causing members of the public and policy makers to falsely perceive them as inconsequential. In a sporting context, sport-originated brain injury (SOBI) may be a more useful term by directly calling the injury what it is: an injury to the brain originating from participation in sport/physical activity. This clearly communicates that an injury to the brain has occurred to athletes who

may tend to underestimate the significance of their injury while also considering the burden experienced by significant number of athletes who suffer prolonged complications. A reason for adopting SOBI instead of mTBI is also the ability to be able to say the word easily by the public (e.g., strains, sprains, and SOBI). SOBI is put forward in this thesis as a potential term to use in the future although a thorough and collaborative process to operationalize and establish the validity and reliability of the term would be necessary before widespread adoption.

This evolution in mindset about the importance of terminology is reflected throughout the chapters of this thesis, as sport-related concussion (SRC) was used in the publication presented in Chapter 2, while sport-related mTBI (SR-mTBI) was used for the publications in Chapters 3 and 5. Finally, in Chapters 6 and 8 sport-originated brain injury (SOBI) was used in place of SR-mTBI. Chapters 4 and 7 focussed on all severities of TBI and a pilot study on healthy individuals, respectively, so sport-specific terminology was unnecessary. SOBI is used throughout the Introduction in Chapter 1, and the Discussion presented in Chapter 9, to describe TBIs (predominantly mTBIs) that occur as result of participation in sport/physical activity. mTBI is used in the thesis when discussing evidence outside of a sport-specific context.

Definitions

Mild traumatic brain injury (mTBI)

Use of mTBI throughout this thesis adheres to the operational definition provided by the World Health Organisation Collaborating Centre for Neurotrauma Task Force on mTBI as follows:

> mTBI is an acute brain injury resulting from mechanical energy to the head from external physical forces. Operational criteria for clinical identification include: (i) 1 or more of the following: confusion or disorientation, loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; (ii) Glasgow Coma Scale score of 13–15 after 30 minutes post-injury or later upon presentation for healthcare. These manifestations of mTBI must not be due to drugs, alcohol, medications, caused by other injuries or treatment for other injuries (e.g., systemic injuries, facial injuries or intubation), caused by other problems (e.g., psychological trauma, language barrier or coexisting medical conditions) or caused by penetrating craniocerebral injury ([9] p. 115).

For the sake of this thesis this definition is extended to include mTBI as a discrete period of rapid stretching and shearing of neuronal and vascular tissues that triggers a complex pathophysiological process which disrupts normal brain function.

Sport-originated brain injury (SOBI)

SOBI refers to all severities of TBI that occur due to participation in sport/physical activity, with the caveat that 98% of SOBIs meet mTBI criteria [10]. We proposed SOBI as an intuitive term that clearly conveys to athletes that they have sustained a brain injury that should be taken seriously, and as an alternative the ambiguity of concussion and issues of labelling an injury as “mild” when working with athletes [11].

Neurophysiological approaches

Neurophysiological approaches is used throughout this thesis as an umbrella term to describe methods of assessing and treating traumatic brain injury that are based on our expanding knowledge of the relationship between pathophysiology and clinical presentation. For example, measurement of heart rate variability during physical exertion is a neurophysiological approach to identifying how abnormalities in autonomic control contribute to exercise intolerance in athletes who have sustained a SOBI compared to uninjured athletes [12]. Observations such as these have led to the identification of sub-symptom threshold aerobic exercise as a neurophysiological approach to reduce signs and symptoms through restoration of autonomic control [13, 14]. There appear to be many more opportunities to use a neurophysiological approach of assessing and treating mTBI/SOBI that may improve clinical practice, a small subset of which are explored in the chapters of this thesis.

Ecological validity

Ecological validity is a key concept that is stressed throughout this thesis, however there is no agreed upon definition of ecological validity. For the purpose of this thesis ecological validity is meant to represent a component of external validity which reflects how the design of a study replicates the environmental conditions where the findings of the study would be expected to have a benefit. Authors have previously criticized the use of ecological validity in this manner because the criteria for determining ecological validity are often poorly defined and lack specificity [15]. The subsequent chapters of this thesis present studies that were designed and conducted in a busy outpatient clinical setting, thus the following operationalization of ecological validity is intended for the generalisability of study findings to similar environments. Studies evaluating assessment methods would be considered ecologically valid for outpatient settings if:

1) the assessment could be conducted/obtained in ≤10 minutes to preserve clinical flow;
2) the assessment was non-invasive (i.e., not a blood or cerebral spinal fluid draw);
3) the assessment demonstrated high sensitivity and specificity without requiring a baseline measurement;
4) the assessment demonstrated high sensitivity and specificity when conducted/obtained 3-14 days post-mTBI/SOBI as this is commonly the time that would elapse between injury and initial clinical presentation;
5) conducting/obtaining the assessment did not require highly specialised training (i.e. clinical EEG).

Biofidelity

Since animal studies are widely reference throughout this thesis it is also pertinent to define biofidelity as the “concordance between specific features of a given animal model and the human disease or condition being modelled” and “goes hand in hand with confirmation of experimental results across different animal models with clinical findings in humans (i.e., validity)” ([16] pg 8).

Chapter 1: Introduction and rationalisation

Traumatic brain injury

Exposure to sudden physical trauma can result in traumatic brain injuries (TBIs) that may have life altering consequences. Damaging forces can be exerted upon the brain directly by the head colliding with another object/surface or when contacted by a projectile; alternatively, indirect forces can also damage brain tissue following blast exposure or when acting upon other areas of the body leading to a whipping action of the neck/head [17-20]. When exposed to these types of forces neuronal and vascular tissues within the brain undergo a discrete period of rapid stretching and shearing. This brief tissue deformation triggers the secondary injury phase of TBI characterised by disrupted neurological homeostasis [21]. The degree of force transmitted through the brain during the primary phase of TBI coupled with the magnitude and duration of neurophysiological disruption in the secondary phase produces a spectrum of injury severity post-TBI. The Glasgow Coma Scale (GCS) is one of the main methods of determining the severity of a brain injury in acute trauma patients immediately following injury [22]. The GCS is scored based on three aspects of responsiveness: eye-opening, motor, and verbal responses [22]. This assessment of a patient's level of consciousness is scored out of 15, with 3 being the lowest and worst possible score and 15 being the best score [22].

Mechanisms of injury such as high-speed motor vehicle collisions or falls from great heights can cause severe TBIs that are often associated with GCS ≤8, skull fracture, intracranial abnormalities, prolonged post-traumatic amnesia and altered mental state, significantly reduced level of consciousness, diffuse axonal injury, and permanent disability or death [20, 22]. Similarly, patients who suffer a moderate TBI may also present structural/intracranial damage, but generally demonstrate less amnesia and altered mental state/consciousness and lower mortality than severe injuries with a GCS between 9 and 12 [20, 22]. While moderate and severe TBI can be associated with catastrophic consequences, they are estimated to represent ≤5% of all TBIs [23]. The majority of TBI patients sustain mild TBI (mTBI) which is generally not associated with skull fracture or positive neuroimaging with no/short-lived loss of consciousness and amnesia and is classified based on a GCS between 13-15 with symptom complaints [20, 22, 24]. In the case of mTBI, brain tissues still endure mechanical deformation and trauma, but mTBI-related complications are generally thought to be associated with disturbances in normal brain function in the absence of noteworthy structural damage.

Injury epidemiology

Epidemiological studies indicate that falls, motor vehicle collisions, assault, blast exposure, and participation in risky play/sport are the most common causes of TBI producing an estimated 27 million TBIs

globally per year [23, 25]. However, this estimate is likely an underrepresentation of the true incidence rate of TBI as mTBIs account for ~95% of all brain injuries. Many individuals who sustain mTBI do not present clinically, thus their injury goes unrecorded [26, 27] and many injuries sustained in polytrauma can be missed due to the focus on more severe injuries such as broken bones. The prevalence of persistent symptoms following mTBI is unclear with estimates ranging from 10-64% [28]. Inconsistencies in estimates of persistent mTBI symptoms within the literature appear to be dependent on the diagnostic criteria used combined with the non-specific nature of mTBI symptomology [28-32].

The burden of mTBI in New Zealand

In a New Zealand population-based longitudinal cohort study, nearly half of mTBIs were associated with significant impairment at one-year post-injury as determined by four or more mild to severe symptoms on the Rivermead Post-Concussion Symptoms Questionnaire [33]. Long-term negative effects of mTBI were detected in a subset of this cohort at four years post-injury when compared to age-sex matched controls [34]. Amongst this cohort, 88.6% of patients had sought medical attention within 24 hours of their injury and still went on to experience ongoing impairments in cognitive function and community participation (i.e., productivity, social relations, and engaging in activities in the community) [34].

The average cost of a moderate/severe TBI claim in New Zealand far exceeds that of a mTBI claim ($36,648 USD versus $4,636 USD) [35]. The volume of mTBI claims combined with the prevalence of prolonged complications associated with mTBI places an inordinate financial burden on the healthcare system (three times the total cost of moderate/severe TBI) and contributes to reduced quality of life for those personally affected [34-37]. While the mortality of mTBI may be low, these figures indicate a need to improve current rehabilitation protocols and clinical management pathways for mTBI patients.

This PhD thesis focussed on SOBIs because of: the high incidence rates in children and young adults [10]; the general desire to return to the activity that caused the SOBI as quickly as possible [8]; and growing concerns about the long-term neurological consequences of exposure to repeated SOBIs [38]. Between March 2010 and the end of February 2011, 291 SOBIs were sustained in the Hamilton and Waikato regions of New Zealand, accounting for 21% of all TBIs recorded within this period [10]. Of the 291 SOBIs, 98% were considered mTBIs and over half (51%) were sustained by youth under the age of 18 years old. For children (<16 years), SOBIs were most often caused by participating in cycling, rugby, football, or swimming; whereas in adults, SOBI was most often reported due to rugby, equestrian activities, or trail riding on motorbikes [10]. Of all recorded SOBIs in the study 46% were associated with risk of further complications [10].

Persistent complications

Attention has been dedicated to the identification of variables that can predict which patients are most likely to experience unfavourable recovery outcomes following mTBI/SOBI. Preinjury history of mental health problems and migraine have been associated with slower recovery trajectories [39]. Males tend to be at a higher risk of suffering a SOBI [10], while females are more likely to report greater symptom burden and experience worse recovery outcomes post-SOBI [39]. Greater acute and subacute symptom burden appears to be a strong predictor of delayed recovery [39]. Delayed presentation of >7days for an examination by a physician is indicative of longer recovery times in children [40], as is poor performance during a tandem stance static balance task [40, 41]. There is evidence to suggest that repeated mTBI, and the proximity of injuries to one another in time, may negatively influence recovery outcomes [42, 43]. High risk activities such as military service and/or collision sports like rugby may expose an individual to a heightened risk of multiple mTBIs, in turn increasing risk of prolonged recovery. Observational cohort studies have also identified that once athletes return to play following SOBI they are more likely to sustain a time-loss musculoskeletal lower extremity injury [44, 45]. While several variables appear to be predictors of longer recovery trajectories, they do not provide explanations as to why some individuals experience rapid and spontaneous clinical recovery following mTBI, while others suffer persistent symptoms.

Persistent symptoms are particularly worrisome in children and adolescents, as some research suggests that children and adolescents experience longer recovery trajectories than adults [46-48]. This seemingly increased vulnerability in youth is hypothesised to be a result of the ongoing development and/or final stages of maturation during childhood and adolescence compounded with the neurophysiological consequences of mTBI [49, 50], although results of studies investigating these hypotheses are inconsistent [39]. Differences in recovery outcomes within these observational studies may also be explained by differences in access to care between the children/adolescents compared to the adults in the samples. Whether due to an interaction with maturational changes and/or access to care, persistent symptoms and impaired neurophysiological processes in children and adolescents could have negative consequences on normal cognitive development [50]. Overall, the occurrence of ongoing mTBI-related symptom burden may lead to difficulty with psychosocial adjustment, deterioration of quality of life, and/or decreased financial independence due to diminished performance or absence from work or school [51]. For these reasons more conservative recommendations are endorsed for children and youth than for adults [52].

In recent years, the long-term consequences of unidentified and mismanaged SOBIs have become a topic of interest within academic literature and mainstream media. This increased attention has led to several high-profile lawsuits wherein players are claiming that teams/leagues in sports such as American football [53], ice hockey [54], and rugby [55] did not adequately communicate or mitigate the risks of playing these sports on long-term neurological health. Some researchers claim that exposure to repeated head/brain

impacts in collision sports directly leads to a progressive neurodegenerative disease called chronic traumatic encephalopathy that manifests in the form of irritability, impulsivity, aggression, depression, short-term memory loss and suicidal behaviour that usually begin 8–10 years after experiencing repetitive SOBI [38]. When considering the literature, these claims of cause and effect extend beyond the available evidence [56-58]. Nevertheless, athletes with a history of repeated impacts to the head/brain are suffering significantly reduced quality of life after retirement [53-55]. Rates of SOBI reporting and diagnosis have significantly increased in recent decades [59, 60] likely due to increased media attention and efforts to increase awareness. Since previous generations of athletes would have been competing at a time when SOBI was not considered a noteworthy injury by players, sporting bodies, and medical professionals they likely would have continued to play after sustaining a SOBI with little or no management and rehabilitation. Accumulation of multiple SOBIs with little time off to from training and competition to recover may have contributed to the chronic burden these athletes are suffering after retirement.

A clear explanation for why people experience persistent complications weeks, months, years, or decades following exposure to SOBI(s) remains elusive. One explanation is that past and current clinical management strategies fail to identify and address functional and neurobehavioural deficits that manifest because of SOBI induced disruptions in neurophysiological homeostasis [61]. Another potential explanation is that current technologies are not sensitive enough to detect damage following impacts that do not cause an athlete to become symptomatic, and an accumulation of these unacknowledged impacts leads to negative recovery outcomes. Improved understanding of how to effectively assess and manipulate the neurophysiological effects of SOBI under clinically realistic conditions may advance the clinical management patients receive. Through improved knowledge clinicians may be able assess, treat, and refer patients more effectively in the acute phase post-SOBI, subsequently reducing the likelihood of suffering another SOBI or musculoskeletal injury and the number of patients experiencing ongoing debilitating complications.

Pathophysiology to symptomology

By understanding injury mechanisms, clinicians are better equipped to manage the underlying issues responsible for the dysfunction and burden experienced by the injured individual. For example, iliotibial band syndrome and patellofemoral pain syndrome are common musculoskeletal injuries in athletes characterised by localised knee pain. While the knee is the site of the pain, biomechanical studies have identified that suboptimal strength and/or motor control of hip/trunk musculature are associated with unfavourable movement strategies which contribute to the development of these injuries [62]. This information helps clinicians direct their efforts towards improving strength and motor control further up the chain rather than solely focussing on the knee pain itself [62]. This example highlights the value of understanding the pathophysiological mechanisms responsible for mTBI symptoms, and that interventions

targeting these mechanisms may improve recovery outcomes. Although, in the case of mTBI, this will be more challenging than the musculoskeletal example provided due to the complexities of the brain.

Our knowledge regarding mTBI pathophysiology has rapidly expanded in recent decades thanks to efforts in the field of basic science. These investigations have identified that the secondary injury phase of mTBI is characterised by: ionic and neurometabolic dysregulation; neurovascular and autonomic uncoupling; axonal and cytoskeletal damage; impaired synaptic plasticity; neuroinflammation; disrupted blood brain barrier permeability and damaged cell membranes; impaired synaptic plasticity; and neuronal apoptosis (see [21] for a recent review detailing TBI pathophysiology).

Kenzie et al. have integrated a wide range of evidence into a causal-loop diagram to visualise factors influencing pathophysiology and recovery following mTBI (Figure 1.1) [61]. This work provides a framework to understand how cellular disruptions may impair neurological networks and subsequently manifest in symptom complaints and deficits at the experiential and social levels [61]. It is not yet well understood how much time is required for the cellular consequences of mTBI to resolve in humans as much of this knowledge was gained from animal models. Disruption to the brain's ionic equilibrium and metabolism typically requires 7-10 days to normalise in rodents, but likely requires more time in humans [42, 63]. It is possible that individuals with longer recovery trajectories suffer longer durations of cellular disruption. Alternatively, cellular disruption may resolve within a similar timeframe across patients, but patients who are slower to recover sustain greater magnitudes of cellular disruption which leads to more severe dysfunction in neurological networks. Identification of methods to understand how neurophysiological dysfunctions associate with clinical outcomes could improve the clinical assessment and management of mTBI.

Clinicians face challenges when trying to effectively assess and manage the return of mTBI patients to everyday activities. Unlike soft tissue or bony injuries, mTBI is commonly described as an "invisible" injury because there is no obvious bruising, swelling, or deformity upon physical examination and standard neuroimaging techniques typically do not detect structural abnormalities [18, 19]. Recent works with human participants using fluid biomarkers and advanced imaging have begun to corroborate aspects of the pathophysiological response after TBI first observed in animal models. Differences in markers of neuroinflammation [64], neuroplasticity [65], and axonal damage [66] have been observed in serum samples collected from athletes following SOBI compared to baseline/controls. MicroRNAs – non-coding RNAs that play a key role in gene expression and regulation of proteins [67] – appear to have promise as a diagnostic biomarkers of mTBI and can be measured non-invasively in saliva [68, 69]. Additionally, functional magnetic resonance imaging or diffusion tensor imaging have been demonstrated to be sensitive to impairments in brain function and neurological signalling following mTBI [13, 70-72].

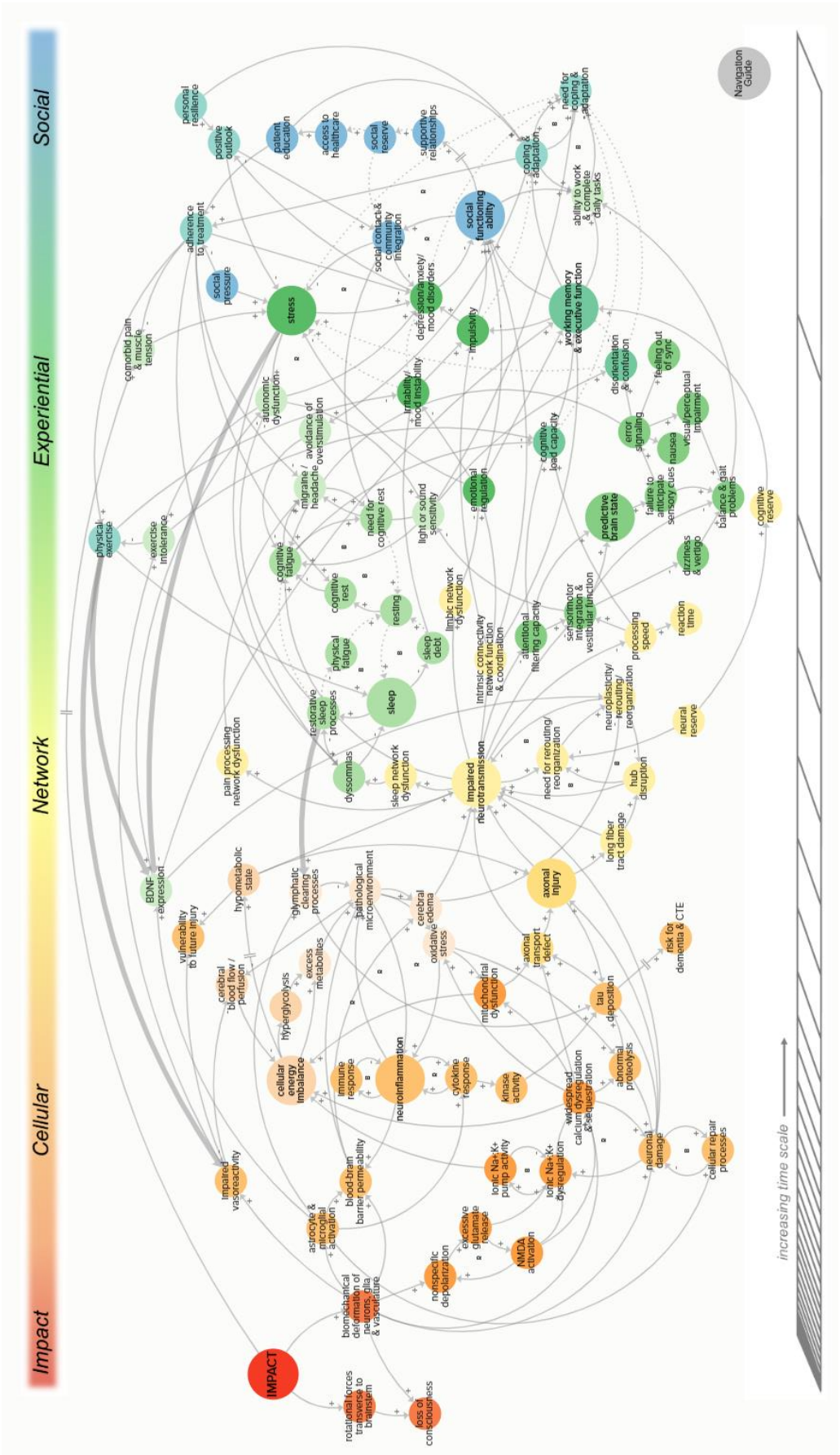


Figure 1.1 Causal-loops of mTBI developed by Kenzie et al. 2018 [61] (permission obtained from corresponding author)

Further development of these functional neuroimaging techniques and fluid biomarkers may become gold-standard measures in mTBI research and further our knowledge of how differences in cellular and network impairments relate to clinical presentation and outcomes. However, these works are still in relatively early stages of research and development. Even if/when these measures are shown to be valid and reliable, issues with accessibility and cost are likely to limit widespread integration within clinical practice. For these reasons, despite several promising candidates, no objective tools or biomarkers have been validated for use in clinical practice for mTBI [43]. This means that diagnosis must be made via clinical examination where self-reported symptoms are one of the key indicators used to guide clinical decisions [39, 43].

Current clinical recommendations

Clinical assessment

Regarding SOBI, current clinical guidelines state that physicians should implement a multi-faceted approach including a comprehensive history and physical examination [43], standardised evaluation of symptoms, attention, concentration and memory using the most recent version of the Sports Concussion Assessment Tool (SCAT-5) [43, 73], neurocognitive testing [43], and exercise stress testing [74] to assist identification of predominant symptom clusters for patients who remain symptomatic 21 days post-injury [75]. The SCAT-5 contains an immediate/on-field component and a clinical/off-field component, but only the clinical elements are described for relevance. The SCAT-5 includes a brief screening of: subjective symptom reports using a Likert scale (0 – no symptom, 6 – severe symptom) across 22 common symptoms; cognitive assessment of immediate memory, concentration, and delayed memory; static postural control; and neurological screening. Although the SCAT-5 is widely recommended for both sideline and clinical use, there are notable limitations when utilised in clinical environments.

First, pre-season baseline testing has been recommended to provide an individualised reference for evaluations occurring after an athlete has sustained a SOBI [76-80]. Financial and logistical barriers make acquisition of baseline testing challenging in community settings [81]. If the athlete is distracted during testing, misunderstands the testing protocol, or deliberately performs poorly during testing the validity of baseline data may be compromised [81]. Additionally, commonly implemented assessments of SOBI such as the SCAT-5 demonstrate sub-optimal test-retest reliability [76]. While acquisition of baseline data is recommended, these concerns about the reliability and validity of baseline assessments coupled with the practical limitations of acquiring this data for large numbers of athletes highlights a need to identify assessments tools that offer clinical utility, sensitivity, and specificity without the need for baseline data. Second, reliance on self-reported symptoms can be problematic because of the non-specific nature of mTBI symptoms and delayed symptom onset in some patients [29, 30, 32, 43, 82-86]. In the case of SOBI, some athletes underreport when they have sustained a SOBI and/or minimise related symptomology [4-8]. The most common reasons athletes might not report a SOBI are: they may not think the injury is serious enough

to need attention; they are motivated to avoid being withheld from training and competition; or they lack the awareness and knowledge to identify that they sustained an SOBI [4, 8]. Finally, the battery of cognitive assessments and balance testing within the SCAT-5 demonstrate ceiling effects and little clinical utility and validity beyond 2-3 days post-injury [73, 87, 88]. While the SCAT-5 is widely used, clinical practice would benefit from the identification of objective and robust methods of assessment that offer a higher degree of reliability, sensitivity, and specificity.

Predominant symptom clusters (PSCs) can be identified by criteria developed by Ellis et al. [75] to identify whether a patient's unresolved symptoms at ≥21-days post-injury have a predominantly physiological, vestibulo-ocular, or cervicogenic origin. These PSCs are determined using the patient's clinical history, results of their clinical examination, and performance on an incremental treadmill stress test following a Balke protocol [75]. Use of this incremental stress testing approach has been shown to be a safe, reliable, and useful method to differentiate the pathophysiological basis for unresolved symptoms [75, 89-91]. Briefly, physiological PSC refers to symptoms due to impairments resulting from alterations in global cerebral metabolism and autonomic uncoupling. Symptoms are often exacerbated by cognitive and physical loading [75]. Physiological PSC patients typically experience symptom exacerbation during treadmill testing [75]. Vestibulo-ocular PSC refers to symptoms due to dysfunction of the vestibulo-ocular system and symptoms are exacerbated by tasks that stress these systems such as reading or balancing [75]. Vestibulo-ocular PSC patients will often not experience symptom exacerbation achieving targeted heart rates of 85% HRmax predicted during a treadmill testing but will present with abnormal saccades, disrupted balance, etc. [75]. Cervicogenic PSC refers to symptoms due to dysfunction within the cervical spine somatosensory system. Symptoms may be exacerbated by rapid head movements and prolonged periods of cognitive or physical activity in poor postural positions [75]. Cervicogenic PSC patients will not typically experience symptom exacerbation during a treadmill test as outlined above but will often experience symptom exacerbation during palpation and/or active/passive movements of the cervical spine [75]. This pathophysiological approach to classifying persistent symptoms provides a framework to direct patients towards evidence-based treatment options that target the underlying cause for their burden [75].

Management and rehabilitation

Less than a decade ago consensus recommendations for the management of acute and persistent mTBI symptoms consisted entirely of cognitive and physical rest; including (but not limited to) no school or work, driving, screen time, chores, physical exercise, or activity that resulted in perspiration [92, 93]. The rationale for this rest-based approach was to provide the brain an opportunity to divert energy towards resolving the ionic and metabolic consequences of mTBI [42, 93]. Cognitive and physical rest was prescribed to individuals experiencing acute and persistent symptoms of mTBI alike, although there was no reported effectiveness of rest beyond the first 24-48 hours post-mTBI [94].

More recently, it has been recommended that clinicians advise patients to engage in cognitive and physical rest for the first 48 hours following mTBI followed by progressive reintroduction of cognitive and physical activities/sensory environments in a manner that does not worsen symptomology [43, 94-97]. Patients typically receive basic education about their injury and recovery expectations in addition to recommendations. If the patient does not positively respond to this approach by 2-3 weeks post-injury further evaluation and treatment options considered.

Once athletes with unresolved SOBI-related symptoms receive an appropriate examination an individualised exercise-based treatment program can be prescribed based on the PSC(s) likely contributing to their delayed symptom resolution [98]. Typically, this program consists of sub-symptom threshold aerobic exercise based on the patient's performance during treadmill testing, in combination with cervicogenic, and/or vestibulo-ocular exercises. Aerobic, cervicogenic, and vestibulo-ocular exercise prescription has been reported to be more effective at attenuating persistent mTBI symptoms than rest-based prescription [13, 14, 94, 96, 99-102]. Targeted cervicogenic and vestibulo-ocular exercise/physiotherapy can address mechanical neck disorders and cervicogenic dizziness as well as central/peripheral vestibular and proprioceptive dysfunction that occurs secondary to SOBI [98, 103-106]. A randomised controlled trial showed that participants with persistent symptoms of dizziness, neck pain and/or headache who received cervical spine and vestibular physiotherapy were nearly four times more likely to be recovered at eight weeks than participants who received range of motion exercises, postural education, and graded physical exertion [106]. Similarly, a randomised controlled trial indicated that early initiation of subsymptom threshold aerobic exercise within the first week post-SOBI was associated with earlier recovery outcomes than participants who performed a stretching program [14]. The benefits of aerobic exercise are hypothesised to provide the appropriate stimulus to create a favourable internal environment to resolve neurophysiological impairments caused by mTBI. The effectiveness of aerobic exercise to reduce persistent mTBI symptoms is still not understood in detail; but it is likely to be because of the effects of aerobic exercise on cognitive and autonomic function, affective symptoms, and regulation of neuroplasticity [107-115]. In parallel to exercise-based interventions, or if their symptom burden does not improve in response to these treatment strategies, athletes may also undergo assessment and receive treatment from a clinical neuropsychologist and/or occupational therapist.

Current rehabilitation model for mTBI/SOBI in New Zealand

In New Zealand the Accident Compensation Corporation (ACC) is the body established to carry out the Accident Compensation Act [116] through accomplishing their vision to "…create a unique partnership with every New Zealander, improving quality of life by minimising the incidence and impact of injury" [117]. The Accident Compensation Act and corresponding Corporation cover the costs of treatment following an accidental injury while also compensating the injured individual at 80% of their salary while they are unable

to work. When an injury occurs the patient can present to a variety of approved healthcare professionals (physicians, physiotherapists, occupational therapists, etc.) who will identify the correct code under which to submit a claim to ACC to cover the costs of the treatment the patient requires. While private practices do exist, this ACC process is how the majority of healthcare practitioners who work with injuries are compensated for their time in New Zealand. Only a physician can diagnose and code an mTBI in New Zealand.

Currently ACC does not have a defined acute model of care for individuals who sustain mTBI/SOBI. Despite the evidence presented in the previous section regarding the importance of early intervention in the first 1-2 *weeks* post-injury, the ACC mTBI service pathway is cumbersome and involves very little intervention in the first 1-2 *months* post-mTBI [118]. A patient suspected to have sustained an mTBI may receive a diagnosis from a general practice physician and brief education about cognitive/physical rest and progressive reintroduction of cognitive and physical activities/sensory environments in a manner that does not worsen symptomology [43, 94-97]. Only once a patient has been suffering from mTBI-related symptomology for several weeks post-injury are further physiotherapy, neuropsychology and/or occupational therapy options considered. These "wait and see" systems were likely developed based on previous estimates that 80-90% of mTBI patients would spontaneously recover in less than two weeks [93, 97]. However, more recent estimates suggest that up to nearly half of all mTBI patients in New Zealand may experience symptoms beyond this milestone (including sport-originated and non-sport injuries) [10, 33]; meaning a large proportion of individuals suffer debilitating symptoms for weeks before they can be enrolled in the ACC service. Due to scheduling constraints several weeks can elapse before patients receive specialist evaluation, and if further referral is required even more time can pass before treatments are initiated. Population and qualitative research has indicated great frustration on the behalf of mTBI patients with the lack of early initiatives and wait times within the current system in New Zealand [34, 119]. This process is the same from all mTBIs whether caused by a workplace injury, fall, sport, assault, or motor vehicle collision.

In an effort to address the shortcomings in the current New Zealand mTBI model, ACC funded a pilot program to evaluate the effectiveness of an evidence-based acute care clinical model for athletes who suffered a SOBI in Auckland, New Zealand. The acute care model adhered to the recommendations described in the previous section of this introduction (additional details can be found in Appendix 1). This pilot took place at Axis Sports Medicine and was led by Axis physicians Dr Stephen Kara and Dr Mark Fulcher, both who have extensive experience working with athletes with SOBI through their extensive experience in sports medicine and roles as team doctors for the Auckland Blues Super Rugby franchise and the New Zealand Men's Football team, respectively. Data collection for Chapters 3, 5, 6, and 8 took place while embedded within the Axis SOBI service to evaluate if neurophysiological approaches to assessing and treating SOBI could further advance current best practice.

Rationale and significance of research

Early and intense delivery of neurorehabilitation has been associated with improved functional recovery following moderate and severe TBI [120]. While current assessment and management recommendations for mTBI/SOBI are more advanced than the recommendations available a decade ago, New Zealand still predominantly adheres to a "wait and see" approach. Objective methods allowing early identification of patients likely to experience longer recovery trajectories who will need targeted treatment is lacking. Accurate and early prediction of these patients would allow for better allocation of resources to those most in need. Clinicians, and their patients, would also benefit from objective tools that classify subgroups based on underlying mechanisms of symptomology, and to understand differences in recovery trajectories. There is no current proactive means of reducing the damaging effects of mTBI in a manner that might improve recovery outcomes and decrease the likelihood of a patient experiencing persistent symptoms. Where current treatment strategies react to the clinical manifestations of the neurophysiological consequences of mTBI, proactive interventions could prevent or reduce the development of symptoms by favourably blunting the cellular damage and dysfunction in the form of neuroinflammation, altered neuroplasticity, blood brain barrier disruption, etc. that characterises the secondary injury phase of mTBI. Improvements in these areas could reduce the number of patients who experience persistent symptom burden and expedite return to normal daily activities and quality of life. Advancements in the field of neurophysiology may hold the key to achieving these objectives through rapidly expanding knowledge about how mTBI triggered disturbances in cellular and network functions relate to and cause experiential and social burden for patients affected by these injuries. Emerging evidence indicates that measures of functional cortical connectivity [121-123], autonomic and postural control [12, 124-128], and biomarkers of neuroplasticity [129] may provide objective insights to assist clinical mTBI diagnosis, management, and monitoring of symptom recovery. A concept diagram illustrating how these neurophysiological approaches may add value to clinical practice is provided in Figure 1.2.

The somatosensory cortex of the brain receives stimuli from the periphery and external environment, processes this stimuli, and rapidly coordinates a motor response [130]. Changes in cortical connectivity and function have been reported in the acute stage of mTBI recovery, and in individuals experiencing persistent symptoms [131, 132]. A non-invasive technique to quantify somatosensory function is to measure the relationship between stimuli applied to sensors in the skin, and the subsequent capacity to respond to stimuli quickly and accurately. Measurement of this somatotopic relationship provides insight into the information processing capacity of the central nervous system. Somatosensory function can be measured by a small, portable, easy to use device called a Brain Gauge developed by Cortical Metrics [121-123, 133-137]. This Brain Gauge is a sophisticated computer mouse, capable of emitting stimuli in the form of vibrations to the fingertips of the second and third digit of the non-dominant hand. The Brain Gauge testing protocol requires approximately 20 minutes and utilises a two-alternative forced choice paradigm to track

an individual's ability to discriminate between two stimuli. The Brain Gauge testing protocol has been reported to be sensitive to differences between individuals with and without mTBI, and tracking mTBI recovery over time [121-123]. Somatosensory assessments delivered by the Brain Gauge appear to be a potentially useful objective means to evaluate the central nervous system following mTBI; although the utility of these assessments to assist clinical decision making has yet to be explored under ecologically valid conditions.

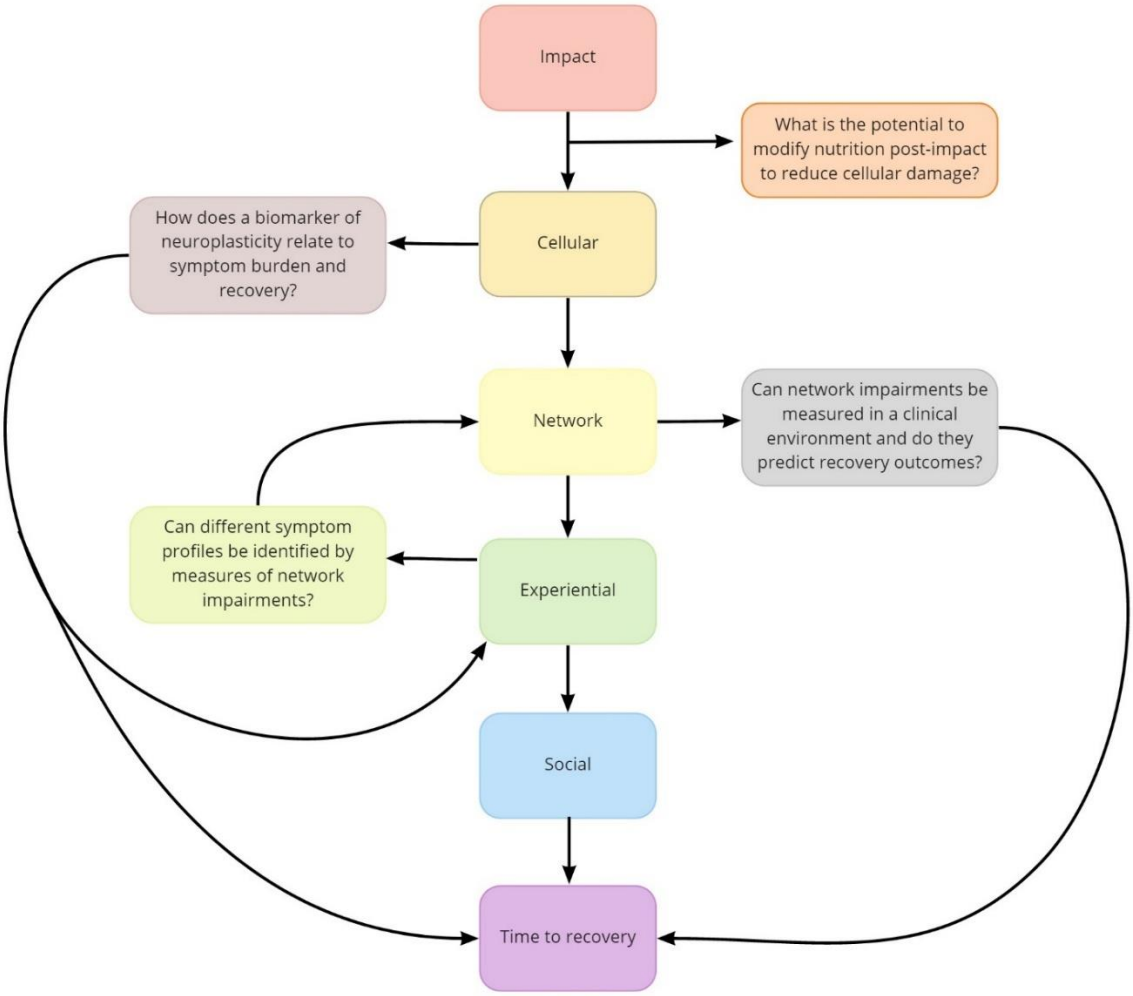


Figure 1.2 Concept diagram depicting how neurophysiological approaches to improve clinical management of SOBI were investigated in this thesis based on the framework provided by Kenzie et al. [61].

The combined usage of treadmill stress testing and PSC criteria allows clinicians to identify subgroups of SOBI patients who require different intervention programs. It is hypothesised that symptoms consistent with physiological PSC are manifestations caused by an uncoupling of the autonomic nervous system and cardiovascular system [12, 61, 75, 124]. Conversely, symptoms that characterise vestibulo-ocular PSC are thought to be due to underlying issues with sensorimotor integration and vestibular function [61, 75, 138,

139]. Measurement of heart rate variability using electrocardiography has identified differences in autonomic regulation during exercise, but not at rest, between athletes who recently sustained a SOBI and healthy controls [12, 124]. Similarly, differences in static/dynamic balance and gait have been reported between healthy controls and injured participants using force plates and accelerometers [127, 128, 140]. These observations highlight that technological methods may be sensitive to impairments not detected by clinical tests, and it is possible that individuals with vestibulo-ocular PSC may display a greater impairment in balance/gait than those with physiological PSC, and vice versa for autonomic regulation. However, these findings were primarily generated with laboratory grade equipment and methodologies which limit their translation. Development of wearable sensors that have been validated against lab equipment offer potential for transition research from the lab to the clinic [141, 142]. The integration of treadmill stress testing within clinical practice represents an opportunity to investigate whether electrocardiogram and accelerometer data acquired by a wearable sensor during testing can discriminate between patients with different PSCs. If this were the case it could significantly assist clinicians when classifying a given patient's PSC.

Neurotrophic factors are a family of proteins that have demonstrated a profound impact on neurons and the central nervous system as a whole [129, 143]. This family of proteins have been shown to be involved in regulating neuroplasticity — the ability of the brain to reorganize neurons throughout life to strengthen existing neuronal connections and create new connections [129, 143, 144]. This formation of new connections can also be extended to include the regeneration of nerve cells after a central nervous system injury. Neurotrophic factors may potentially serve as biomarkers to understand differences in mTBI recovery outcomes. Of the neurotrophic factors, brain-derived neurotrophic factor (BDNF) plays a key role in regulating neuroplasticity and its known functions demonstrate potential as a non-pharmacological intervention to benefit impaired neurological abilities [145]. BDNF can be collected either through blood serum or saliva samples. Decreased levels of BDNF have been reported in neurodegenerative diseases such as Alzheimer's Disease and Huntington's Disease, and in neuropsychiatric conditions such as depression and schizophrenia [129, 146]. To date, there have been limited studies investigating changes in BDNF concentrations in human subjects after acute mTBI and over time throughout mTBI recovery. Prior investigations on animal models of TBI reported that low levels of BDNF within brain tissue were associated with decreased cognitive performance compared to animals with higher tissue levels of BDNF [129]. The association between changes in BDNF concentrations and symptom reports following mTBI does not appear to have been explored in human subjects. Salivary-BDNF may represent a non-invasive option to expedite our understanding of how a patient's underlying neuroplastic environment relate to recovery and symptom burden.

Preliminary findings also suggest modification of dietary intake in the form of fasting [147] or a ketogenic diet [148] and/or supplementing with anti-oxidants [149], creatine [150], or polyunsaturated fatty acids

[151] demonstrate potential to proactively attenuate the damaging effects of secondary injury following mTBI. Yet there are no reviews which consolidate this information to determine which nutrition strategy/strategies and methods of implementation would be most beneficial for mTBI/SOBI patients. While the rationales and initial findings for these assessment methods and potential nutrition interventions appear promising, the translational potential and clinical utility of these candidates are unknown and require investigation.

Questions, approach taken, and structure of the thesis

The overall aim of the thesis was to evaluate how a diverse collection of evolving neurophysiological approaches to assessing and managing mTBI might translate from bench to bedside for SOBI. The two main research questions were "what factors influence time to recover following SOBI?" and "what is the translational potential of neurophysiological approaches to advance clinical management of SOBI?"

Approach

Due to the complexity of mTBI many researchers focus their efforts in a specific subdiscipline to gain intimate knowledge about one aspect of pathophysiology, epidemiology, psychological burden, etc. However, holistic research initiatives identifying how to link these valuable works together require attention to translate this knowledge to the benefit of mTBI patients. This PhD concentrated on evaluating how/if a diverse collection of evolving neurophysiological approaches to assessing and managing mTBI might translate from bench to bedside. The purpose of structuring the thesis in this way was to develop a diverse technical skillset and multidisciplinary understanding of what is required to improve translation of promising findings from highly controlled studies into real-world clinical environments.

Structure

This document follows a manuscript structure and is comprised of seven studies to address the objectives and research questions of this thesis (Figure 1.3). The manuscript for each study represents an article that has been accepted in, submitted to, or formatted for relevant peer-reviewed journals. A linking paragraph is provided between Chapters to develop an overarching narrative throughout the thesis. This linking paragraph is accompanied by bullet points detailing new technical skills and expertise gained through conducting each study. While chapters were originally formatted for different journals, referencing formatting has been standardised throughout the thesis for consistency.

The studies within this thesis are presented in two separate sections, with the studies within each section building towards answering the two main research questions of this PhD. Section 1 is comprised of Chapters 2 and 3 and builds towards answering the question "what factors influence time to recovery following SOBI?". Before investigating the translational potential of neurophysiologically-based approaches

it is necessary to establish the strengths, weaknesses, and gaps in current clinical practice to serve as a benchmark for comparison. Chapter 2 presents a case study of an individual experiencing persistent debilitating symptoms seemingly related to a history of SOBI and highlights the consequences that can occur when patients do not receive comprehensive and thorough assessments. Chapter 3 is a retrospective cohort study using prospectively collected data from a dedicated SOBI clinic implementing current best practice recommendations (details in Appendix 1). These data were analysed to determine which aspects of initial clinical assessment best predict recovery trajectory.

Section 2 consists of two parts encapsulating Chapters 4 to 8 which aimed to answer "what is the translational potential of neurophysiological approaches to advance clinical management of SOBI?". Exercise has become a cornerstone in the management of athletes with SOBI, but little is known about interventions that proactively target the secondary injury phase of TBI in a manner that would assist recovery. Some symptoms (i.e. headache or sleep disruption) can be managed with pharmaceuticals, but there is no pharmaceutical agent that can blunt the pathophysiological mechanisms that propagate symptomology. Lifestyle factors such as diet and nutrition play crucial roles in maintaining neurological function and overall brain health. In this regard, modification of dietary intake and/or nutritional supplementation post-TBI demonstrate potential to attenuate damaging effects of secondary injury following TBI by simultaneously acting upon multiple neurophysiological pathways. In Section 2 Part A the first systematic review to consolidate the available evidence describing how nutritional interventions can improve neurophysiological impairments following TBI is presented in Chapter 4. Only animal studies have evaluated these relationships with no studies conducted to date that have evaluated neurophysiological outcomes in response to nutrition interventions in humans, likely due to invasiveness, costs, and logistics. Therefore, Section 2 Part B (Chapters 5, 6, 7, and 8) evaluated the translational potential of neurophysiologically-based methods to assess impairments and track recovery post-SOBI under ecologically valid conditions. Identification of ecologically valid objective assessments that can assist diagnostic decision making, predicting recovery outcomes, subgroup classification, and understanding differences in symptom resolution are essential to improving more accurate and proactive clinical assessment and to evaluating the efficacy of novel interventions (Figure 1.2). Chapter 5 evaluated the clinical utility of tactile somatosensory assessments to assist clinicians when making SOBI diagnosis and management decisions. Chapter 6 investigated whether a machine learning algorithm could accurately classify SOBI patients with physiological PSC versus vestibular PSC using accelerometer and electrocardiogram data collected by a wearable sensor during treadmill stress testing. Chapter 7 consisted of a pilot study on healthy individuals to investigate trends in intra-individual variations in salivary-BDNF to understand if saliva may be a practical biological sample for the measurement of BDNF in clinical populations. Chapter 8 built on the findings of Chapter 7 by assessing salivary-BDNF concentrations at multiple timepoints over the course of clinical

recovery and how BDNF relates to subjective symptom reports at each timepoint in patients presenting with SOBI to a specialised outpatient clinic.

At the outset of this PhD the plan was to link Chapters 2-8 together with an eighth study that would evaluate the feasibility of integrating a nutritional intervention (as determined by Chapter 4) into current clinical best practice for the management of SOBI. A research protocol was developed (Appendix 2: Protocol for ω-3 supplementation feasibility study, logistics were in order, ethical approval had been obtained (Appendix 3), and the study was set to launch in late April 2020 but was derailed because of the COVID-19 pandemic. Due to the uncertainty around when data collection could take place because of lockdown protocols, combined with the body of work already conducted, a decision with the supervision team was made to omit the feasibility study from this thesis.

Finally, Chapter 9 presents a general discussion summarising the overall findings of this thesis. Contributions to the literature, limitations, and future directions are discussed and a personal reflection of the PhD process is provided.

Preface

Chapter 1: Introduction and Rationalisation

Section 1: What factors influence time to recovery following SOBI?

Chapter 2: Case study

- **McGeown, J. P.**, Hume, P. A., Kara, S., Neary, J. P., & Gardner, W. (2019). Is it really the result of a concussion? Lessons from a case study. *Sports Med Open,* 5(1), 8. https://doi.org/10.1186/s40798-019-0181-4

Chapter 3: Retrospective cohort study

- **McGeown, J. P.**, Kara, S., Fulcher, M., Crosswell, H., Borotkanics, R., Hume, P. A., . . . Theadom, A. (2019). Predicting Sport-related mTBI Symptom Resolution Trajectory Using Initial Clinical Assessment Findings: A Retrospective Cohort Study. *Sports Medicine.* https://doi.org/10.1007/s40279-019-01240-4

Section 2: What is the translational potential of neurophysiological approaches to advance clinical management of SOBI?

Chapter 4: Systematic Review

- **McGeown, J. P.,** Hume, P. A., Theadom, A., Quarrie, K. L., & Borotkanics, R. (2021). Nutritional interventions to improve neurophysiological impairments following traumatic brain injury: A systematic review. *Journal of Neuroscience Research*, 99(2), 573-603. https://doi.org/10.1002/jnr.24746

Chapter 5: Clinical cohort study

- **McGeown, J. P.,** Hume, P. A., Kara, S., King, D., & Theadom, A. Preliminary evidence for the clinical utility of tactile somatosensory assessments of sport-related mTBI.
  Under review – *Sports Medicine - Open*

Chapter 6: Cross-sectional study

- **McGeown, J. P.,** Hume, P. A., Theadom, A., Kara, S., & Russell, B. A deep learning approach to classifying sport-originated brain injury subgroups using wearable sensor data acquired during exercise stress testing: A pilot study.
  Intended journal for publication – *Artificial Intelligence in Medicine*

Chapter 7: Experimental study

- **McGeown, J. P.,** Hume, P. A., Quarrie, K.L., Theadom, A., & Dulson, D.K. Shortcomings of saliva as a practical means to measure intra-individual variation of BDNF – A pilot study.
  Submitted to – *Scandinavian Journal of Clinical and Laboratory Investigation*

Chapter 8: Clinical cohort study

- **McGeown, J. P.,** Hume, P. A., Quarrie, K.L., Theadom, A., Kara, S., & Dulson, D.K. (2021). Does salivary-BDNF relate to symptom burden over the course of clinical recovery following sport-originated brain injury?
  Submitted to – *Neurorehabilitation and Neural Repair*

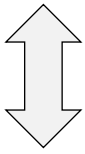
Chapter 9: Summary and Conclusions

Figure 1.3 Flow of thesis.

Table 1.1 Research key points and links between chapters.

Chapter and Title	Chapter Content - Question / Rationale / Findings
1 **Introduction and Rationalisation.**	**Main Questions of the Thesis:** 1. What factors influence time to recovery following SOBI? 2. What is the translational potential of neurophysiological approaches to advance clinical management of SOBI? **Secondary Questions of the Thesis:** • What is the underlying source of chronic symptom complaints for an individual with a history of multiple SOBIs? • Which items in the initial clinical assessment of SOBI predicted whether an athlete became asymptomatic in less than 14 days? • What nutritional interventions introduced post-TBI demonstrate the greatest potential to serve as proactive interventions to blunt the neurophysiological consequences of TBI? • Do tactile somatosensory assessments demonstrate potential clinical utility to assist SOBI diagnosis, prognosis, and recovery decisions? • Can a deep learning approach accurately classify SOBI patients with predominantly physiological versus predominantly vestibulo-ocular symptoms using wearable sensor data collected during the Buffalo Concussion Treadmill Test? • How do timing of sampling and delays in storage influence intra-individual salivary-BDNF protein concentrations in healthy participants? • How do salivary-BDNF concentrations relate to subjective symptom reports over the course of clinical recovery in a cohort of SOBI patients presenting to a specialised outpatient clinic?

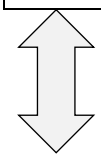
Section 1. Factors that influence time to recovery post-SOBI

Chapter and Title	Chapter Content - Question / Rationale / Findings
2 **Is it really the result of concussion? Lessons from a case study.**	**Question:** • What is the underlying source of chronic symptom complaints for an individual with a history of multiple SOBIs? **Rationale:** • Some individuals with a history of SOBI report symptom complaints and functional deficits for months or years post-injury. There is a need to understand underlying factors that contribute to these persistent complaints. **Approach:** • Case study. **Findings:** • Despite a history of multiple SOBIs, Participant A's persistent symptom reports were associated with peripheral vestibular dysfunction and otolithic dysfunction seemingly unrelated to his SOBI history. **Novel Contributions:** • Case study showcasing that increased awareness surrounding SOBI can lead to lazy diagnostic approaches for individuals with a history of SOBI who are suffering symptoms that are comorbid with other conditions. • Highlighting current lack of diagnostic precision for SOBI-related symptoms that are also comorbid with other conditions. • Example of level of thoroughness and interdisciplinary collaboration required to identify and treat the most likely source of symptoms.
 *Link between Chapters 2 and 3:*	Having identified the consequences of inadequate assessment and the benefits of a thorough multi-disciplinary approach, the next step was to identify what components of initial clinical assessment at a specialised SOBI clinic predict which patients are most likely to experience longer recovery trajectories.

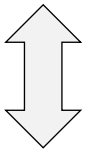
Chapter and Title	Chapter Content - Question / Rationale / Findings
3 **Predicting SR-mTBI recovery trajectory using initial clinical assessment findings.**	**Question:** • Which items in the initial clinical assessment of SOBI predicted whether an athlete became asymptomatic in less than 14 days? **Rationale:** • Some SOBI patients experience symptoms for weeks or months post-injury, while others recover in less than two weeks. • Symptom burden is a key criterion for determining clinical recovery. • A model that can predict which patients are likely to take >14 days to recover based on initial assessment findings would assist allocation of resources to patients most likely to require them. **Approach:** • Retrospective analysis of prospectively collected data. Development and validation of prognostic model. **Findings:** • 50% of SOBI patients experienced symptoms >14 days. • Identification of the predominant symptom cluster during initial clinical assessment was predictive of SOBI symptom resolution. • A prognostic discriminant model consisting of participant sex, predominant symptom cluster, and Positive Symptom Total, classified >14-day vs ≤14-day symptom resolution trajectories with 76-81% accuracy. **Novel Contributions:** • This is the first athlete patient cohort assessed using predominant symptom cluster classification criteria during the *initial assessment* of SOBI (rather than at 21 days post-injury for participants with unresolved symptom complaints). • Identification of predominant symptom clusters associated with worse recovery outcomes. • One of few studies that presented model performance on training data as well as validation data. • Modeling may assist better prognosis during initial clinical consultation and result in earlier initiation of referrals and treatment modalities for individuals predicted to experience > 14-day symptom resolution; potentially facilitating improved SOBI recovery times.
Link between Sections 1 and 2:	Having developed a model that accurately predicts which patients are likely to experience longer recovery trajectories and require early access to resources and interventions, it was important to identify potential interventions that might proactively reduce the overall neurophysiological damage caused by SOBI.

Section 2. Translational potential and clinical utility of neurophysiological approaches to advance management of SOBI

Chapter and Title	Chapter Content - Question / Rationale / Findings
4 **Nutritional interventions to improve neurophysiological impairments following traumatic brain injury: A systematic review.**	**Question:** • What nutritional interventions introduced post-TBI demonstrate the greatest potential to serve as proactive interventions to blunt the neurophysiological consequences of TBI? **Rationale:** • Lifestyle factors such as diet and nutrition play crucial roles in maintaining neurological function and overall brain health. • Modification of dietary intake and/or nutritional supplementation post-TBI demonstrate potential to attenuate the damaging effects of secondary injury following TBI by simultaneously acting upon multiple neurophysiological (NP) pathways. • An added advantage of nutritional interventions (NUTs) is the "over-the-counter" accessibility of food and supplements which could benefit patients post-TBI as an affordable means to alter the secondary damage of TBI when availability of medical services may be limited. **Approach:** • Systematic review **Findings:** • No studies have evaluated the effect of a NUT on NP outcomes of TBI in humans. • The overall reporting of methods and results of included pre-clinical studies was poor, limiting the biofidelity of the evidence. Therefore, these limitations prohibit a decisive recommendation about which NUTs will benefit clinical TBI patients. • Based on animal studies, anti-oxidants, branched chain amino acids, and ω-3 polyunsaturated fatty acids appear to be promising candidates, but more research is needed to determine translational potential. **Novel Contributions:** • First review to systematically consolidate all evidence for all non-invasive nutritional interventions and their effects on neurophysiological outcomes after TBI. • Identification of methodological limitations that need to be addressed in future research to increase likelihood of translation. • Recommendations on ways to address methodological limitations and which NUTs appear to be the most promising candidates for potential translation.
Link between Chapters 4 and 5:	Having identified the strengths and weaknesses of the literature for NUTs for TBI provides a platform to begin to explore the feasibility of conducting clinical trials to determine if such interventions can complement and improve clinical practice. Yet, no studies have evaluated how NUTs influence NP outcomes in TBI patients. This is likely due to costs, logistics, and ethical considerations of measuring NP outcomes in humans. There is a need to identify objective, accurate, affordable, and ecologically valid NP measures for SOBI trials Such measures would aid clinical decision making while also enabling the evaluation of NUTs for TBI patients.

Chapter and Title	Chapter Content - Question / Rationale / Findings
5 **Preliminary evidence for the clinical utility of tactile somatosensory assessments of sport-related mTBI.**	**Questions:** • Do tactile somatosensory assessments demonstrate potential clinical utility to assist SOBI diagnosis, prognosis, and recovery decisions? **Rationale:** • After mTBI somatosensory function (the brains ability to gather sensory information, process the information, and coordinate a motor response) can be impaired. • The Brain Gauge is a quick somatosensory assessment that may be a useful metric to objectively assess neurophysiological function in a clinical setting. • No studies have evaluated the utility of Brain Gauge somatosensory assessments to provide objective information that might assist diagnostic and management decisions for SOBI patients under ecologically valid conditions. **Approach:** • Clinical cohort study. **Findings:** • The Brain Gauge assessments evaluated poorly discriminated between cases belong to the SOBI sample or a healthy reference sample. • These assessments also did not provide prognostic value to identify participants with different recovery trajectories. • Two of three assessments evaluated appear to lack sensitivity to differentiate individuals who recently sustained SOBI performance consistent with healthy of individuals. While the third suggested neurophysiological abnormalities persist beyond clinical recovery. **Novel contribution:** • First study to independently investigate clinical utility of Brain Gauge assessments under ecologically valid conditions. • Presented a novel approach to pool and simulate a healthy comparison group using control data from previous investigations. • There is limited initial evidence for the use of BG-SA to assist diagnostic decision making or to predict SOBI recovery trajectory under ecologically valid conditions. • Findings do not provide sufficient justification to recommend the allocation of time and resources to conduct the Brain Gauge assessments evaluated to assist clinical management of SOBI patients at this time.
 *Link between Chapters 5 and 6:*	Previous reports suggested Brain Gauge somatosensory assessments could be a useful tool to aid diagnostic and prognostic decision making for clinicians working with SOBI patients. Our results suggest limited diagnostic and prognostic value of the somatosensory assessments evaluated when administered under ecologically valid conditions. The addition of wearable sensors and machine learning algorithms to established clinical tests may assist accurate identification of subgroups of SOBI patients who require different management plans.

Chapter and Title	Chapter Content - Question / Rationale / Findings
6 **A deep learning approach to classifying sport-originated brain injury subgroups using wearable sensor data acquired during exercise stress testing: A pilot study.**	**Question:** • Can a deep learning approach accurately classify SOBI patients with predominantly physiological versus predominantly vestibulo-ocular symptoms using wearable sensor data collected during the Buffalo Concussion Treadmill Test (BCTT)? **Rationale:** • Although injury mechanisms of SOBI may be similar across patients, it is becoming increasingly clear that patients cannot be treated as one homogenous group as several predominant symptom clusters (PSCs) have been identified, each requiring specific and individualised treatment plans. • Exercise stress testing using a treadmill has become a key clinical assessment of SOBI and classification of PSCs, but still relies on clinical experience and honest symptom reporting. • This established testing procedure provides an opportunity to acquire physiological (electrocardiogram) and biomechanical (accelerometer) data during treadmill testing using a wearable sensor. • There is potential for machine learning algorithms, specifically deep learning techniques, to support medical decision making by automatically detecting the most important features related to patient outcomes. • The capacity of a deep learning model trained using time series signals from ECG and accelerometry during gait to classify PSC subgroups in SOBI patients has not been previously explored. **Approach:** • Cross-sectional study. **Findings:** • Utilising a leave-one-out cross-validation approach, moderate levels of physiological versus vestibulo-ocular PSC classification accuracy were observed when a convolutional neural network was trained using accelerometry and electrocardiography data acquired during a BCTT. • Our results provide proof of concept that incorporation of wearable sensors during BCTT and deep learning techniques have potential to assist decision making for clinicians working with SOBI patients. **Novel contribution:** • This is the first study to explore whether a deep learning approach could accurately classify PSCs in SOBI patients using sensor data collected during BCTTs. • Overall, these findings suggest that with further research the addition of wearable sensors during clinical tests like the BCTT, combined with deep learning models, may have clinical utility to assist clinicians when classifying PSCs in SOBI patients. • Recommendations for future studies to determine potential generalizability of these initial findings.



Link between Chapters 6 and 7:

A combination of wearable sensors and deep learning techniques appear to be a promising means of supporting the development of individualised treatment plans that could easily fit within clinical practice. Identification of a non-invasive biomarker than provides insight regarding differences in recovery outcomes would be valuable. Preliminary pilot work is needed to understand the potential to acquire such a biomarker under ecologically valid conditions.

Chapter and Title	Chapter Content - Question / Rationale / Findings
7 **Shortcomings of saliva as a practical means to measure intra-individual variation of BDNF – A pilot study.**	**Question:** • How do timing of sampling and delays in storage influence intra-individual salivary-BDNF protein concentrations in healthy participants? **Rationale:** • Brain derived neurotrophic factor (BDNF) serves as a biomarker of neuroplasticity – the brains ability to adapt and change throughout life and after injury. • BDNF is typically measured in serum or plasma collected by venipuncture but can also be measured non-invasively via saliva. • Acquisition of blood or saliva samples to measure BDNF typically occurs under strict conditions which are not practically feasible in many clinical settings. • A non-invasive and practical means to assess BDNF under ecologically valid clinical conditions would provide a useful tool to understand how neuroplasticity is affected by neurological conditions (i.e., mTBI), and how intra-individual changes in BDNF relate to recovery outcomes. • It is necessary to investigate how sampling at different times of day and delays in storage might affect interpretation of an individual's salivary-BDNF concentration from one timepoint to the next. **Approach:** • Experimental study **Findings:** • Findings from healthy participants suggest that measurement of BDNF protein concentrations via saliva is not a valid or practically advantageous means of accurately comparing intra-individual changes in BDNF from one timepoint to another. • While collecting saliva by passive drool offers a non-invasive means of measuring BDNF this advantage appears to be offset by the risk of sampling error during collection. **Novel contribution:** • Results should not discourage future research into the potential clinical applications of BDNF as a biomarker to advance understanding of neurological conditions; our findings highlight that the best method to measure BDNF in ecologically valid environments remains elusive. • Future studies should replicate the experiments within this study using venous and capillary blood samples or salivary-microRNAs related to BDNF expression to determine if clearer intra-individual trends in BDNF variation can be observed.
Link between Chapters 7 and 8:	This pilot study with healthy individuals highlights some potential issues that prevent intra-individual comparisons of salivary-BDNF from one timepoint to another when sampled under clinically representative conditions. These findings indicate that group-level analysis is a more appropriate starting point to evaluate whether salivary-BDNF concentrations relate to symptom reports over the course of clinical recovery following SOBI.

Chapter and Title	Chapter Content - Question / Rationale / Findings
8 **Does salivary-BDNF relate to symptom burden over the course of clinical recovery following sport-originated brain injury?**	**Question:** • How do salivary-BDNF concentrations relate to subjective symptom reports over the course of clinical recovery in a cohort of SOBI patients presenting to a specialised outpatient clinic? **Rationale:** • BDNF serves as a biomarker of neuroplasticity – the brains ability to adapt and change throughout life and after injury. • Previous research indicates diagnostic and prognostic utility of serum-BDNF in a sample of TBI patients presenting to hospital on day of injury. • Fear of needles in the general population and logistical challenges of acquiring blood samples are considerable barriers to furthering our knowledge of the role BDNF plays in mTBI/SOBI recovery outcomes. • Since BDNF can also be quantified in saliva, this provides an alternative to allow greater sampling to expedite our knowledge of how BDNF changes over the course of clinical recovery after SOBI. • No study has attempted to measure BDNF via saliva in a clinical setting under real-world conditions; nor evaluated whether any relationships exist between salivary-BDNF concentrations and SOBI-related symptom reports over the course of clinical recovery. **Approach:** • Clinical cohort study **Findings:** • Our results do not show any relationships between salivary-BDNF concentrations (measured via passive drool under ecologically valid conditions) and SOBI-related symptom reports measured across multiple timepoints over the course of clinical recovery. • The most plausible explanation for these unexpected findings is the inherent risk of sampling error when measuring BDNF in saliva. • For this reason, we do not recommend investment of time and resources towards the quantification of BDNF in saliva in future studies. **Novel contribution:** • First study to evaluate salivary-BDNF in a clinical population under real-world conditions. • First study to evaluate changes in salivary-BDNF concentrations in a clinical cohort with SOBI. • Given the abundance of research demonstrating BDNF-mediated neuroplasticity in neurological conditions, concluding that no relationship exists between BDNF and symptom burden over the course of clinical recovery following mTBI/SOBI seems premature.

Chapter	Chapter Content - Conclusions
9 **Summary and Conclusions**	• Insufficiencies in clinical pathways appear to be the greatest modifiable factor that influences recovery outcomes post-SOBI. • An acute model of care delivered by clinicians who are experienced with SOBI can significantly reduce the number of patients who go on to experience prolonged symptoms. • Patients with vestibulo-ocular symptomology appear particularly vulnerable to negative outcomes. • Figures claiming the majority of SOBI patients will be recovered by 14-days post-injury are out of date and require updating as it appears closer to 50% of patients will experience symptoms beyond this timeframe. • Overall, current best practice relies heavily on clinical experience/training and honest symptom reporting on behalf of the patient and would benefit from more objective methods to assist clinical decision making. • Nutritional interventions appear to have merit as a potential method to reduce the secondary damage of mTBI/SOBI in a manner that might reduce the amount of time required to recover but the biofidelity of the animal models providing this evidence is low • Neither salivary-BDNF or Brain Gauge somatosensory assessments appear to offer utility to improve clinical assessment of SOBI, nor to evaluate the potential benefit of a nutritional intervention. • The potential value of using wearable sensors and machine learning algorithms to collect and analyse objective data during an already established clinical test is the most promising finding but requires a great amount of development. • Overall, the evidence does not suggest that nutritional interventions or neurophysiological assessments will be coming to the aid of clinicians and their SOBI patients in the near future. • Future studies need to prioritise study designs that emphasise biofidelity and ecological validity so that findings are more likely to translate. • In the interim, responsibility falls on healthcare organisations, educational institutes, and sporting bodies to improve the education, standards, and pathways moving forward to reduce the burden caused by persistent complications following SOBI.

Research publications resulting from this doctoral thesis

Section 1: Understanding sport-related mTBI patients recovery outcomes

Chapter 2: McGeown, J. P., Hume, P. A., Kara, S., Neary, J. P., & Gardner, W. (2019). Is it really the result of a concussion? Lessons from a case study. *Sports Med Open, 5*(1), 8. https://doi.org/10.1186/s40798-019-0181-4

Chapter 3: McGeown, J. P., Kara, S., Fulcher, M., Crosswell, H., Borotkanics, R., Hume, P. A., . . . Theadom, A. (2019). Predicting Sport-related mTBI Symptom Resolution Trajectory Using Initial Clinical Assessment Findings: A Retrospective Cohort Study. *Sports Medicine*. https://doi.org/10.1007/s40279-019-01240-4.

Section 2: Translational potential and clinical utility of neurophysiological approaches

Chapter 4: McGeown, JP, Hume, PA, Theadom, A, Quarrie, KL, Borotkanics, R. Nutritional interventions to improve neurophysiological impairments following traumatic brain injury: A systematic review. *J Neurosci Res*. 2021; 99: 573– 603. https://doi.org/10.1002/jnr.24746

Other publications since starting the Doctor of Philosophy

McGeown, J., Hume, P. A., & Hardaker, N. (2019). Sport originated brain injury (SOBI): An update for physiotherapists. *New Zealand Physiotherapy Journal, 8*, 20-21.

Hume, P. A., King, D., **McGeown, J.**, & Theadom, A. (2019). Sports-related concussion, mild traumatic brain injury or sport-originated brain injury (SOBI): A more useful term. *New Zealand Journal of Sports Medicine*. *45*(2), 64-67.

Kara, S., Crosswell, H., Forch, K., Cavadino, A., **McGeown, J.**, & Fulcher, M. (2020). Less Than Half of Patients Recover Within 2 Weeks of Injury After a Sports-Related Mild Traumatic Brain Injury: A 2-Year Prospective Study. *Clin J Sport Med, 30*(2), 96-101.

Manuscripts submitted for publication

Chapters 5: McGeown, J. P., Hume, P. A., Kara, S., King, D., Theadom, A. Preliminary evidence for the clinical utility of tactile somatosensory assessments of sport-related mTBI. *Submitted to Sports Medicine – Open*

Chapter 7: McGeown, J.P., Hume, P.A., Quarrie, K.L., Theadom, A., Dulson, D. Shortcomings of saliva as a practical means to measure intra-individual variation of BDNF – A pilot study. *Submitted to Scandinavian Journal of Clinical an Laboratory investigation*

Chapter 8: McGeown, J.P., Hume, P.A., Quarrie, K.L., Theadom, A., Kara, S., Dulson, D. Assessing the relationship between symptom burden and salivary-BDNF over the course of clinical recovery following sport-originated brain injury. *Submitted to Neurorehabilitation and Neural Repair*

Conference and workshop presentations

McGeown, J. (2018) "Implications for biomechanists: Research needed to help address mild traumatic brain injury," *ISBS Proceedings Archive*: Vol. 36: Iss. 1, Article 255

Hume, P. A., **McGeown, J.**, & Fulcher, M. (2018, 4-7th October 2018). *Sports-related concussion - cases and management workshop*. presented at the meeting of the 2018 Annual Scientific Meeting New Zealand and Australian Associations of Musculoskeletal Medicine, Heritage Hotel, Auckland

McGeown, J. P. (2019). Key components of knowledge transfer for concussed athletes. *Sports Medicine New Zealand – Auckland Branch.* Sports concussion assessment and management. A practical workshop and seminar series, August 31st, 2019.

McGeown, J.P. & Kara, S. (2019). Sports-related concussion - A new model of care. Axis Sports Medicine Clinic - An ACC collaborative pilot. Geneva Healthcare Brainstorm International Concussion Conference 2019, Auckland.

McGeown J.P. (2020). Predicting recovery outcomes after sport-related mTBI. AUT Traumatic Brain Injury Network Public Forum,

McGeown, J.P. (2020). Neurophysiological abnormalities exist beyond clinical recovery following sport-related mTBI. Sport Medicine New Zealand North/South Conference 2020, Tauranga.

Hume, P. A., M. Bussey, **J. McGeown,** N. Draper and K. Quarrie (2020). Invited Concussion Panel: Should contact in sports be limited for children to reduce SOBI? Sport and Exercise Science New Zealand. Christchurch, SESNZ.

Section 1:
Factors that influence time to recovery post-SOBI

Chapter 2: Is it really the result of concussion? Lessons from a case study.

This chapter comprises the following paper published in *Sports Medicine – Open:* **McGeown, J. P.**, Hume, P. A., Kara, S., Neary, J. P., & Gardner, W. (2019). Is it really the result of a concussion? Lessons from a case study. *Sports Med Open, 5*(1), 8.

Author contribution

McGeown, J. P. 80%, Hume, P. A. 5%, Kara, S. 5%, Neary, J. P. 5%, Gardner, W. 5%

Overview

Background: Within the last two decades, attitudes have shifted from considering sports-related concussion as an insignificant minor injury with no long-term repercussions to a potentially serious brain injury garnering attention from media, clinicians, researchers, and the general public. **Objectives**: To conduct a case study to determine the underlying cause of persistent issues suspected to be associated with a history of sports-related concussion. **Protocol**: Participant A underwent neurophysiological testing following the Neary protocol (assessment of cerebrovascular and cardiovascular variables), comprehensive concussion assessment at a dedicated sports concussion clinic (history, neurological assessment, cervical spine screening, vestibulo-ocular screening, SCAT-5, and exercise testing), referral to a neurologist, structural MRI scan, and referral for specialised assessment at a dedicated dizziness and balance centre. **Results**: Despite a history of multiple sports-related concussions, Participant A's persistent symptom reports were associated with peripheral vestibular dysfunction and otolithic dysfunction seemingly unrelated to his concussion history. **Discussion**: Lessons from Participant A's case study showed that on-going symptoms that patients may associate with the effects of concussions may instead be due to unrelated causes that share similar symptomology. **Conclusion**: This research exemplifies the importance of a multi-disciplinary assessment using a repeated testing protocol.

Introduction

In recent years, the acute and long-term effects of sports-related concussion (SRC) have garnered increased concern and attention from researchers, medical practitioners, media reporters, athletic organisations administrators, and the general public. Within the last two decades, attitudes have shifted from considering SRC as an insignificant minor injury with no long-term repercussions to a potentially serious brain injury. Changes in perceptions have been driven by rapidly evolving evidence within the literature regarding SRC epidemiology, underlying mechanisms of SRC, symptoms, assessment, rehabilitation/return-to-play, and potential long-term repercussions of a history of SRC [43]. This evolving evidence has facilitated substantial rule changes in a variety of sporting codes to decrease the likelihood of an athlete sustaining an impact that may result in SRC and persistent concussion symptoms (PCS). For example, New Zealand's Accident Compensation Corporation (ACC) has worked together with Auckland University of Technology's (AUT) Sport Performance Research Institute New Zealand (SPRINZ), NZ Rugby Union, NZ Rugby League, NZ Football, and NZ Netball staff to develop and release national guidelines for sport concussions as part of the ACC SportSmart initiative [152]. The intent of these national guidelines is to standardise how to recognise SRC, remove athletes from play, refer the athlete to proper medical attention, and how to appropriately return athletes to school/work and subsequent reintegration to sport and activity following SRC [152].

World Rugby, NZ Rugby, and AUT conducted the inaugural NZ RugbyHealth project to explore the longer-term impacts of playing rugby on general health and cognitive function. Since the initiation of the NZ RugbyHealth project the investigation into the effects on general health and cognitive function from playing rugby has expanded into a Global RugbyHealth Research Programme (GRHRP). This GRHRP involves collaboration between researchers in New Zealand, the United Kingdom, Canada, Australia, and the United States of America, to better understand the long-term health of retired rugby players. Published findings [153] from the NZ RugbyHealth project generated considerable media attention worldwide resulting in an increased awareness within the general public of the potential effects of a history of SRC sustained from participating in different rugby codes [10, 33]. This media attention led Participant A, age 40, to contact NZ RugbyHealth project principal investigator Professor Patria Hume of AUT. Participant A shared his history of 10 self-reported SRCs that he sustained from rugby, skiing, and mountain biking, and explained that he felt as though he was suffering from the long-term consequences of his SRCs on a regular basis, and thus possibly experiencing PCS [98]. Participant A described a history of symptoms he had been experiencing over the last eight years including: pressure headaches, nausea without vomiting, feeling foggy, dizziness, feeling as if the room was spinning/tilting, and sporadic severe "head spins" lasting 15-60 seconds that would leave him feeling disoriented. Furthermore, Participant A noted that sleep, stress reduction, hydration, and quality nutrition aided in improving these spontaneous incidents of dizziness and disorientation. Prior to learning about the RugbyHealth Project, Participant A had made efforts to try and determine the cause of the problems he was experiencing by organising referral for a structural MRI and

consultations with an otolaryngologist, respiratory physician, and orthopaedic surgeon. The findings from the MRI and specialist consultations all came back negative. Participant A was informed he was in good health and did not receive any explanation for the symptoms he was suffering. Feeling frustrated due to the apparent absence of a solution for his symptoms, Participant A expressed to Professor Hume his desire to get involved with brain health research. Participant A wanted answers to his own questions, but also wanted to benefit others who may be going through a similar situation.

Professor Hume informed Participant A of an upcoming sabbatical research visit from GRHRP co-principal investigator Professor Patrick Neary from the University of Regina in Canada. Professor Hume informed Participant A that the purpose of Professor Neary's sabbatical was to share and establish his Neary protocol for brain health assessment in New Zealand. The protocol, which has been published in separate components [124, 125, 154-157] enables the neurophysiological assessment of an athletes' history of SRC and current brain health. Given Participant A's self-reported background, he was advised he would qualify as a participant for testing using the Neary protocol. Participant A enthusiastically agreed to participate and was scheduled to be the first New Zealand participant to be assessed using the Neary protocol. AUT ethics #18/45 was gained to enable Participant A to be a case study to evaluate if the neurophysiological assessment battery could detect impaired functions suspected to underlie persistent SRC symptoms.

Protocol

Athletes who experience persistent symptoms after SRC typically experience these symptoms as a result of one or more underlying impairments in normal physiological, vestibulo-ocular, and/or cervical spine function as a result of the initial SRC [75, 158]. In more rare cases, persistent complaints may be due to neuropathological changes, excitoimmunotoxicity, and/or in rare instances by chronic traumatic encephalopathy [42, 43, 159, 160]. The majority of persistent symptoms occur as a consequence of physiological dysfunction following SRC [161]. The Neary protocol is a battery of tests (requiring approximately 60 minutes to administer) that assess neurophysiological responses under a variety of conditions including: in a resting seated position; during changes in posture; during a cognitively and visually demanding "Where's Wally" task; while undergoing repeated 20 second breath holds; and during a 15 minute cycle exercise test with workload increases every five minutes. During this testing protocol measures of heart rate variability, cerebrovascular reactivity, and respiratory gas exchange are collected in parallel throughout the duration of the protocol [124, 125, 154]. The Neary protocol is sensitive to changes in these neurophysiological functions following acute SRC, allowing for the identification of atypical physiological responses likely underlying symptoms subjectively reported by concussed athletes [124, 125, 154]. The GRHRP has implemented the Neary protocol in Canada and the UK from May – December 2017 to investigate if differences in neurophysiological responses could be observed between retired rugby players and athletes from non-contact sports with no history of SRC.

Participant A underwent the first Neary protocol testing in New Zealand at SPRINZ located within AUT Millennium on March 7th, 2018. Participant A also had a second testing session on March 20th, 2018 to ascertain if any changes in his neurophysiological responses were observed between assessments.

Recent recommendations from Ellis et al. [75, 98] suggested that isolated dysfunction of the vestibular, ocular, and/or cervical spine neurological sub-system may be the cause of PCS when impaired neurophysiological function is not apparent. The results of Participant A's Neary protocol assessments did not reveal any notable impairments in neurophysiological functions; indicating the possibility that his symptoms could be a product of vestibulo-ocular and/or cervicogenic dysfunction secondary to SRC as described by Ellis et al [98]. To further investigate what might be causing Participant A's symptoms, he was scheduled for a medical consultation at a dedicated concussion clinic. Assessments included:

- clinical examination (consisting of a thorough medical and concussion history),
- physical examination (involving neurological assessment and signs of autonomic dysfunction, cervical spine examination),
- vestibular screening (using vestibulo-ocular motor screening tools),
- neurocognitive assessment (using SCAT 5 testing),
- balance and gait assessment (using Balance Error Scoring System and Tandem Gait 3m Walk time), and
- treadmill exercise testing (using a modified Balke protocol as per the Buffalo Concussion Treadmill Test) [89].

Participant A performed within normative data limits on all assessment tests. During the medical history component of the consultation Participant A reported unilateral tinnitus on the right that arose after pistol shooting approximately 8 years ago. All of Participant A's SRCs took place before the age of 30, and Participant A reported that symptoms of these SRCs resolved within days or weeks after the injury. Participant A's current symptoms began when he was 33 years old. The symptoms recalled from his previous SRCs did not present with similarities to his current symptom complaints.

The conclusion was that the current symptoms appeared to be consistent with a peripheral vestibular cause rather than persistent complications of SRC; due to the lack of a temporal relationship between the onset of his symptoms and the previous concussions. Participant A was therefore referred for medical review with a neurologist. The neurologist ordered an updated structural MRI scan and conducted a further neurological assessment. Both the MRI and neurological assessment were normal (except for a minor note of fluid trapped in the inner ear). Participant A exhibited normal neck movements, carotid artery responses, reflexes, tone, strength, coordination, and a negative Dix-Hallpike test. Participant A did not present with typical features of Meniere's disease or benign positional vertigo. Participant A's reports of imbalance and intermittent dizziness appeared to be related to the right inner ear and the neurologist noted that these results were suggestive of disembarkment syndrome and that peripheral vestibular disturbance was the likely cause. Participant A was referred for further assessment and management at a specialised dizziness and balance centre where he underwent assessments administered by both a vestibular therapist and

vestibular audiologist. The specialists noted normal dynamic visual acuity. However, unilateral tinnitus and an asymmetric hearing profile in combination with otolithic dysfunction as the main finding of the assessment were confirmed during a gait assessment wherein large head movements reproduced some of Participant A's symptoms. This response to head movements during walking indicated stress on the otolithic organ was responsible for increased symptomology. Accordingly, Participant A received an exercise programme to increase demand on the otolith to promote adaptation to improve, and hopefully permanently resolve, the cause of his symptoms. Follow-up appointments at the dizziness and balance centre were planned to progress and review the effect of the exercise programme every 3-4 weeks.

Discussion

Current consensus indicates that the majority of individuals with SRC will experience complete symptom resolution within 10-14 days of their injury [43]. However, little is known about the long-term effects of SRC, and estimates suggest between 20-40% of individuals will experience persistent complications for weeks or months following SRC [33, 162]. A major challenge when working with individuals who have had a history of SRC is the comorbidity shared between symptoms of SRC and an extensive array of other conditions such as: migraine, depression, mental health disorders, learning disabilities, sleep disorders, or (in Participant A's case) peripheral vestibular dysfunction and tinnitus [93, 98]. Further increasing this challenge is the "invisible" nature of SRC and these other comorbid conditions; meaning these conditions are unlike a broken bone or a soft tissue injury that can easily be confirmed or ruled out using validated clinical tests, x-ray, or MRI. This is especially evident in cases where an individual who has a history of SRC is reporting persistent issues for months or years since their most recent SRC. How is the patient or the clinician supposed to know if the reported persistent symptoms are due to SRC, a comorbid condition sharing symptoms with SRC, or a combination of the two? To date, differentiating between symptoms of SRC and other comorbid conditions relies heavily on the level of experience and expertise of the medical professional(s) assessing these athletes. While media attention surrounding the long-term effects of SRC led to the series of events resulting in Participant A's eventual diagnosis, we feel as though it is within reason to suggest his peripheral vestibular dysfunction and otolithic dysfunction were unrelated to his history of SRC based on the findings of his clinical evaluations in addition to temporal relationship between his last SRC and the onset of his symptoms. However, this cannot be concluded with absolute certainty due to the current limited understanding of long-term consequences associated with SRC.

In the past SRCs were not considered serious injuries, therefore were not assessed and monitored thoroughly by medical professionals throughout the athlete's recovery. The absence of monitoring might increase the risk of secondary injury and potential long-term deficits and complications for an athlete [33, 42, 43, 153, 159]. Largely due to improved media reporting of scientific studies, and case studies of current and retired players having sustained concussion, there is increased awareness of the danger of SRC.

Athletes may no longer be underestimating the severity of SRC injury. However, fear of persistent symptoms associated with SRC such as memory issues, headaches, dizziness etc., may lead to athletes with a history of SRC (and clinicians treating these athletes) to inappropriately conclude all symptoms or impairments are manifestations due to concussion. These preconceptions and premature conclusions before a thorough interdisciplinary assessment can be conducted could be detrimental to the long-term health of the athlete by missing the true cause of reported symptoms. The findings of Participant A's case suggest that a comprehensive and systematic collaborative effort by an interdisciplinary team may be essential to determine the cause of persistent symptom reports in current or retired athletes with a history of SRC. There is no individual testing procedure that can determine the origin of persistent SRC or non-SRC symptoms; rather, an exhaustive process of elimination is necessary to rule out potential causes of symptoms until only the most logical and realistic cause(s) are left. This process of elimination approach is the only method at this point in time to identify and diagnose these types of "invisible" pathologies. In New Zealand the public healthcare model includes ACC, which allows injured patients to access necessary medical services for little to no personal financial cost. Even within this healthcare model, it took several weeks to coordinate appointments with all the medical professionals involved in the assessment and management of Participant A. In regions without public healthcare, individuals of low socio-economic status may struggle to afford and access the necessary services they require for their injury. Therefore, there is a need, both in New Zealand and globally, to develop and optimize interdisciplinary clinical models to streamline the management of patients with complex injuries such as SRC and related comorbidities. While this approach requires a substantial amount of communication and coordination between multiple healthcare disciplines, this effort is paramount to ensure proper management for individuals presenting with comorbid symptoms that may or may not be related to SRC. Once a final diagnosis has been made then it is possible to design and administer an individualised treatment program to address any underlying impairments causing the patient distress. Without this comprehensive and collaborative process, individuals may continue to suffer from their respective afflictions, potentially impacting their mental and social well-being as well as their ability to perform at school or work [43, 163]. Governing and medical organisations must embrace this challenge to ensure patients with serious injuries/conditions do not slip through the cracks of the healthcare system.

The introduction of the Neary protocol to New Zealand was a key component in the process of understanding the potential pathophysiological mechanism(s) responsible for Participant A's symptoms. Participant A did not demonstrate any abnormalities in neurophysiological function during the physical elements of the Neary protocol. Additionally, Participant A did not struggle with the cognitively demanding "Where's Wally" component of the Neary protocol, nor did he report difficulties coping with cognitive load during his day to day life or at work. Participant A's unremarkable findings from his two Neary protocol assessments were the first step in the identification of peripheral vestibular dysfunction and otolithic

dysfunction responsible for his symptoms. Intolerance to physical and cognitive loading is indicative of a symptomatic SRC patient, and improvements in tolerance are used as clinical markers of recovery. In contrast, a symptomatic patient who exhibits normal tolerance to physical and cognitive loading indicates that referral for assessment by a specialised health-care professional may be required to identify the source of symptomology [98, 164]. This may include referral to:

- a neurologist, for a comprehensive evaluation of the central and peripheral nervous systems;
- a psychologist, to screen for any mental health or mood disorders;
- a neuropsychologist, to gauge cognitive function and performance;
- and/or a vestibular therapist to assess central and peripheral vestibular systems.

Participant A's history and early clinical evaluations did not indicate that referral to a neuropsychologist or a psychologist was necessary; however, this may not be the case for other individuals suffering from non-specific symptomology which may or may not be related to SRC. Therefore, stressing the need for individualised management on a case by case basis.

The GRHRP has three research clinics currently (Canada, UK and now New Zealand) to collect Neary protocol data from retired rugby players with no history of SRC, retired rugby players with a history of multiple SRCs, and retired athletes who engaged in non-contact sports with no history of SRC. The aim of this international project is to determine if any differences in neurophysiological responses during the Neary protocol are present between these groups of athletes, and if differences are observed, do these differences relate to symptoms or impairments reported by the athletes. The GRHRP has preliminary pilot data collected in the UK to suggest that regional differences in the pre-frontal cortex exist between normal healthy control participants (without a history of SRC) and retired rugby players. Future findings from this GRHRP investigation into neurophysiological responses will enhance our current understanding of the long-term effects of playing rugby and/or a history of SRC on how the brain regulates physiological functions. While the Neary protocol was designed to detect changes in neurophysiological responses following SRC, the present case study suggests the Neary protocol may be an objective method to assist in discerning whether persistent issues reported by an individual with a history of SRC are due to SRC or potentially a comorbid cause i.e., otolithic dysfunction. However, we provide a caveat that additional testing using blood pressure monitoring (which was not performed on Participant A) is necessary to confirm whether pressure alleviation occurred during the time of his testing sessions [124]. The potential discriminatory utility of the Neary protocol would require additional research. Nonetheless, improved objective screening protocols may help clinicians decide whether there were physiological contributions to reported symptoms. The overall knowledge gained from the ongoing GRHRP project will benefit athletes, clinicians, and researchers by assisting in guiding the progression of future research and clinical practice.

Conclusions

Lessons from Participant A's case study show that on-going symptoms that patients may associate with the effects of concussions, may not be related to concussion. In Participant A's case an eventual diagnosis of peripheral vestibular dysfunction and otolithic dysfunction was made. Increased awareness and changes in

attitudes/policies in recent years has enabled a major leap forward in terms of protecting the short and long-term health of athletes following SRC. Nevertheless, caution must be exercised when assessing and managing an individual with a history of SRC. This caution is necessary to confirm that all possibilities for contributing factors have been considered for SRC symptoms that are comorbid in nature with a variety of other conditions. Lack of a thorough assessment for athletes with a history of SRC who present with symptoms that are comorbid with conditions unrelated to SRC may result in missing the true cause of these symptoms. By missing the true contributing cause of symptoms this may lead to the athlete experiencing prolonged issues for weeks, months, or years. To overcome these challenges a clinical model involving the coordination and communication of a collaborative interdisciplinary team of experts is essential to ensure the patient receive the best and most appropriate care [98].

Personal development as a researcher resulting from Chapter 2

- Understanding the pros and cons of raising awareness with a complicated issue such as SOBI.
- How to work within a patient-centred multi-disciplinary research/medical team.
- First exposure to a different medical system to what I have been previously accustomed to in Canada.
- Differences in conducting research in New Zealand versus in Canada.
- Further motivation to identify more objective assessments and earlier interventions to reduce the likelihood persistent complications for an individual with a history of SOBI.
- The challenges caused by the comorbidity of many SOBI symptoms with other conditions.

Link between Chapters 2 and 3

Chapter 2 provides a narrative describing how inadequate clinical assessment can lead to a patient with a history of SOBI to experience chronic issues that may last for years. The comorbidity shared between symptoms of SOBI and an extensive array of other conditions such as: migraine, depression, mental health disorders, learning disabilities, sleep disorders, or vestibular conditions present a major challenge when working with individuals who have a history of SOBIs. This is especially evident in cases where an individual who has a history of SOBI is reporting persistent issues for months or years since their most recent SOBI. Without objective clinical tools, how is the patient or the clinician supposed to differentiate whether the reported persistent symptoms are due to SOBI, a comorbid condition sharing symptoms with SOBI, or a combination of the two? While the Neary protocol did not detect any physiological abnormalities, it did lead to ruling out a physiological cause for Participant A's symptom complaints which triggered the subsequent referrals for more thorough and multi-disciplinary assessment. Nonetheless, Participant A experienced debilitating symptoms from a cause seemingly unrelated to his history of SOBI for years before receiving any intervention. The most logical solution to this issue is better acute management of SOBI, rather than letting months/years elapse before initiating treatment. Chapter 3 of this thesis analysed retrospective data collected from a dedicated SOBI clinic that was piloting the effectiveness of acute management for SOBI based on up-to-date recommendations from the research. Chapter 3 specifically focussed on developing a prognostic model using data acquired during the initial clinical assessment to predict which SOBI patients were more likely to experience longer recovery outcomes.

Chapter 3: Predicting SR-mTBI recovery trajectory using initial clinical assessment findings.

This chapter comprises the following paper published in *Sports Medicine:* **McGeown, J. P.**, Kara, S., Fulcher, M., Crosswell, H., Borotkanics, R., Hume, P. A., Quarrie, K.L., Theadom, A. (2019). Predicting Sport-related mTBI Symptom Resolution Trajectory Using Initial Clinical Assessment Findings: A Retrospective Cohort Study. *Sports Medicine*. https://doi.org/10.1007/s40279-019-01240-4.

Author contribution

McGeown, J. P. 65%, Kara, S. 5%, Fulcher, M. 5%, Crosswell, H. 5%, Borotkanics, R. 5%, Hume, P. A. 5%, Quarrie, K. L. 5%, Theadom, A. 5%

Overview

Objectives: To identify which aspects of initial clinical assessment for sport-related mild traumatic brain injury (SR-mTBI) predict whether an athlete achieves symptom resolution within 14 days of the injury. **Research design:** Retrospective cohort study using prospectively collected data. **Methods:** Clinical assessment data were collected from 568 patients diagnosed with SR-mTBI at a single medical clinic between February 2017 and December 2018. Demographic data, medical history, SCAT-5 testing, and physician notes were included in the data set. Data were processed and analysed to identify a shortlist of predictor variables to develop a logistic regression model to discriminate between SR-mTBI symptom resolution that occurred in ≤14-days or >14-days. The data were randomly divided into model development and validation subsamples. The top 15 models were analysed to determine the predictor variables to be included in the final logistic regression model. The final model was then applied to the validation subsample. **Results:** Half of the athlete participants in this study experienced >14-day symptom resolution. The final logistic regression model included sex, symptom reporting at initial assessment and presentation with a physiological predominant symptom cluster. The model accounted for 0.90 and 0.85 of the area under the curve and predicted recovery trajectory with 81% and 76% accuracy for the training and validation subsamples, respectively. **Conclusions:** Being female, reporting a higher Positive Symptom Total at initial assessment, and being less likely to have a physiological predominant symptom cluster at initial assessment predicted >14 versus ≤14-day SR-mTBI symptom resolution with a high level of accuracy.

Introduction

Sport-related concussion (SRC) results from direct or indirect biomechanical forces to the head, neck, or body during participation in sport and exercise, resulting in stretching and shearing of neurons [17, 43]. This biomechanical event triggers a neuropathophysiological cascade resulting in secondary impairment in regular brain function [42, 63]. While SRC was the term used in the 2016 consensus statement [43], recent guidelines [3] recommended adoption of the term mild traumatic brain injury (mTBI) rather than concussion within clinical practice, therefore mTBI or sports-related mTBI (SR-mTBI) is used herein. Current evidence suggests most adults will achieve spontaneous clinical recovery within 10-14 days (28 days for children) after the biomechanical event causing SR-mTBI [43, 75, 93]. Definitions of clinical recovery appear to differ across studies, although symptom resolution appears consistently as one of the key components of determining clinical recovery [39, 43, 165, 166]. Estimates of persistent symptoms beyond 10-14 days following mTBI in sporting and non-sporting contexts have varied from 10-15% [75, 93] to 40-50% within the literature [33, 166]. Acute symptoms at 14 days post-mTBI are predictive of long-term recovery that may require weeks, months, or years to resolve [167]. Based on current evidence, 14 days post-mTBI serves as a meaningful clinical milestone.

Clinicians face challenges when trying to effectively manage and return SR-mTBI patients to everyday activities. Sport-related mTBI presents as an “invisible injury” where external signs such as bruising, swelling, or deformation as seen with soft tissue or bony injuries are not present. Standard neuroimaging techniques do not detect abnormalities following mTBI. To date no objective tools or biomarkers have been validated for use in clinical practice for mTBI [43]. Current clinical guidelines state that physicians should implement a multi-faceted approach to assessing SR-mTBI patients including a comprehensive history and physical examination [43], standardised evaluation of symptoms, attention, concentration and memory using the most recent version of the Sports Concussion Assessment Tool (SCAT-5) [43, 73], exercise stress testing for symptomatic participants beyond 10-14 days post-injury [74], classification of persistent symptoms into predominant symptom clusters (PSC) [75], and neuropsychological testing [43], There is a need to identify aspects of these assessment tools that can be used in prognostic models to predict recovery trajectories for athletes based on information collected during initial clinical assessment of SR-mTBI. An accurate model may potentially assist clinicians in being able to give patients a better prognosis at the initial consultation. The purpose of this study therefore was to identify which items in the initial clinical assessment predicted whether an athlete symptomatically resolved in less than 14 days.

Methods

Study design

This retrospective cohort study used prospectively collected data from a single medical clinic between February 2017 and December 2018.

Participants

The 568 patients diagnosed with SR-mTBI by a Sport and Exercise Medicine Physician who participated in the current study sustained their injury during sport. Participants were treated and monitored by a dedicated SR-mTBI service from initial clinical assessment until clinical recovery and subsequent discharge. The sample in this study included both males and females; as well as children, adolescents, and adults from a variety of sporting codes. Institutional (ACC and AUT 18/46) ethics committee approvals were obtained for this investigation and this study was performed in accordance with the ethical standards of the Declaration of Helsinki. Participants consented to have their clinical data used for research and publication. All clinical data collected from patients and analysed for this study were de-identified to protect the confidentiality of the study participants.

Clinical SR-mTBI variables

Demographic information

Age, sex, years of education, the sport/activity resulting in mTBI, number of days between occurrence of SR-mTBI and initial assessment, number of days missed from work and/or school, and history of previous TBI (with or without formal diagnosis from a physician) information were collected from participants via self-report.

Symptom quantification and management

All participants completed the full SCAT-5 (22 item Symptom Scale; Standardised Assessment of Concussion – SAC; modified Balance Error Scoring System – mBESS) during initial clinical assessment. Participant symptoms were quantified during clinical assessments using the Symptom Scale within the SCAT-5 producing a Positive Symptom Total (PST), and a Symptom Severity Score (SSS). A Global Severity Index (GSI = SSS ÷ 22) and Positive Symptom Distress Index (PSDI = SSS ÷ PST) were calculated [168] to produce weighted composite scores for self-reported symptoms. Following initial assessment, participants received education and written guidance regarding controlled cognitive and physical loading to address symptoms and were scheduled for a follow-up consultation approximately day-14 post-injury independent of the timing of their initial appointment. Participants who were still symptomatic at the follow-up visit underwent the Buffalo Concussion Treadmill test [74, 89, 90], upper cervical spine assessment, and Vestibular/Oculomotor Screening (VOMS) assessment [169]. An individualised treatment program was

designed and administered, based on clinical impairments of these systems, in combination with sub-symptom threshold aerobic exercise to address symptoms. Additional follow-up assessments were scheduled every two weeks while participants underwent treatment, re-evaluating symptoms and progression of treatment regimen until participants became asymptomatic.

Clinical observations

All participants underwent a comprehensive clinical examination by a Sport and Exercise Medicine Physician. Examination results were represented by the categorical variables: PSC, presentation of autonomic dysfunction, and any pre-diagnosed psychological condition/illness (attention deficit hyperactivity disorder, anxiety, depression, or any other mental health conditions). The physician applied the criteria published by Ellis et al. [75] to identify whether a predominantly physiological, vestibulo-ocular, or cervicogenic origin PSC appeared to be contributing to the participant's symptom reporting and presentation during the first clinical visit. Briefly, physiological PSC refers to symptoms due to impairments resulting from alterations in global cerebral metabolism. Symptoms are often exacerbated by cognitive and physical loading [75]. Physiological PSC patients will experience symptom exacerbation during a treadmill test [75]. Vestibulo-ocular PSC refers to symptoms due to dysfunction of the vestibulo-ocular system and symptoms are exacerbated by tasks that stress these systems such as reading or balancing [75]. Vestibulo-ocular PSC patients will often not experience symptom exacerbation achieving targeted heart rates of 85% HRmax predicted during a Buffalo Concussion Treadmill Test but will present with abnormal saccades, disrupted balance, etc. [75]. Cervicogenic PSC refers to symptoms due to dysfunction within the cervical spine somatosensory system. Symptoms are exacerbated by rapid head movements and prolonged periods of cognitive or physical activity in poor postural positions [75]. Cervicogenic PSC patients will not typically experience symptom exacerbation during a treadmill test as outlined above but will often experience symptom exacerbation during palpation and/or active/passive movements of the cervical spine [75]. Participants were classified as mixed PSC if their clinical presentation was not clearly associated with one PSC.

Outcome variables

The study outcome variable was SR-mTBI symptom resolution trajectory using the symptoms assessed via the SCAT-5 [73]. Two groups (>14-days or ≤14-days) were defined based on whether participants were asymptomatic after or at/before 14 days. Some participants became asymptomatic within ≤14-days of their injury, but due to scheduling limitations, were not evaluated by a physician until beyond 14 days post-injury. In this scenario, the physician recorded the participant's self-reported days until asymptomatic within the dataset (i.e., follow up assessment 16 days post-injury, symptom free at day 12 post-injury, therefore days until asymptomatic = 12).

Statistical analyses

Participant demographic information, SCAT-5 performance, and clinical observations obtained during initial SR-mTBI assessment were extracted and used as predictor variables. Differences between continuous variables for >14-day and ≤14-day groups were evaluated using two sample Kolmogorov-Smirnov tests due to a lack of normality. Comparisons were made between males and females within each respective group using the same method. Group comparisons of categorical variables were accomplished using Chi square or Fisher's Exact tests. Relationships between clinical assessment variables were evaluated using Pearson product moment correlations. Due to the variable nature of clinical data, a priori α was set to 0.05 for comparisons.

The prospectively collected clinical data were used to train and validate a logistic model capable of predicting SR-mTBI participants who had higher odds of experiencing >14-day symptom resolution. The data were randomly split with 50/50 probability into training and validation subsamples. To examine predictors of ≤14 or >14-day symptom resolution following SR-mTBI, multivariable logistic regression models were developed following a defined methodological approach [170] using the training subsample. Collinearity was accounted for in development of the model combinations by eliminating all models that contained two predictor variables sharing a relationship >0.4 [171]. All variable combinations containing 2-6 predictor variables were generated, producing 14,160 models. A short-list of the top 15 models was determined by a mutual balance between area under the curve (AUC) and Akaike information criterion. Predictor variables for the final training model were selected based on clinical relevance and evidence from the literature, while taking consistency of β-coefficient and standard error values across the short-list of models into account. The final model was chosen to include the least number of variables in order to increase clinical simplicity/utility and avoid overfitting. The optimal multivariable model was then applied to the validation subsample, evaluating model performance on a new dataset. Goodness-of-fit for each subsample was evaluated using Hosmer and Lemeshow tests. Sensitivity, specificity, AUC, percent correctly classified, and receiver operating characteristic (ROC) analyses were derived for both the training and validation subsample. Finally, model results from the training and validation subsamples were compared using Delong's ROC curve AUC comparison. Statistical analyses were performed using Python v3.6 and RStudio v1.1.383.

Results

Descriptive statistics and results of between group comparisons are summarised in Table 3.1, with initial assessment data split by sex, and symptom resolution in Table 3.2. Results are presented as Mean ±SD, or as Median [IQR] for selected variables where extreme values skewed the Mean in a way that was not representative of the group. Frequencies (%) are reported for categorical variables. Correlational heatmaps of continuous variables during initial clinical assessment of SR-mTBI are illustrated in Figure 3.1.

Demographic information

Of the 568 participants there were 133 females (23%) and 274 participants under 18 years old (48%). Participants with >14-day symptom resolution had an average age of 20.4 ±9.1 years compared to 20.0 ±8.0 years for those with symptom resolution in ≤14-days (p = 0.195). Days until initial assessment was higher in the >14-day group (9.6 ±6.9) than the ≤14-day group (7.8 ±4.9; p = 0.001), as was days until asymptomatic (>14-day: 25.0 [19.0; 38.0]; ≤14-day: 7.0 [3.0; 12.0]; p = <0.001). Both groups self-reported a history of approximately one previous mTBI (>14-day: 0.9 ±1.6; ≤14-day: 0.9 ±1.4; p = 1.000).

SCAT-5

There were no differences in initial SAC scores between ≤14-day and >14-day, or between males and females within the groups. Initial PST (>14-day: 14.6 ±5.3; ≤14-day: 6.1 ±5.5; p = <0.001), initial SSS (>14-day: 30.0 [18.0; 50.0]; ≤14-day: 6.0 [2.0; 15.0]; p = <0.001), initial GSI composite (>14-day: 1.6 ±1.0; ≤14-day: 0.5 ±0.6; p = <0.001), and initial PSDI composite (>14-day: 2.3 ±0.8; ≤14-day: 1.3 ±0.8; p = <0.001) were higher in the >14-day group compared to the ≤14-day group. There were no differences in initial PST between males and females within the >14-day and ≤14-day groups. Initial SSS scores were higher in >14-day females (38.5 [24.0; 59.0]) than in >14-day males (28.0 [17.0; 46.5]; p = 0.010), similarly females (11.0 [5.0; 24.0]) reported higher initial SSS than males (5.0 [2.0; 14.0]; p = 0.142) in the ≤14-day group.

Clinical observations

The majority (85%) of all participants (n = 568) were identified as experiencing symptoms of a predominantly physiological origin. Of the 291 >14-day participants, 77% of participants displayed a physiological PSC, 11% a vestibulo-ocular PSC, 3% a cervicogenic PSC, and the remaining 9% a mix of PSCs. In contrast, 94% of ≤14-day participants (n = 277) demonstrated a physiological PSC, 3% vestibulo-ocular, 3% cervicogenic, and 1% mixed PSCs. Rugby accounted for 60% of all SR-mTBIs in this study (301 Rugby Union and 37 Rugby League). Autonomic dysfunction was observed in 41% of >14-day participants against 9% in the ≤14-day group. Psychological modifiers were reported by 19% of participants in the >14-day group compared to only 10% of participants in the ≤14-day group.

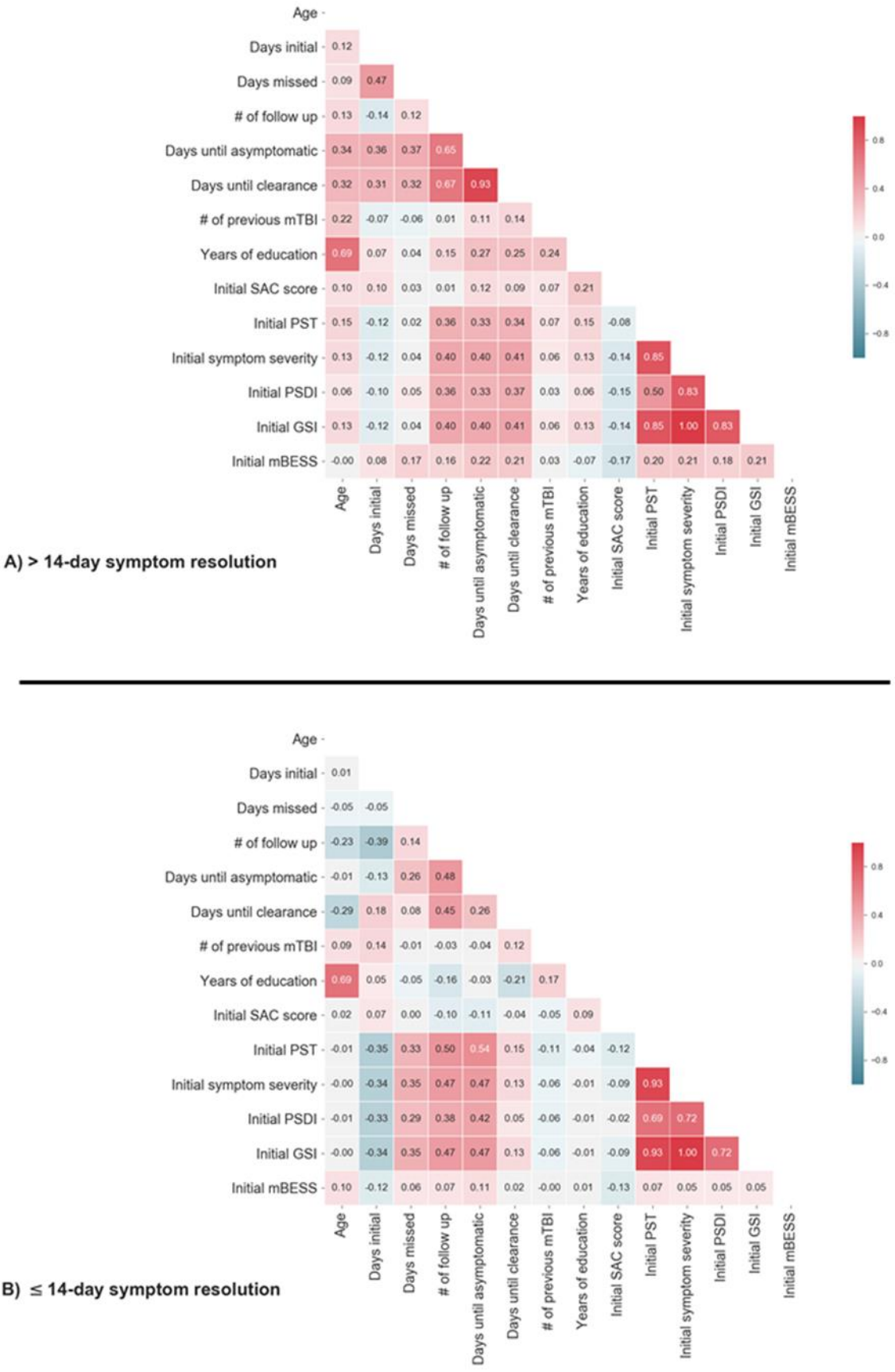


Figure 3.1 Heatmaps of initial assessment continuous variables split by a) ≤14-day and b) >14-day SR-mTBI symptom resolution (numerical values presented within cells are Pearson's r values of bivariate relationships).

Table 3.1 Descriptive statistics and symptom resolution comparisons of clinical SR-mTBI assessment variables.

	Total (n=568)	**Resolution >14-days (n=291)**	**Resolution ≤14-days (n=277)**	***p***
Demographics				
Age*	20.2 ±8.6	20.4 ±9.1	20.0 ±8.0	0.195
Days until initial assessment*	8.7 ±6.0	9.6 ±6.9	7.8 ±4.9	**0.001**
Days missed from work/school*	2.1 ±4.0	3.1 ±5.1	1.1 ±1.8	**<0.001**
# of follow up visits*	1.9 ±1.3	2.6 ±1.4	1.1 ±0.7	**<0.001**
Days until asymptomatic†	15.0 [7.0; 25.0]	25.0 [19.0; 38.0]	7.0 [3.0; 12.0]	**<0.001**
Days until full clearance†	24.0 [18.0; 37.0]	35.0 [26.0; 49.0]	18.0 [17.0; 23.0]	**<0.001**
# of previous mTBI*	0.9 ±1.5	0.9 ±1.6	0.9 ±1.4	1.000
Years of education*	12.7 ±3.3	12.8 ±3.3	12.5 ±3.2	0.664
SCAT-5				
Initial SAC score*	26.5 ±2.6	26.2 ±3.0	26.8 ±2.1	0.622
Final SAC score*	26.9 ±2.5	26.9 ±2.4	26.9 ±2.6	1.000
Initial PST*	10.5 ±6.8	14.6 ±5.3	6.1 ±5.5	**<0.001**
Final PST*	2.4 ±3.0	3.2 ±3.2	1.5 ±2.6	**<0.001**
Initial symptom severity†	17.0 [5.0; 36.0]	30.0 [18.0; 50.0]	6.0 [2.0; 15.0]	**<0.001**
Final symptom severity†	1.0 [0.0; 4.0]	2.0 [1.0; 6.0]	1.0 [0.0; 2.0]	**<0.001**
Initial PSDI composite*	1.8 ±0.9	2.3 ±0.8	1.3 ±0.8	**<0.001**
Final PSDI composite*	0.7 ±0.7	0.9 ±0.6	0.6 ±0.7	**<0.001**
Initial GSI composite*	1.1 ±1.0	1.6 ±1.0	0.5 ±0.6	**<0.001**
Final GSI composite*	0.1 ±0.2	0.2 ±0.2	0.1 ±0.1	**<0.001**
Initial mBESS score*	3.8 ±3.1	4.3 ±3.6	3.2 ±2.5	**0.017**
	Frequency (%)	**Frequency (%)**	**Frequency (%)**	***p***
Age				
Under 13	44 (8)	18 (6)	26.0 (9)	**0.006**
13 to 17	230 (41)	136 (47)	94.0 (34)	
18 and over	294 (52)	137 (47)	157.0 (57)	
Sex				
Female	133 (23)	100 (34)	33.0 (12)	**<0.001**
Male	435 (77)	191 (66)	244.0 (88)	
Predominant Symptom Cluster				
Physiological	482 (85)	223 (77)	259.0 (94)	**<0.001**
Vestibulo-ocular	40 (7)	33 (11)	7.0 (3)	
Cervical	17 (3)	10 (3)	7.0 (3)	
Mixed	29 (5)	25 (9)	4.0 (1)	
Sport				
Rugby Union	301 (53)	114 (39)	187.0 (68)	**<0.001**
Rugby League	37 (7)	17 (6)	20.0 (7)	
Football (Soccer)	67 (12)	43 (15)	24.0 (9)	
Field Hockey	14 (2)	12 (4)	2.0 (1)	
Netball	10 (2)	8 (3)	2.0 (1)	
Other	139 (24)	97 (33)	42.0 (15)	
Autonomic Dysfunction‡				
Not present	423 (75)	171 (59)	252.0 (91)	**<0.001**
Present	142 (25)	118 (41)	24.0 (9)	
Psychological Modifier‡				
Not present	483 (85)	234 (81)	249.0 (90)	**0.004**
Present	82 (14)	55 (19)	27.0 (0.10)	

Data are presented as * Mean±SD, † Median [25th percentile; 75th percentile], or as Frequency (%).
‡ Frequency sum does not equal column total due to missing data.
SAC: Standardised Assessment of Concussion; mBESS: modified Balance Error Scoring System; PST: Positive Symptom Total score; PSDI: Positive Symptom Distress Index; GSI: Global Severity Index.

Table 3.2 Summary of sex differences for SCAT-5 and clinical evaluation findings collected during initial SR-mTBI assessment.

	Female >14-days (n=100)	**Male >14-days (n=191)**		**Female ≤14-days (n=33)**	**Male ≤14-days (n=244)**	
			p			***p***
SCAT-5						
Initial SAC score*	26.4 ±3.7	26.1 ±2.5	0.299	27.4 ±1.4	26.8 ±2.2	0.230
Initial PST*	15.5 ±5.2	14.2 ±5.3	0.234	8.6 ±6.5	5.8 ±5.3	0.387
Initial symptom severity†	38.5 [24.0; 59.0]	28.0 [17.0; 46.5]	**0.010**	11.0 [5.0; 24.0]	5.0 [2.0; 14.0]	0.142
Initial PSDI composite*	2.5 ±0.8	2.2 ±0.8	**0.001**	1.6 ±0.8	1.3 ±0.8	0.070
Initial GSI composite*	1.9 ±1.0	1.5 ±0.9	**0.010**	0.8 ±0.8	0.5 ±0.6	0.142
Initial mBESS*	4.5 ±3.4	4.2 ±3.7	0.935	3.7 ±2.8	3.1 ±2.5	0.905
	Frequency (%)	**Frequency (%)**	***p***	**Frequency (%)**	**Frequency (%)**	***p***
Age						
Under 13	2.0 (2)	16.0 (8)	**0.022**	0 (0)	26.0 (11)	NC
13 to 17	42.0 (42)	94.0 (49)		14.0 (42)	80.0 (33)	
18 and over	56.0 (56)	81.0 (42)		19.0 (58)	138.0 (57)	
Predominant Symptom Cluster						
Physiological	72.0 (72)	151.0 (79)	0.475	28.0 (85)	231.0 (95)	NC
Vestibulo-ocular	14.0 (14)	19.0 (10)		3.0 (9)	4.0 (2)	
Cervical	3.0 (3)	7.0 (4)		2.0 (6)	5.0 (2)	
Mixed	11.0 (11)	14.0 (7)		0 (0)	4.0 (2)	
Sport						
Rugby Union	17.0 (17)	97.0 (51)	NC	6.0 (18)	181.0 (74)	NC
Rugby League	4.0 (4)	13.0 (7)		4.0 (12)	16.0 (7)	
Football (Soccer)	13.0 (13)	30.0 (16)		8.0 (24)	16.0 (7)	
Field Hockey	6.0 (6)	6.0 (3)		1.0 (3)	1.0 (0)	
Netball	8.0 (8)	0 (0)		2.0 (6)	0 (0)	
Other	52.0 (52)	45.0 (24)		12.0 (36)	30.0 (12)	
Autonomic Dysfunction‡						
Not present	51.0 (52)	120.0 (63)	0.074	27.0 (82)	225.0 (93)	0.083
Present	48.0 (48)	70.0 (37)		6.0 (18)	18.0 (7)	
Psychological Modifier‡						
Not present	76.0 (76)	158.0 (83)	0.225	30.0 (91)	219.0 (90)	0.865
Present	24.0 (24)	31.0 (16)		3.0 (9)	24.0 (10)	

Data are presented as * Mean±SD, † Median [25th percentile; 75th percentile], or as Frequency (%).
‡ Frequency sum does not equal column total due to missing data.
NC Statistic not calculable due to zeros in cells
SAC: Standardised Assessment of Concussion; PST: Positive Symptom Total score; PSDI: Positive Symptom Distress Index; GSI: Global Severity Index; mBESS: modified Balance Error Scoring System.

Table 3.3 Final multivariable model summary and prediction performance of SR-mTBI symptom resolution for two unique samples.

	Training subsample (n= 262)					Validation subsample (n= 295)				
Variable	**Z**	***p***	**OR**	**Std. Err**	**95% CI**	**Z**	***p***	**OR**	**Std. Err**	**95% CI**
Female Sex	2.58	**0.010**	2.93	0.42	1.32 - 6.77	1.90	0.057	2.07	0.38	0.99 - 4.45
Physiological Predominant Symptom Cluster	-1.33	0.185	0.46	0.59	0.14 - 1.41	-3.04	**0.002**	0.26	0.45	0.1 - 0.6
Initial Positive Symptom Total score	8.27	**0.000**	1.33	0.03	1.25 - 1.43	7.90	**0.000**	1.24	0.03	1.18 - 1.31
Model accuracy	**Value**	**95% CI**				**Value**	**95% CI**			
AUC	0.90	0.86-0.94				0.85	0.81-0.90			
Sensitivity	0.80					0.77				
Specificity	0.82					0.75				
Correctly classified,	81	76-86				76	71-81			
Model evaluations										
Hosmer and Lemeshow goodness-of-fit test	$\chi^2 = 8.67$, df = 8, $p = 0.371$					$\chi^2 = 7.71$, df = 8, $p = 0.462$				
Delong's ROC curve AUC comparison	$D = 1.58$, $p = 0.114$									

Predictors of symptom resolution

Initial assessment data from the 568 participants was used to train and validate a logistic regression model to predict ≤14-day versus >14-day symptom resolution groups. Correlational evaluation of continuous variables indicated that only initial PST, SSS, and GSI shared a correlation coefficient >0.4 with number of days until asymptomatic within the complete dataset and ≤14-day sub-group; while none of these variables exceeded r = 0.4 within the >14-day sub-group. The model results for the training and validation subsamples are presented in Table 3.3. Results of ROC analysis are displayed in Figure 3.2. Hosmer and Lemeshow goodness-of-fit test results within both the training ($p = 0.371$) and validation subsamples ($p = 0.462$), did not indicate concerns with overall model fit. The final model included participant's PSC, initial PST, and sex as predictor variables of ≤14 versus >14-day symptom resolution groups. The AUC was 0.90 and 0.85, with 81% and 76% of participants correctly classified within the training and validation subsamples, respectively.

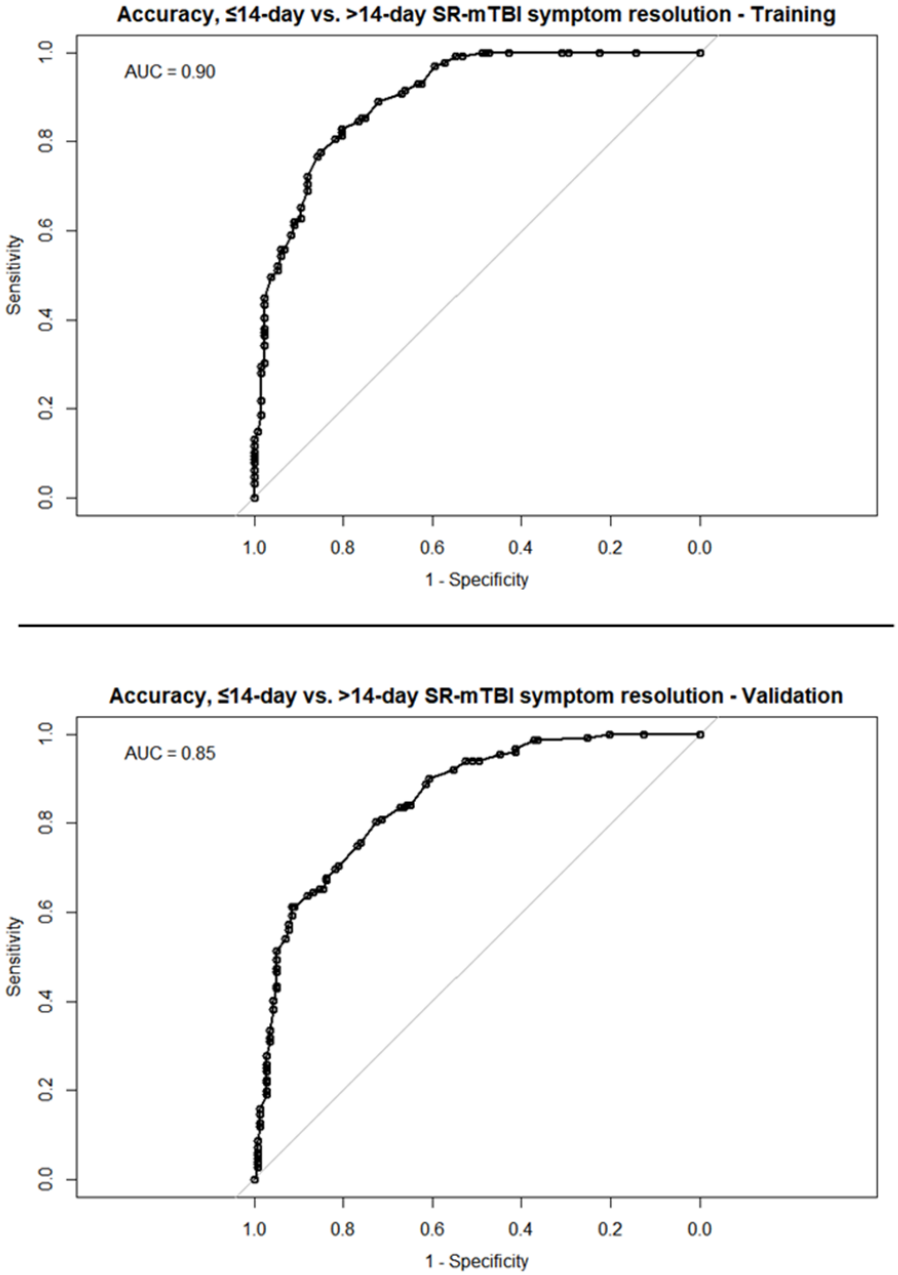


Figure 3.2 ROC curves for final logistic regression model applied to training (top) and validation (bottom) subsamples to predict >14-day versus ≤14-day SR-mTBI symptom resolution.

Discussion

Sex, symptom burden, and predominant symptom cluster

In the current study, female sex was disproportionately associated with >14-day symptom resolution outcomes compared to males (Table 3.2), which is in agreement with existing data relating to clinical recovery predictors [39]. Females accounted for 23% of participants within the current cohort, yet 34% of participants who experienced >14-day symptom resolution were female. Early evidence has suggested that widely reported sex differences in mTBI recovery may be explained by anatomical [172], hormonal [173, 174], and biomechanical [172] factors. At initial assessment, >14-day participants indicated greater PST and

SSS's than ≤14-day participants. Consistent with previous reports [175], female participants within our cohort demonstrated greater initial symptom burden than males in both >14-day and ≤14-day groups. However, females have been documented to report higher symptoms than males during baseline testing [176]. In contrast, there were no differences in SAC scores between sexes and >14-day and ≤14-day groups at initial assessment. The observed differences in mBESS scores between >14-day and ≤14-day groups are likely trivial due to the ceiling effect and questionable validity of the mBESS [87]. Current guidelines recommend evaluating SR-mTBI with the full SCAT-5 (Symptom Scale; SAC; mBESS) during both sideline and during subsequent clinical evaluations [73]. Compared to pre-season baseline testing, reduced SAC scores at the time of SR-mTBI and 3 hours post-injury have been reported, however, SAC performance returns to baseline levels within 2-3 days post-injury [88]. Our findings highlight limited clinical utility of the SAC and mBESS when administered during initial clinical assessment of SR-mTBI approximately one week after the injury. While it did provide a useful framework to guide management, SAC scores did not change as a participant's symptoms resolved and did not help predict symptom resolution or assist clearance for return to sport. Approximately 10 minutes was required to administer the SAC and mBESS components of the SCAT-5, which has been shown to be a barrier to use in primary care settings [177]. Based on existing evidence, continued administration of the Symptom Scale and removal of the SAC and mBESS for initial clinical assessments of SR-mTBI that occur more than two days post-injury may be considered to streamline clinical flow.

To our knowledge, this is the first athlete patient cohort assessed using the PSC classification criteria [75] during the *initial assessment* of SR-mTBI (rather than at 21 days post-injury for participants with unresolved symptom complaints). Participants who presented with complaints consistent with a vestibulo-ocular and/or cervical PSC were more likely to experience >14-day symptom resolution trajectories than participants presenting with symptoms consistent with physiological cause (i.e., symptoms exacerbated by cognitive and/or physical activity). This reinforces evidence that identification of vestibulo-ocular abnormalities during initial assessment is predictive of >14-day symptom resolution from SR-mTBI [165, 178]. Therefore, we recommend integration of experienced vestibulo-ocular physiotherapists into multi-disciplinary clinical models within institutions tasked with managing SR-mTBI patients. Initiation of appropriate vestibulo-ocular treatment sooner post-injury may reduce the proportion of patients who recover in >14-days but requires further research.

The recovery prediction model

In this study, a multivariable prognostic model to predict SR-mTBI symptom resolution trajectory with high accuracy was trained and validated using prospectively collected data following current clinical guidelines. Analysis demonstrated that females, participants with a higher initial PST, and cervicogenic and/or vestibulo-ocular PSC were at higher odds of >14-day SR-mTBI symptom resolution. Initial PST was included

in the final model over SSS and GSI/PSDI composite scores as the strongest predictor of symptom resolution trajectory. Based on this observation, number of initial symptoms endorsed may be more meaningful for predicting symptom resolution trajectory than the severity of symptoms. However, from a clinical perspective, continued use of GSI and PSDI composite scores is recommended to provide clinicians with a brief summary of symptom burden in an easily interpreted format.

Interestingly, patient age was not included in the final model as a significant covariate despite commonly cited reports that children and adolescents take longer to experience symptom resolution than adults [43, 75, 93]. A 2017 meta-analysis of predictors of clinical recovery reported that out of 31 studies (of varying size and quality) evaluating age as a predictor, seven studies concluded that younger age was predictive of worse outcomes while the remaining 24 studies concluded the contrary [39]. The potential contribution of age to symptom resolution trajectories remains unclear and warrants further exploration.

Implementing this model into clinical practice may assist clinicians to identify patients at heightened risk of >14-day SR-mTBI symptom resolution. A quick and accurate means to predict SR-mTBI symptom resolution trajectory during initial assessment, provides clinicians with valuable information to proactively tailor the patient's management plan earlier in the recovery timeline than is currently possible. The clinicians involved with this cohort are highly experienced and trained in the management of SR-mTBI, allowing them to effectively identify the participant's PSC based on the clinical evaluation and Symptom Scale scores. Model accuracy may suffer if clinicians lack education and experience in the management of SR-mTBI. Model accuracy also relies on patient compliance, because athletes have been documented to under-report symptoms following SR-mTBI [6]. If a patient deliberately minimises their symptoms during the initial clinical assessment the accuracy of the model will diminish. There is a need to identify feasible objective clinical measures to assess mTBI to compensate for situations where clinicians lack experience and/or patient symptom disclosure is not trustworthy. Ongoing and future investigations into the measurement and clinical utility of biomarkers [179-181], neurophysiological parameters [124, 154, 182-185], biomechanics [127, 128, 186, 187], and somatosensory function [121, 122] may identify the optimal test(s) to further enhance current clinical assessment and management of SR-mTBI. Furthermore, more research is needed to evaluate generalisability of the current model and whether the model may be useful in primary care settings such as an emergency department to identify patients that may need early referral to a multi-disciplinary service.

If future research determines sufficient generalisability and transferability of the current model it may be applied to assist clinicians with the identification of patients likely to require >14-days to achieve symptom resolution. This may aid clinicians, particularly those less experienced in the management of SR-mTBI, by supporting or refuting their initial clinical impressions to assist in determining the optimal management plan. For example, if the managing clinician suspects the patient is likely to experience symptom resolution

in <14-days while the model predicts >14-day resolution, this may lead the clinician to take a more conservative management strategy with the patient by ordering additional assessments (i.e., treadmill testing and/or comprehensive vestibulo-ocular evaluation) and scheduling referrals to specialised services earlier post-injury. Presently, in this scenario these assessments and referrals likely would not be initiated until 14 days following SR-mTBI when the patient's symptoms remain unresolved at this milestone. By the time these services are scheduled the patient would have been experiencing symptoms for a month or more before appropriate treatment strategies are initiated, which may result in substantial time missed from work/school/sport and expose the patient to considerable amounts of psychosocial stress. This becomes a greater consideration if the patient lives in an area where these services are unavailable and may be required to travel to receive the specialised management they require. Additionally, with many soft tissue and bony injuries it is possible to establish recovery timeline expectations early in the recovery process with the use of sensitive and specific tests. Establishing these expectations is not yet possible for clinicians working with SR-mTBI. It is conceivable that the open-ended nature of when to expect symptom resolution contributes to psychosocial stress for the patient. Therefore, this model may also benefit patients by providing an indication for when to anticipate symptom resolution in order to set recovery expectations early in the recovery process to combat psychosocial burden during SR-mTBI recovery. Of course, no model can replace quality clinical judgement and decision making, however, predictive models can complement clinical practice by providing practitioners with more information to guide their decisions.

Limitations

Interpretation of the study findings should consider several limitations. No data were available from the day the SR-mTBI occurred for each participant (apart from self-report of the event) as data included in this study were collected approximately 5-10 days post-injury. Only PST and SSS of the Symptom Scale and SAC total score were recorded from the SCAT-5 due to database limitations. The model building process included an automated component to narrow down the strongest predictors of SR-mTBI symptom resolution trajectory which may have yielded over-optimistic findings [188]. A defined methodological approach was used that balanced model performance and fit to reduce the risk of overfitting as is common in automated modeling. Data were randomly split into training and validation subsamples, to evaluate the model's performance on separate data to which it was trained on. The prognostic model trained and validated within this study was from data collected at a single clinic. Further model external validation is necessary to determine the full extent of the model's generalisability. Model implementation is limited to clinics with experienced clinicians trained to identify the participant's PSC. Model accuracy relies on the self-reported symptom profile provided by the patient with SR-mTBI.

Conclusions

Sex, initial PST, and PSC were variables collected during initial clinical assessment of SR-mTBI that demonstrated strong predictive capacity of >14 versus ≤14-day symptom resolution trajectories post-mTBI. Based on inclusion of PSC in the final model, a recommendation for clinicians is to utilise the PSC classification criteria at the first clinical SR-mTBI assessment. Exploration of the feasibility of emerging objective measures to further optimise clinical SR-mTBI assessment merits future research.

Personal development as a researcher resulting from Chapter 3

- Introduction to computer programming.
- Learning how to code in Python and RStudio.
- First experience conducting thorough exploratory data analysis.
- Development of data cleaning skillset and pipelines in Python Pandas.
- Importance of database structure for data quality.
- How to execute statistical modelling in RStudio.

Link between Section 1 and Section 2 Part A (Chapter 4)

Based on the findings of Section 1, one of the biggest factors that influence time to recovery post-SOBI is whether a patient with a history of SOBI receives a thorough assessment that identifies their specific treatment needs. Using data collected during the initial clinical assessment of SOBI the prognostic model in Chapter 3 enabled early prediction of patients likely to experience prolonged recovery so that earlier referral to specialist services can take place and resources can be allocated to those most in need. Early and accurate delivery of established treatment strategies (i.e., sub-symptom threshold aerobic exercise, vestibulo-ocular physiotherapy, etc.) should reduce the time patients require to recover and return to normal activities following SOBI. However, current treatment modalities react to functional and neurobehavioural deficits (i.e., mood instability, impaired cognition and memory, confusion, intolerance to cognitive and physical loading, vestibulo-ocular deficiencies, and sleep disturbances, etc.) which seemingly develop as consequences of cellular damage and dysfunction triggered by the secondary injury phase of TBI. There is a need for both effective treatments to address burden experienced by the patient, and proactive interventions that lessen the magnitude of cellular damage that manifests as symptoms. Advances in our knowledge of the neurophysiological consequences of TBI provide targets for proactive interventions to blunt the degree of cellular damage caused by SOBI in a manner which could subsequently reduce the proportion of patients who go on to experience prolonged recovery. Currently, there is no pharmacological method to achieve this proactive approach to improving recovery outcomes following TBI since the consequences of TBI do not occur in a single cellular pathway. For this reason, it is thought that nutritional interventions may serve as proactive methods to reduce the damaging effects post-TBI by acting upon multiple physiological pathways. Chapter 4 presents the first systematic review to consolidate available evidence for post-TBI nutritional interventions and their associated effects on neurophysiological outcomes in both animal and human studies. A model like the one presented in Chapter 3 could identify SOBI patients with a higher likelihood of experiencing prolonged recovery enabling investigations into the effectiveness of nutritional interventions to attenuate the neurophysiological consequences of SOBI in a manner that positively alters a patient's recovery trajectory.

Section 2:
Translational potential and clinical utility of neurophysiological approaches to advance management of SOBI

Chapter 4: Nutritional interventions to improve neurophysiological impairments following traumatic brain injury: A systematic review.

This chapter comprises the following paper published in *Journal of Neuroscience Research:* **McGeown, J. P.**, Hume, P. A., Theadom, A., Quarrie, K. L., & Borotkanics, R. (2021). Nutritional interventions to improve neurophysiological impairments following traumatic brain injury: A systematic review. *Journal of Neuroscience Research*, 99(2), 573-603. https://doi.org/10.1002/jnr.24746

Author contribution

McGeown, J. P. 80%, Hume, P. A. 5%, Theadom, A. 5%, Quarrie, K. L. 5%, Borotkanics, R. 5%

Overview

Traumatic brain injury (TBI) accounts for significant global health burden. Effects of TBI can become chronic even following mild injury. There is a need to develop effective therapies to attenuate the damaging effects of TBI and improve recovery outcomes. This literature review using a-priori criteria (PROSPERO; CRD42018100623) summarised 43 studies between January 1998-July 2019 that investigated nutritional interventions (NUT) delivered with the objective of altering neurophysiological (NP) outcomes following TBI. Risk of bias was assessed for included studies, and NP outcomes recorded. The systematic search resulted in 43 out of 3,748 identified studies met inclusion criteria. No studies evaluated the effect of a NUT on NP outcomes of TBI in humans. Biomarkers of morphological changes and apoptosis, oxidative stress, and plasticity, neurogenesis, and neurotransmission were the most evaluated NP outcomes across the 43 studies that used 2,897 animals. The risk of bias was unclear in all reviewed studies due to poorly detailed methodology sections. Taking these limitations into account, anti-oxidants, branched chain amino acids, and ω-3 polyunsaturated fatty acids have shown the most promising pre-clinical results for altering NP outcomes following TBI. Refinement of pre-clinical methodologies used to evaluate effects of interventions on secondary damage of TBI would improve the likelihood of translation to clinical populations.

Introduction

Traumatic brain injury (TBI) has been referred to as a silent epidemic, with an estimated age standardised global incidence rate of 369 per 100,000 person-years and an estimated incidence of 27 million new TBIs per year [23, 189]. The true incidence of TBI may be even higher due to reporting and recording limitations [26]. Injury burden is undeniable; yet due to intricate TBI pathophysiology, clinical strategies to attenuate damage and assist recovery across the TBI spectrum remain elusive. Mild, moderate, and severe TBIs are characterised by a primary biomechanical event wherein force is transmitted, injuring the brain as a consequence of events such as a fall, motor vehicle accident, assault, blast exposure, or sport-related activity [25]. TBI severity is primarily determined using the Glasgow Coma Score (assessment of level of consciousness). Additionally, moderate and severe TBIs are typically associated with skull fracture and/or positive neuroimaging findings indicating structural damage [18, 19]. Mild TBI (mTBI) represents the majority (95%) of TBIs and is generally classified based on a Glasgow Coma Score between 13-15, symptom complaints, and the absence of both skull fracture and positive neuroimaging [18, 19, 27]. Within minutes of the traumatic event (primary injury) neurological homeostasis is disrupted representing the secondary injury phase of TBI. Neurophysiological (NP) consequences of TBI can include: ionic and neurometabolic dysregulation; neurovascular and autonomic uncoupling; axonal and cytoskeletal damage; impaired synaptic plasticity; neuroinflammation; disrupted blood brain barrier (BBB) permeability and damaged cell membranes; and neuronal apoptosis (see [21] for a recent review detailing TBI pathophysiology).

Improved understanding of NP consequences of TBI provide targets for therapeutic interventions to improve recovery outcomes [19, 21]. On this basis, targeted exercise prescription has been shown to be an effective intervention to address symptom burden and functional deficits that commonly occur as a consequence of TBI [13, 75, 96, 98, 105, 158, 190-197]. Some symptoms such as headache complaints, sleep disruption, and/or mood disorders, that can occur secondary to TBI, have been managed with prescription pharmaceuticals (although greater clarity regarding efficacy of these approaches is needed) [198-202]. However, there is no intervention to proactively target the secondary injury phase of TBI in a manner that promotes earlier resolution of a range of symptom complaints and deficits observed clinically [203].

Lifestyle factors such as diet and nutrition play crucial roles in maintaining neurological function and overall brain health [204]. In this regard, modification of dietary intake and/or nutritional supplementation post-TBI demonstrate potential to attenuate damaging effects of secondary injury following TBI by simultaneously acting upon multiple NP pathways. An advantage of nutritional interventions (NUT) is the “over-the-counter” accessibility of affordable food and supplements which could potentially benefit patients post-TBI to alter the secondary damage of TBI when availability of medical services may be limited. Modifying nutrition is potentially useful for enhancing resilience to the damaging effects of TBI for at risk

populations (i.e., military personnel and athletes participating in high-risk sports) [205, 206]. Due to scalability and implementation limitations, the preventative role of nutrition is beyond the scope of the current review.

Cellular damage and dysfunction that characterises the secondary injury phase of TBI manifest clinically as an array of functional and neurobehavioural deficits [42, 61]. Previous reviews in the field of TBI have not summarised relevant NUT literature both comprehensively and systematically, or have focussed on interventions that target observable clinical symptoms [207-215]. This review evaluated the evidence for treatments to reduce the physiological causes of these clinical symptoms through exploring the effect of NUTs on NP outcomes.

Aim

This review aimed to systematically consolidate evidence from animal and human studies, evaluating NUTs administered post-TBI and their subsequent effects on NP outcomes.

Methods

We conducted this review according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and followed clear a priori criteria registered with the International Prospective Register of Systematic Reviews (PROSPERO; CRD42018100623). Review methodology was developed according to Population, Intervention, Comparator, and Outcome (P.I.C.O.) guidelines (Table 4.1).

Search strategy and study selection criteria

Table 4.1 outlines search terms to identify studies investigating the influence of nutritional and dietary manipulation interventions on NP outcomes post-TBI. We filtered titles and abstracts for nutritional and diet-related terms within 10 words either side of TBI-related keywords. When permitted by database functionality, additional filters were applied to limit search results to peer-reviewed studies, available in full-text, and published in English. Our systematic database search identified 4,148 articles (Figure 4.1) available online and published between January 1998 to July 2019 through MEDLINE [EBSCO] (n = 652), Web of Science (n = 608), Scopus (n = 639), CINAHL [EBSCO] (n = 451), SportDiscus [EBSCO] (n = 245), PsycINFO [OVID] (n = 1,489), and Cochrane Library [Wiley] (n = 62). Reference lists of included articles were reviewed but did not identify any additional results. Once duplicate references were removed, screening for eligibility by JM of titles, abstracts, and full-texts of 3,748 studies was completed. A 10% random check of references was performed at each screening stage by PH. Table 4.1 provides detailed inclusion and exclusion criteria used to guide study selection.

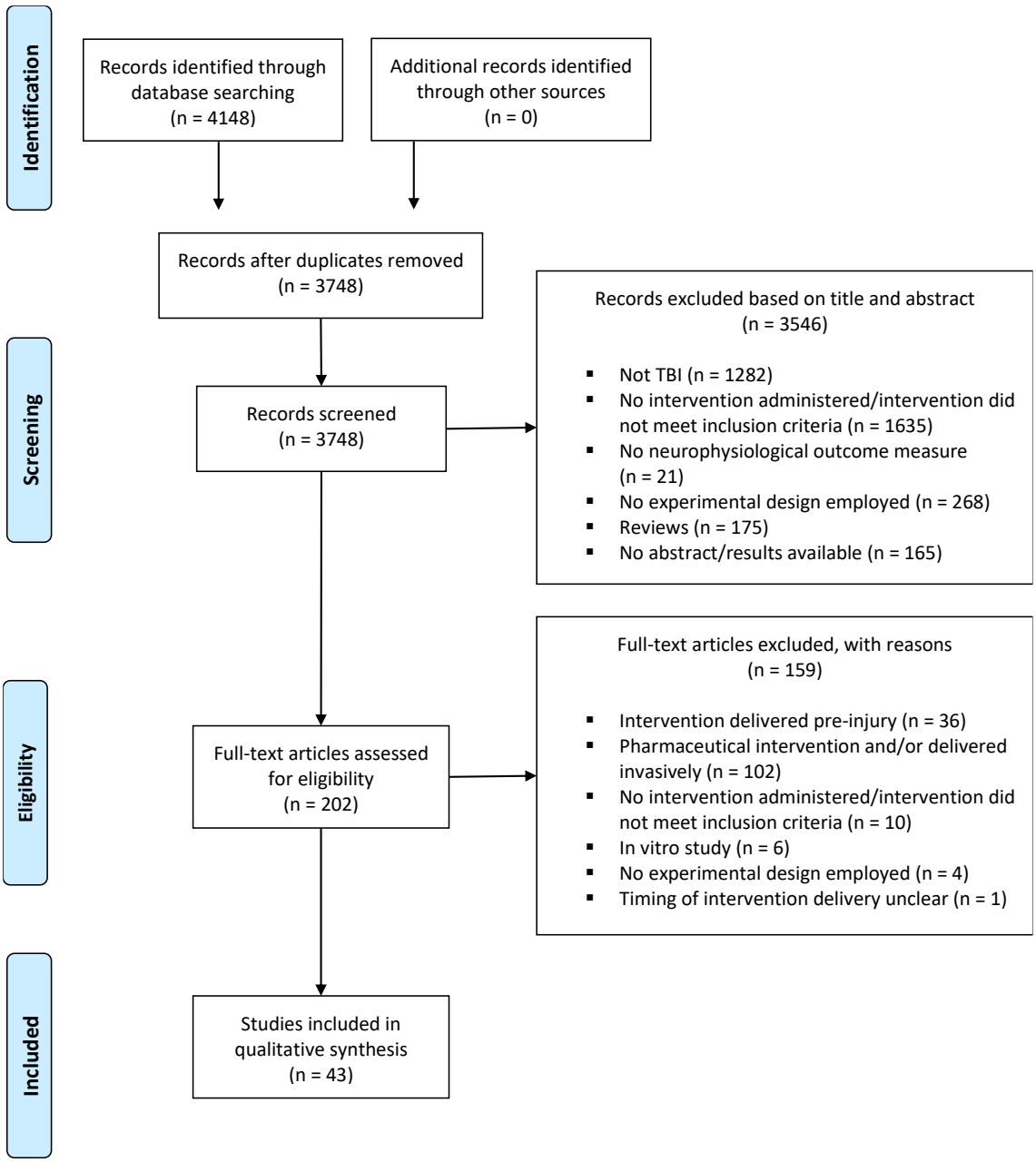


Figure 4.1 Prisma flowchart.

Quality assessment

Only pre-clinical animal studies met all inclusion criteria for this review, so quality and risk of bias (RoB) was assessed for included studies using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) RoB tool [216]. The SYRCLE RoB tool adapted the Cochrane Collaboration RoB tool [217] for randomised controlled trials (RCTs) using human subjects adjusting for aspects of bias within animal studies. The ten signalling questions used within SYRCLE's RoB tool, and respective results for included studies are presented in Table 4.2. Quality assessment of all included studies was performed by JM with a 10% random check by PH.

Table 4.1 Search strategy and study eligibility criteria.

Systematic search strategy		
TBI-related search terms	postconcussion or "post-concussion" or concussion or tbi or mtbi or "traumatic brain injur*" or "cortical impact" or "fluid percussion" or "acceleration injury"	
The nutrition & diet-related search terms below were queried within 10 words before or after TBI-related search terms		
Nutrition & diet-related search terms	diet* or supplement* or "neuroprotective agent*" or creatine or antioxidant* or "anti-oxidant*" or "fatty acid*" or vitamin* or nutri* or nutraceutical or keto* or "amino acid*" or "complementary and integrative medicine" or "complementary and alternative medicine"	
P.I.C.O. framework & study selection criteria		
	Exclusion criteria	**Inclusion criteria**
Participants/population Humans and/or mammals that have sustained TBI	In vitro models of brain/neuronal/axonal injury Spinal cord injury Human subjects with TBI on life support, in a comatose state, and/or subjects that are paralysed	Mild, moderate, and severe TBI Single or repetitive impacts/injuries In vivo investigations including: 1) Clinical studies with human subjects and/or 2) Pre-clinical studies on animal models of TBI
Intervention Nutrition and/or dietary manipulation interventions administered *post-injury* with the aim to attenuate impaired neurophysiological functions due to TBI	Nutrition/dietary interventions administered pre-TBI Acute lifesaving interventions Administration of nutrition supplement via invasive means such as intravenous, intraperitoneal, intrathecal, or intracerebroventricular/intracerebral injections Nutrition/dietary interventions targeting the gut/intestines after severe TBI Enteral feeding for paralysed/comatose subjects Pharmaceutical/drug/hormone interventions	Nutrition and/or dietary manipulation interventions administered non-invasively* post-TBI *exception made for nutrients/supplements delivered via gavage to animal models to ensure dosage compliance
Comparators/controls Pre-injury baseline measures, current best practice, regular diet, no treatment, sham injury, control, or placebo group	Absence of comparison group	Presence of comparison group
Outcomes Measures of neurophysiological function related to secondary injury phase of TBI	Neurocognitive, neuropsychological, behavioural, or clinical outcome measures only	Minimum of one objective outcome measure to quantify neurophysiological outcomes and/or consequences of TBI including: morphological changes, cell death/apoptosis/cell survival, energy metabolism, mitochondrial function, oxidative stress, glial activation, immune response, BBB integrity, cell membrane homeostasis, plasticity, neurotransmission, and/or neurogenesis

Data extraction and synthesis

Studies meeting inclusion criteria were recorded and curated for: study characteristics (study type, number of study groups, method of TBI induction, TBI severity and mortality rate); animal characteristics (species, sample size, sex, age, and weight); intervention details (type of intervention, controls used, timing of initiation of intervention, intervention dosage and duration of intervention); and outcomes (NP outcomes evaluated, biomarkers used, and behavioural/neurocognitive outcomes). Efforts were made to contact study authors to acquire any key information that was missing or unclear in published articles. A summary of study characteristics (Table 4.3), summary of NP outcomes (Table 4.4) and details of NP and behavioural outcomes (Table S1) are provided.

Results

Article selection and quality assessment

Application of inclusion and exclusion criteria to titles and abstracts of search resulted in 201 full-text articles that were screened for eligibility; 43 journal articles [147-150, 218-256] met all eligibility criteria. Most studies (28/43) were conducted since 2013, with 4-7 new studies being released each year since 2016. All studies were pre-clinical animal studies with 27/43 studies clearly stating that randomisation took place. RoB was generally unclear across all studies (Table 4.2) due to limited methodological detail, which failed to address several criteria within the SYRCLE RoB tool such as: “5) Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?” (unclear in 40 of 43 studies); and “6) Were the animals selected at random for outcome assessment?” (unclear in 30 of 43 studies).

Table 4.2 Included studies RoB evaluated using the SYRCLE tool.

	1) Was the allocation sequence adequately generated and applied?	2) Were the groups similar at baseline or were they adjusted for confounders in the analysis?	3) Was the allocation to the different groups adequately concealed during?	4) Were the animals randomly housed during the experiment?	5) Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	6) Were the animals selected at random for outcome assessment?	7) Was the outcome assessor blinded?	8) Were incomplete outcome data adequately addressed?	9) Are reports of the study free of selective outcome reporting?	10) Was the study apparently free of other problems that could result in a high risk of bias?
Prins et al. 2005	UC	Yes	UC	UC	UC	UC	UC	UC	UC	UC
Davis et al. 2008	UC	Yes	UC	UC	UC	No	UC	No	No	UC
Hu et al. 2009a	Yes	Yes	UC	UC	UC	UC	Yes	UC	Yes	UC
Hu et al. 2009b	Yes	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Prins et al. 2009	Yes	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Toklu et al. 2009	Yes	Yes	UC	UC	UC	UC	Yes	UC	Yes	UC
Bailes & Mills 2010	UC	Yes	UC	No	UC	No	UC	UC	Yes	UC
Cole et al. 2010	Yes	Yes	UC	UC	UC	UC	Yes	No	Yes	UC
Schwartzkroin et al. 2010	Yes	Yes	UC	UC	UC	UC	Yes	No	Yes	UC
Sharma et al. 2010	Yes	Yes	UC	UC	UC	No	UC	UC	Yes	UC
Deng-Bryant et al. 2011	UC	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Mills et al. 2011	UC	Yes	UC	UC	UC	No	UC	UC	Yes	UC
Shin & Dixon 2011	Yes†	Yes	UC	UC	UC	UC	No†	UC	Yes	UC
Wu et al. 2011	UC	Yes	UC	UC	UC	No	UC	UC	Yes	UC
Saraiva et al. 2012	Yes†	Yes	UC	UC	UC	UC	Yes†	UC	Yes	UC
Itoh et al. 2013	Yes	Yes	UC	UC	UC	Yes	UC	UC	Yes	UC
Lim et al. 2013	Yes	Yes	UC	UC	Yes	UC	Yes	UC	Yes	UC
Kumar et al. 2014	Yes	Yes	UC	No	UC	UC	UC	Yes	Yes	UC
Wu et al. 2014	UC	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Zhao et al. 2014	Yes	Yes	UC	UC	UC	Yes	Yes	Yes	Yes	UC
Ozbal et al. 2015	Yes	Yes	UC	UC	UC	UC	Yes	Yes	Yes	UC
Wei et al. 2015	Yes	Yes	UC	UC	UC	UC	Yes	Yes	Yes	UC
Cheng et al. 2016	Yes	Yes	UC	UC	Yes	Yes	Yes	Yes	Yes	UC
Greco et al. 2016	Yes	Yes	UC	UC	UC	UC	No†	Yes	Yes	UC
Schober et al. 2016	UC	Yes	UC	No	UC	Yes	Yes	Yes	Yes	UC
Su et al. 2016	UC	Yes	UC	UC	UC	No	UC	Yes	Yes	UC
Wang et al. 2016a	Yes	Yes	UC	UC	UC	UC	UC	Yes	Yes	UC
Wang et al. 2016b	Yes	Yes	UC	UC	Yes	UC	Yes	Yes	Yes	UC
Xing et al. 2016	Yes	Yes	UC	UC	UC	Yes	UC	UC	Yes	UC
Ji et al. 2017	UC	Yes	UC	No	UC	UC	Yes	No	Yes	UC
Jiang et al. 2017	UC	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Liu et al. 2017	Yes	Yes	Yes	UC	UC	UC	Yes	No	Yes	UC
Xu et al. 2017	Yes	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Zhu et al. 2017	Yes	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Elliott et al. 2018	Yes	Yes	UC	UC	UC	UC	Yes	No	Yes	UC
Özevren et al. 2018	UC	Yes	UC	UC	UC	UC	UC	Yes	Yes	UC
Wang et al. 2018	Yes	Yes	UC	UC	UC	UC	UC	UC	Yes	UC

Xie et al. 2018		Yes	Yes	UC	UC	UC	No	UC	UC	Yes	UC
Zhang et al. 2018		Yes	Yes	UC	No	UC	UC	Yes	UC	Yes	UC
Gerbatin et al. 2019		Yes	Yes	UC	UC	UC	UC	Yes	UC	Yes	UC
Krishna et al. 2019		UC	Yes	UC	UC	UC	UC	UC	No	Yes	UC
Rubovitch et al. 2019		UC	Yes	UC	UC	UC	UC	UC	UC	Yes	UC
Thau-Zuchman et al. 2019		Yes	Yes	UC	UC	UC	Yes	Yes	Yes	Yes	UC
Column totals	Yes	29	43	1	0	3	6	18	12	43	0
	No	0	0	0	5	0	7	2	7	0	0
	Unclear	14	0	42	38	40	30	23	24	0	43

Note: † = information acquired by contacting corresponding author, UC = unclear in published article.

We qualitatively appraised studies, aggregating evidence to identify strengths, weaknesses, and trends suggesting preliminary positive intervention effects from animal studies within this review while identifying gaps in knowledge. Through identifying these trends we can begin to develop explanatory theories about how NUTs may facilitate improved recovery outcomes post-TBI; while generating hypotheses to justify and inform the feasibility of translating this evidence to clinical patients [257, 258].

TBI induction and animal characteristics

TBI induction

Across the 43 included studies (Table 4.3), TBIs were produced in 2,897 animals (more animals were used but the sample size in several studies was unclear) using controlled cortical impact (16 CCI studies), fluid percussion injury (12 FPI studies), or weight drop techniques (15 studies). Generally, Feeney's weight drop model (5/15) and CCI are implemented to mimic a mainly focal brain injury that might occur after a fall, motor vehicle accident (MVA), or sport-related TBI [259]. Marmarou's weight drop model (6/15) produced mainly diffuse brain injury like that following a fall or MVA. To achieve mixed brain injury (with focal and diffuse consequences) FPI is commonly delivered [259]. No studies used a blast wave model which would mimic TBI commonly suffered by deployed military personnel [259, 260]. Animals were anesthetised in 42 studies prior to delivery of TBI. Craniotomy or skull surgical alteration occurred in 34/43 studies. The head of animals was secured during TBI induction in 15/43 studies, while freedom of head movement was unclear or not reported in 27/43 studies (9 CCI, 10 FPI, 8 weight drop).

TBI severity

TBI induction methods (Table 4.3) inflicted mTBI in nine studies (5 FPI, 4 weight drop), moderate TBI in nine studies (1 CCI, 6 FPI, 2 weight drop), and severe TBI in four studies (1 CCI, 3 weight drop). In 23 studies TBI severity was not clearly described (15 CCI, 2 FPI, 6 weight drop). Given TBI severity was a key variable of interest, study authors were contacted for information, or recently released classification criteria was used to infer TBI severity in Table 4.3 [259, 261]. One study intentionally compared outcomes after moderate and severe TBI [220], otherwise investigations focused on evaluating a NUT following a specific severity of

TBI. Two studies evaluated a NUT following repetitive TBI [240, 246]. In both studies animals suffered three TBIs by either FPI or Marmarou weight drop to produce repetitive mild and severe TBIs, respectively.

Animal characteristics

Most studies investigated NUTs in rat models of TBI (29 used Sprague Dawley rats; six used Wistar rats), the remaining studies used mice (six used C57BL/6 mice, two used ICR mice; Table 4.3) as an animal model of TBI. No interventions were evaluated for utility across multiple species using comparable methodologies. Fasting was studied in both mice and rats following TBI, however, TBI induction and severity modeling was considerably different between studies preventing results comparison. TBI was modelled in only male rats/mice except for one study [248] that included male and female Sprague Dawley rats to evaluate DHA supplementation after FPI.

Animal maturity

Age and maturity of rodents varied across included studies (Table 4.3). Animal age was commonly indicated based on the number of days post-natal (PND) at which animals were subject to TBI or sham injury. Insights into the interaction of age and TBI are gained by conducting experiments with animals at differing stages of maturity. Adulthood in rats and mice is considered to begin after PND-56 and PND-42, respectively [262]. Included studies used PND-7 to 17 and PND-35 to 55 to define immature/juvenile and adolescent rats, respectively. Juvenile and immature rats were used in three studies, pre-adolescent/adolescent rats in eight studies, and adult rats in 18 studies. All mice studies used late adolescent and adult mice. Animal weight was the only measure of maturity reported in 12 studies. Due to a variety of unreported environmental factors that could affect weight, animal age could not be inferred in these studies. Four studies compared how a ketogenic diet attenuated NP impairments post-TBI between multiple age groups (primarily pre-adolescent rats versus adult rats), and identified a trend for the benefits of ketogenic diet post-TBI being age specific and limited to pre-adolescent rats [148, 222, 232, 233].

Table 4.3 Study design and intervention details.

Author (year) *country*	Animal study type (# of study groups)	Method of TBI delivery, TBI severity, Mortality rate	Animal details (N)	Intervention	Controls	Initiation of intervention (duration of intervention)	Dosage
Anti-oxidants							
Toklu et al. (2009) *Turkey*	Random pre-clinical (n = 4)	MIAI, Mild, UC	300-350g; Wistar rats (64)	α-lipoic acid via gavage	Sham + vehicle; sham + α-lipoic acid; TBI + vehicle;	First dose 30 min post-injury then 1 dose 24 hr later (48 hr)	100 mg/kg per day
Ji et al. (2017) *China*	Pre-clinical (n = 3)	M.A. Flierl weight drop model, Mod, 8%	28-32g; ICR mice (UC)	Astaxanthin diluted in olive oil (1 mL/kg) via gavage	Sham + olive oil; TBI + olive oil	First dose 30 min post-injury then 1 dose per 24 hr until sacrifice (7 days)	75 mg/kg per day
Jiang et al. (2017) *China*	Pre-clinical (n = 7)	CCI, Mod*, NR	Adult; 200-240g; SD rats (UC)	Catechin supp via gavage at 5 dosages	Sham; TBI + vehicle	One dose per day until sacrifice (24 hr or 28 days)	Dose 1: 1 mg/kg Dose 2: 5 mg/kg Dose 3: 10 mg/kg Dose 4: 20 mg/kg Dose 4: 30 mg/kg
Krishna et al. (2019) *USA*	Pre-clinical (n = 3)	FPI, Mod, NR	PND-70; SD rats (UC)	5% Blueberry enriched diet	Sham + Std diet; TBI + Std diet	Immediately post-injury (14 days)	Fed ad libitum
Itoh et al. (2013) *Japan*	Random pre-clinical (n = 3) **	CCI, Mod to severe*, NR	PND-42; 120-140g; Wistar rats (123)	EGCG dissolved in drinking water	Sham + regular drinking water; TBI + regular drinking water	Immediately post-injury (24 hr, 72 hr, 7 days)	0.1% EGCG drinking water; Drank ad libitum
Cheng et al. (2016) *China*	Random pre-clinical (n = 6)	CCI, Mod to severe*, 7%	PND-56 to PND-84; 20-26g; 151 wild type, 31 Nrf2 knockout; C57BL/6 mice (182)	(-)-epicatechin supp via gavage in low, moderate, high dose	Sham + vehicle; TBI + vehicle	First dose 3 hr post-injury then 1 additional per 24 hr for 3 or 7 days (72 hr dosage differences or 7 days moderate dose only)	Low (-)-epicatechin: 5 mg/kg/day; Moderate (-)-epicatechin: 15 mg/kg/day; High (-)-epicatechin: 45 mg/kg/day
Ozbal et al. (2015) *Turkey*	Random pre-clinical (n = 3)	Weight drop, NR, NR	Juvenile; PND-7; 20-30g; Wistar rats (42)	α-lipoic acid via gavage	Sham + Std diet; TBI + Std diet	First dose 30 min post-injury then 1 additional dose 24 hr later (48 hr)	100 mg/kg per day
Wei et al. (2015) *China*	Random pre-clinical (n = 5)	Feeney weight-drop, NR, NR	250-280g; SD rats (150)	α-lipoic acid in low and high dose via gavage	Sham + vehicle; sham + α-lipoic acid; TBI + vehicle;	First dose 30 min post-injury then 1 dose 24 hr later (48 hr)	Low α-lipoic acid: 20 mg/kg; High α-lipoic acid 100 mg/kg

Branched chain amino acids							
Lim et al. (2013) *USA*	Random pre-clinical (n = 3)	FPI, Mild, 0%[†]	PND-35 to PND-49; 20-25g; C57BL/J6 mice (19)	Tap water containing valine, leucine, and isoleucine	Sham + tap water; TBI + tap water	Immediately post-injury (4 weeks)	100 mM of each BCAA dissolved in tap water; Drank ad libitum
Elliott et al. (2018) *USA*	Random pre-clinical (n = 3)	FPI, Mild, 0%[†]	PND-35 to PND-49; 20-25g; C57BL/J6 mice (24)	Tap water containing valine, leucine, and isoleucine	Sham + tap water; TBI + tap water	Initiated 48 hr post-injury (5 days)	100 mM of each BCAA dissolved in tap water; Drank ad libitum (approx 3-5 mL/day)
Cole et al. (2010) *USA*	Random pre-clinical (n = 4)	Lateral FPI, Mild to Mod, 0%	PND-35 to PND-49; 20-25g; C57BL/6 mice (UC)	Tap water containing valine, leucine, and isoleucine; or tap water containing phenylalanine	Sham + tap water; TBI + tap water	Initiated 48 hr post-injury (5 days)	100 mM of each BCAA dissolved in tap water; Drank ad libitum
Creatine							
Saraiva et al. (2012) *Brazil*	Pre-clinical (n = 2)	FPI, Mod[†], 18.07%[†]	Adult; 250-300g; Wistar rats (~24 to 40)	Creatine supp via gavage	Sham + vehicle; sham + creatine supp; TBI + vehicle	First dose 30 mins post-injury then 1 dose per 24 hr until sacrifice (72 hr)	300 mg/kg/day
Gerbatin et al. (2019) *Brazil*	Random pre-clinical (n = 4)	FPI, Mod, 18.07%	PND-90; Wistar rats (166)	Creatine supp via gavage	Sham + Std diet; sham + creatine supp; TBI + Std diet	Initiated 7 days post-injury then 1 dose per 24 hr until sacrifice (4 weeks)	300 mg/kg/day
Fasting & caloric restriction							
Liu et al. (2017) *China*	Random pre-clinical (n = 3)	Weight drop, Mild, NR	PND-84; C57BL/6 mice (45)	Caloric restriction	TBI + normal caloric intake; TBI + high energy intake	Feeding initiated 6-10 hr post-injury (24 hr, 48 hr, 35 days, or 37 days)	High energy: normal caloric intake x 1.3; Caloric restriction: 0.7 x normal caloric intake; Fed ad libitum according to energy ratio; Intermittent fasting: alternating 24 hr periods with/without access to food; Caloric restriction fed ad libitum according to energy ratio: 10% restriction week 1, 20% restriction week 2, 30% restriction week
Rubovitch et al. (2019) *Israel*	Pre-clinical (n = 7)	Weight drop, Mild, NR	PND-42 to PND-49; ICR mice (177)	Intermittent fasting or caloric restriction	Sham + Std caloric intake; sham + fasting; sham + caloric restriction; TBI + Std caloric intake	Immediately post-injury (30 days)	

							3, 40% restriction until sacrifice
Davis et al. (2008) *USA*	Pre-clinical (n = 5) **	CCI, Mod and severe, NR	Adult; SD rats (30)	Fasting	TBI + regular ad libitum feeding	Immediately post-injury (24 hr or 48 hr)	No caloric intake for designated time period
Ketogenic diet							
Zhang et al. (2018) *China*	Random pre-clinical (n = 3)	FPI x 3, Mild, NR	PND-60; 100-140g; SD rats (72)	Ketogenic diet	TBI + Std diet; Sham + Std diet	Immediately post-injury (7 days)	Fed ad libitum
Prins et al. (2005) *USA*	Pre-clinical (n = 20)	CCI, Mod*, NR	PND-17 (n = 27), PND-35 (n = 29), PND-45 (n = 27), PND-65 (n = 26); SD rats (109)	Ketogenic diet	TBI + Std diet; Sham + Std diet	Immediately post-injury (1 hr, 6 hr, 24 hr, 7 days)	Ketogenic diet: 8.4% protein, 78.8% fat, 0.8% carbohydrate, 5% fibre, 3.8% ash; Standard diet: 18.6% protein, 6.2% fat, 59.8% carbohydrate, 4.5% fibre; 6.02% ash; Fed ad libitum
Prins et al. (2009) *USA*	Random pre-clinical (n = 7)	CCI, Mod*, NR	Pre-adolescent (PND-35); Adult (PND-70); SD rats (90)	Ketogenic diet	PND-35 sham + Std diet; PND-35 sham + ketogenic diet; PND-35 TBI + Std diet; PND-70 sham + Std diet; PND-70 sham + ketogenic diet	Immediately post-injury (7 days)	Ketogenic diet: 8.4% protein, 78.8% fat, 0.8% carbohydrate, 5% fibre; Standard diet: 18.6% protein, 6.2% fat, 59.8% carbohydrate, 4.5% fibre; Fed ad libitum
Deng-Bryant et al. (2011) *USA*	Pre-clinical (n = 8)	CCI, Mod*, NR	Pre-adolescent (PND-35); Adult (PND-70); SD rats (UC)	Ketogenic diet	PND-35 sham + Std diet; PND-35 sham + ketogenic diet; PND-35 TBI + Std diet; PND-70 sham + Std diet; PND-70 sham + ketogenic diet; PND-70 TBI + Std diet	Immediately post-injury (6 hr or 24 hr)	Ketogenic diet: 8.4% protein, 78.8% fat, 0.8% carbohydrate, 5% fibre; Standard diet: 18.6% protein, 6.2% fat, 59.8% carbohydrate, 4.5% fibre; Fed ad libitum
Schwartzkroin et al. (2010) *USA*	Random pre-clinical (n = 3)	Lateral FPI, Mod, NR	PND 56; SD rats (55)	Ketogenic diet	Sham + Std diet; TBI + Std diet	Immediately post-injury (3 weeks)	Ketogenic diet: 8% protein, 79% fat (>29% saturated fats), 0.76% carbohydrates, 12%

							water/fiber/ash; Fed ad libitum
Greco et al. (2016) *USA*	Random pre-clinical (n = 6)	CCI, Mod†, 0%†	PND-35 (n = 36), PND-70 (n = 18); SD rats (54)	Ketogenic diet	PND-35 sham + Std diet; PND-35 TBI + Std diet; PND-70 sham + Std diet; PND-70 TBI + Std diet	Immediately post-injury (6 hr or 24 hr)	Ketogenic diet: 8.4% protein, 78.8% fat, 0.8% carbohydrate, 5% fibre; Standard diet: 18.6% protein, 6.2% fat, 59.8% carbohydrate, 4.5% fibre; Fed ad libitum
Hu et al. (2009a) *China*	Random pre-clinical (n = 4)	Feeney weight-drop, UC, NR	130-140g; SD rats (80)	Ketogenic diet	Sham + Std diet; Sham + ketogenic diet; TBI + Std diet	Immediately post-injury (72 hr)	Ketogenic diet: protein 17.4%, fat 69.8%, carbohydrate 0%; Fed ad libitum
Hu et al. (2009b) *China*	Random pre-clinical (n = 5)	Feeney weight-drop, UC, NR	PND 35; SD rats (25)	Ketogenic diet	Sham + Std diet; Sham + ketogenic diet; TBI + Std diet	Immediately post-injury (72 hr)	Ketogenic diet: protein 17.4%, fat 69.8%, carbohydrate 0%; Fed ad libitum
Multi-supplement							
Wu et al. (2014) *USA*	Pre-clinical (n = 5)	FPI, Mod, NR	200-240g; SD rats (UC)	Curcumin enriched diet, DHA enriched diet, or curcumin + DHA enriched diet	Sham + Std diet; TBI + Std diet	Immediately post-injury (14 days)	Curcumin: 500 ppm enriched chow; DHA: 1.2% DHA enriched chow; Fed ad libitum
Thau-Zuchman et al. (2019) *UK*	Random pre-clinical (n = 3)	CCI, Mod†, 0%†	PND-70 to PND-84; 22-27g; C57BL/J6 mice (30)	Fortasyn Connect multi-supplement enriched diet	Sham + Std diet; TBI + Std diet	Immediately post-injury (10 weeks)	Fed ad libitum
Polyunsaturated fatty acids							
Wu et al. (2011) *USA*	Pre-clinical (n = 3)	FPI, Mild, NR	200-240g; SD rats (36)	DHA enriched diet	Sham + Std diet; TBI + Std diet	Immediately post-injury (12 days)	1.2% DHA enriched chow; Fed ad libitum
Su et al. (2016) *China*	Pre-clinical (n = 4) **	Feeney weight drop, Mod, 3.7%	250-300g; SD rats (101**)	Moderate (1:5) or high (1:1) ω-3/ω-6 ratio PUFA oil via gavage	Sham + "western" diet (low ω-3/ω-6 PUFA ratio; 1:30); TBI + "western" diet	Immediately post-injury then 1 dose per 24 hr until sacrifice (72 hr, 5 days, 7 days, or 19 days)	All animals fed same fat-free chow and 5.5 mL/kg PUFA oil mixture: Low ratio - 4% fish oil + 96% linoleic acid; Moderate ratio: 21% fish oil + 79% linoleic acid; or High ratio: 58% fish oil + 42% linoleic acid

Shin & Dixon (2011) *USA*	Pre-clinical (n = 4)	CCI, Mod to severe†, 0%	~PND-60†; 275-300g; SD rats (~20to 24†)	Fish oil supp via gavage	Sham + olive oil; sham	Immediately post-injury then 1 dose per 24 hr until sacrifice (7 days)	1.5 mL/day containing 360 mg EPA & 240 mg DHA
Schober et al. (2016) *USA*	Pre-clinical (n = 3)	CCI, Mod to severe*, 1.5-3.0% across groups	Immature; PND17; SD rats (400)	DHA enriched diet	Sham + Std diet; TBI + Std diet	Immediately post-injury (24 hr, 48 hr, 72 hr, or 50 days)	0.1% DHA enriched chow; Fed ad libitum
Bailes & Mills (2010) *USA*	Pre-clinical (n = 4)	MIAI, Severe*, 0%	Adult; 350-400g; SD rats (40)	Low or high DHA enriched diet	Sham + Std diet; TBI + Std diet	Immediately post-injury (30 days)	Low DHA: 10 mg/kg/day; High DHA: 40 mg/kg/day; Fed ad libitum
Mills et al. (2011) *USA*	Pre-clinical (n = 4)	MIAI, Severe, 0%	Adult; 350-400g; SD rats (40)	Low or high fish oil (EPA + DHA) supp via gavage	Sham + Std diet; TBI + Std diet	Initiated 24 hours post-injury then 1 dose per 24 hr until sacrifice (30 days)	Low fish oil: 10 mg/kg/day High fish oil: 40 mg/kg/day
Zhu et al. (2017) *China*	Random pre-clinical (n = 4)	FPI, UC, NR	PND-49; 300-500g; SD rats; 40 male, 40 female (80)	Low or high DHA supp via gavage	Sham + Std diet; TBI + Std diet	First dose 30 min post-injury then 1 dose per 24 hr until sacrifice (15 days)	Low DHA: 370 mg/kg/day; High DHA: 740 mg/kg/day
Traditional Eastern medicine							
Özevren et al. (2018) *Turkey*	Pre-clinical (n =3)	MIAI, Mild, 0%	280-330g; SD rats (36)	Ganoderma lucidum supp via gavage	Sham + saline vehicle; TBI + saline vehicle	First dose 30 min post-injury (7 days)	20 mL/kg/day
Sharma et al. (2010) *USA*	Random pre-clinical (n = 4)	FPI, Mod, NR	~PND-60; SD rats (24)	Curcumin enriched diet	Sham + Std diet; sham + curcumin enriched diet; TBI + Std diet;	Immediately post-injury (14 days)	Chow enriched with 500 ppm of curcumin; Fed ad libitum
Wang et al. (2016a) *China*	Random pre-clinical (n = 4)	CCI, Mod to severe*, ~10%	PND-56 to PND-70; 200-250g; SD rats (96)	HYSA supp via gavage in low and high dosage	Sham; TBI + saline vehicle	Dose ~20 min post-injury (Single dose post-injury)	Low HSYA: 10 mg/kg; High HSYA: 30 mg/HSYA
Wang et al. (2016b) *China*	Random pre-clinical (n = 3)	CCI, Mod to severe*, 10%	Adult; 220-280g; SD rats (88)	RZD supp via gavage	Sham; TBI + vehicle	First dose 4 hr post-injury then one dose per 24 hr until sacrifice (14 or 24 days)	20 mg/kg/day
Xing et al. (2016) *China*	Random pre-clinical (n = 6)	CCI, Mod to severe*, NR	200-250g; SD rats (182)	XFZY supp via gavage in low and high dosage	Sham + saline vehicle; TBI + saline vehicle; sham + low XFZY dose;	First dose immediately post-injury then 1 dose per 24 hr until sacrifice (24 hr, 72 hr, 7 days, 14 days, 21 days)	Low XFZY: 9 g/kg; High XFZY: 18 g/kg

					sham + high XFZY dose		
Xu et al. (2017) *China*	Random pre-clinical (n = 6)	CCI, Mod to severe*, NR	200-300g; SD rats (108)	Rhubarb (low, moderate, high) or rhein (single dose) supp via gavage	Sham; TBI + vehicle	Immediately post-injury (8, 12, 24 hr)	Low rhubarb: 3 g/kg; Moderate rhubarb: 6 g/kg ; High rhubarb: 9 g/kg; Rhein: 12 mg/kg
Xie et al. (2018) *China*	Random pre-clinical (n=3)	CCI, Mod to severe*, NR	PND-42 to PND-56; 220-250g; SD rats (15)	AR-RAS herbal formula supp via gavage	Sham + saline vehicle; TBI + saline vehicle	First dose ~45 min post-injury then 1 dose per 24 hr until sacrifice (14 days)	3.24 g/kg/day
Kumar et al. (2014) *India*	Random pre-clinical (n = 5) **	MIAI, Severe*, NR	Adult; 250-300g; Wistar rats (50**)	Panax ginseng suspended in CMC via oral route	Sham + CMC vehicle; TBI + CMC vehicle	Initiated 14 days post-injury (14 days)	Panax ginseng: 0.5 mL/100g of bodyweight/day; suspended in CMC delivered via oral route
Wang et al. (2018) China	Random pre-clinical (n = 3)	MIAI x 3, Severe, NR	PND70 to PND-77; 260-300g; SD rats (30)	Berberine supp via gavage	Sham + saline vehicle; TBI + saline vehicle	First dose 24 hours post-injury then 1 dose per 24 hr until sacrifice (4 weeks)	200 mg/kg of bodyweight/day
Zhao et al. (2014) *China*	Random pre-clinical (n = 5)	Feeney weight drop, UC, NR	250-280g; SD rats (50)	MSD supp via gavage in low, moderate, and high dosage	Sham + distilled water; TBI + distilled water	First dose 6 hr post-injury then 2 doses per 24 hr until sacrifice (24 hr, 72 hr, 5 days, 14 days)	Low MSD: 0.5 mL/200g; Moderate MSD: 1.0 mL/200g; High MSD: 2.0 mL/200g

Note: * = Inferred based on available information and criteria summarised by [259, 261], ** = Additional groups and were present in study but were excluded due to being outside scope of review, † = information acquired by contacting corresponding author, Mod = moderate TBI, NR = not reported in published article and not obtained by contacting author, supp = supplementation, UC = unclear in published article and not obtained by contacting author, Std = standard.

Nutritional interventions and neurophysiological outcomes

Nutritional interventions

Nutritional interventions were administered to animals by supplementing a standard diet with a specific nutrient or natural compound, by directly manipulating macro-/micronutrient constituents of the animal's diet, or by controlling availability of food (Table 4.3). Supplementation allowed for controlled and precise nutrient dosage to animals, while manipulating diet/water permitted animals to feed/drink ad libitum when food/water was available. Nutritional interventions described by included studies fell within one of eight distinct themes which were:

1. natural compounds with known anti-oxidant properties [149, 225, 226, 230, 251, 253-255];
2. branched chain amino acids (BCAA) [219, 221, 228];
3. creatine [150, 249];
4. fasting and caloric restriction [147, 220, 229];
5. ketogenic diet (KD) [148, 222-224, 232, 233, 235, 246];
6. multi-supplements [239, 243];
7. ω-3 polyunsaturated fatty acids (PUFAs) [218, 234, 237, 238, 242, 248, 252]; or
8. natural compounds used in traditional eastern medicine [227, 231, 236, 240, 241, 244, 245, 247, 263].

Neurophysiological outcomes

Biomarkers of morphological changes and apoptosis (24 studies), plasticity, neurotransmission, and neurogenesis (18 studies), indicators of oxidative stress (18 studies), cellular energy imbalance (12 studies), cytokines representative of immune response post-TBI (11 studies), BBB integrity (11 studies), cell membrane homeostasis (8 studies) and glial activation (6 studies) were NP outcomes evaluated (Table 4.4 and Table S1). Additionally, 29/43 studies included neurocognitive and/or behavioural outcome measures. Neurocognitive and behavioural outcomes were outside the focus of this review and are only discussed in subsequent sections if relevant. An overview of results for each study are available in Table S1.

Anti-oxidants

Eight studies administered compounds with known anti-oxidant properties to animals by gavage (an orally inserted tube to administer food/supplement directly to stomach) or by enriching the animal's diet/drinking water with the compound. Anti-oxidant compounds α-lipoic acid (ALA) [230, 251, 254], catechin [255], (-)-epicatechin[253], and astaxanthin [225] were delivered via oral gavage. In the remaining studies, animals received (–)-epigallocatechin gallate (EGCG) dissolved in drinking water [149], and a diet enriched with blueberries [226]. In each study the intervention was initiated within three hours of TBI. In 7/8 anti-oxidant studies, delivered compounds were associated with decreased markers of oxidative stress versus control. Two studies reported reduced BBB disruption following ALA delivery. Both ALA and astaxanthin demonstrated capacity to attenuate morphological changes and apoptosis versus control post-TBI. Phenols (catechin and EGCG) elicited an anti-apoptotic benefit. Increased markers of plasticity were reported following a blueberry enriched diet and astaxanthin supplementation versus control. Specifically, two doses of ALA after weight-drop TBI reduced BBB disruption, apoptosis, and oxidative stress within the first 48 hours after injury, although longer term outcomes were not explored [230, 251, 254]. Collectively, animals gavaged with phenols (catechin and EGCG) beginning the same day as moderate to severe TBI displayed less apoptotic damage and oxidative stress and increased BBB integrity compared to controls [149, 253, 255].

Branched chain amino acids

Three studies provided tap water mixed with BCAAs valine, leucine, and isoleucine to be drank ad libitum by mice post-TBI [219, 221, 228]. Two studies provided BCAA infused water 48 hours post-injury while the third made BCAAs available immediately post-injury. Only biomarkers representative of neurotransmission were measured post-TBI; where administration of BCAAs (valine, leucine, and isoleucine dissolved in tap water and drank ad libitum beginning 48 hours after mild to moderate FPI and lasting for five days) improved synaptic efficacy [219, 221]. These BCAA associated benefits supported the restoration of excitatory (glutamatergic) and inhibitory (GABAergic) neuron balance post-TBI. Greater excitatory-inhibitory balance was related to improved regulation of sleep and arousal/wakefulness versus control at four weeks post-FPI in two studies [221, 228].

Creatine

Two studies interrogated the effect of creatine supplementation, delivered by oral gavage, on NP outcomes post-TBI [150, 249]. Creatine reduced oxidative stress and cellular energy imbalance [249] and there was reduced cells lost within the hippocampus and increased inhibitory neuron action versus control [150]. Increased inhibitory action associated with creatine supplementation also had a protective effect against drug-induced seizures (to simulate epileptic seizures post-TBI) versus control in one study [150].

Fasting and caloric restriction

Two studies investigated fasting and caloric restriction in isolation [220, 229], while a third study evaluated both interventions to determine if one was more efficacious post-TBI [147]. Rats that were acutely fasted for 24 hours after induction of moderate TBI demonstrated increased tissue sparing along with reduced oxidative stress and cellular energy imbalance compared to controls [220]. Tissue sparing was not observed at 48 hours following moderate TBI, nor at either timepoint for severe TBI. Data were not available for oxidative stress and cellular energy imbalance outcomes following 48 hours of fasting. Adult mice assigned to caloric restriction demonstrated reduced glial activation, preserved cell density, and increased autophagic activity post-TBI compared to controls [229]. Mice assigned to a high energy diet (130% of normal caloric intake) did not demonstrate any of the same benefits as seen following caloric restriction [229]. Both chronic intermittent fasting and caloric restriction (Table 4.3 shows specific parameters) improved neurometabolic outcomes in the cortex of mice with TBI 30 days post-injury. Only intermittent fasting produced this neurometabolic benefit within the hippocampus for TBI versus control [147].

Ketogenic diet

The ketogenic diet represents the most researched NUT to attenuate secondary damage after TBI as it was reported for eight studies. In all studies, KD increased metabolism of β-hydroxybutyrate, reducing neuronal and glial reliance upon glucose as a primary fuel source. Four of eight KD studies compared effects of this diet post-TBI between pre-adolescent and adult rats [148, 222, 232, 233]. Following moderate TBI,

adolescent rats receiving a KD demonstrated a reduction in cellular energy imbalances and markers of apoptosis [148, 222-224, 232, 233]. However, these NP benefits did not appear to extend to adult animals. The remaining KD studies only measured post-TBI outcomes in adult rats and presented mixed results in regard to anti-apoptotic, preservation of BBB integrity, and mitochondrial energy metabolism [223, 224, 235, 246]. Whilst KD was associated with reduced glial activation versus control, this finding was not replicated across multiple studies for pre-adolescent or adult rats [235].

Multi-supplement

Two studies [239, 243] provided multi-supplements as the intervention. Mice that were provided with 10 weeks of a multi-supplement enriched diet (Fortasyn® Connect – containing DHA, EPA, uridine monophosphate (UMP), choline, folic acid, vitamins B12, B6, C, and E, and selenium) exhibited reduced lesion size and glial activation compared to controls [239]. Mice on the multi-supplement diet had improved cell membrane homeostasis and increased markers of plasticity and neurogenesis [239]. While these positive outcomes associated with a Fortasyn® Connect enriched diet were encouraging, it was unclear which constituents within the supplement facilitated these outcomes. Both a curcumin enriched diet and a DHA enriched diet reduced oxidative stress, supported cell membrane homeostasis, and increased markers of plasticity post-TBI in rats compared to control at two weeks post-injury [243]. All these benefits were observed to a greater extent when curcumin and DHA were combined within the same diet, suggesting an additive advantage of combining these supplement [243].

ω-3 polyunsaturated fatty acids

Of seven studies investigating PUFAs, three studies provided rats with a docosahexaenoic acid (DHA) enriched diet [218, 234, 242] while the others delivered DHA [248], fish oil [237, 252], or a mixed ω-3/ω-6 PUFA oil [238] via gavage. Treatment with PUFAs was initiated immediately post-injury in six studies, with the remaining study delaying initiation until 24 hours post-injury [252]. Delivery of ω-3 PUFAs demonstrated capacity to attenuate apoptosis and oxidative stress, while promoting cell membrane homeostasis and BBB integrity, and upregulate markers of plasticity across the TBI spectrum [218, 234, 238, 242, 243, 248, 252]. These ω-3 PUFA associated NP benefits between 3-50 days post-TBI followed both an enriched diet fed ad libitum or delivery via gavage. Delivery of DHA reduced post-TBI apoptosis in three [218, 234, 248] of four studies [252] evaluating this outcome. Furthermore, rats supplemented with 1:1 ω-3/ω-6 ratio PUFA oil exhibited reduced oxidative stress compared to control [238], and following a DHA enriched diet in two studies [234, 242]. Similarly, 1:1 ω-3/ω-6 ratio PUFA oil supplementation resulted in reduced BBB disruption [238], and two studies [218, 242] showed a DHA enriched diet assisted cell membrane homeostasis. Two studies observed improved neurotransmission due to a DHA enriched diet or fish oil supplementation [218, 237]. DHA reduced the number of injured axons in two studies [218, 252], however, there were mixed results of a DHA-related benefit on neurotransmission measured by magnetic resonance imaging in one study [234]. The potential influence of DHA/fish oil dosing was considered in

three studies [218, 248, 252]; a low and high dose of DHA improved apoptosis [218, 248] and cell membrane homeostasis while reducing axonal damage [218, 252] compared to control, with the higher dose demonstrating greater benefit than the low dose in two studies [218, 248]. In an experiment where ω-3/ω-6 ratios where compared, rats that received a high ratio (1:1) demonstrated reduced BBB disruption post-TBI compared to "western diet" (1:30) controls. In contrast, delivery of a moderate ω-3/ω-6 ratio (1:5) did not alter BBB disruption versus the same controls [238].

Traditional Eastern medicine

Ten distinct traditional eastern compounds were examined for post-TBI utility, including: curcumin [236], panax ginseng [227], modified Shengyu decoction (MSD; multi-herbal supplement) [247], Hydroxysafflor Yellow A (HYSA; flavonoid extracted from Safflower) [241], Xuefu Zhuyu decoction (multi-herbal supplement) [245], ganoderma lucidum (mushroom polysaccharides) [231], berberine (compound within Barberry roots) [240], Astragali Radix and Radix Angelica Sinensis (AR-RAS; multi-herbal supplement) [244], rhubarb and rhein (an active compound within rhubarb) [250], and Rhizoma drynariae (RZD; an active compound with roots of Drynaria Fortunei) [256]. Rats that fed ad libitum for two weeks on a curcumin enriched diet, beginning immediately after moderate FPI, displayed reduced oxidative stress whilst markers of cell membrane homeostasis and neuroplasticity were amplified at 14 days post-injury [236, 243]. Overall, the traditional eastern interventions demonstrated capacity to influence multiple aspects of secondary injury post-TBI in a beneficial manner compared to control. Due to heterogeneity and lack of replication for 9/10 traditional eastern interventions, results were not summarised; however, for an overview of these benefits please see Table 4.4 and Table S1.

Table 4.4 Summary of biomarkers assessed and neurophysiological outcomes

	Intervention specifics	Biomarkers measured	Neurophysiological outcome evaluated		Brief summary of results & main takeaways
Anti-oxidants					
Toklu et al. (2009)	α-lipoic acid (ALA) delivered via gavage	Evans blue extravasation, brain water content	Blood brain barrier integrity	↑	ALA ↓ oxidative stress, ↓ blood brain barrier disruption, & ↓ immune response vs control @ 48hr post-injury
		Luminol & lucigenin chemiluminescence, TBARS, GSH, MPO	Oxidative stress	↓	
		Na-K-ATPase activity	Cellular energy imbalance	↓	
		TNF-α, IL-1β	Immune response	↓	
Itoh et al. (2013)	EGCG dissolved in drinking water	8-OHdG, 4-HNE positive cells; & MDA levels	Oxidative stress	↓	EGCG ↓ oxidative stress & ↓ apoptosis vs control @ pid 1 & 3; EGCG ↓ glial activation vs control @ pid 7
		NeuN, ssDNA, Bcl-2 positive cells	Morphological changes & apoptosis	MIX	
		GFAP positive cells	Glial activation	↓	
Ozbal et al. (2015)	α-lipoic acid (ALA) delivered via gavage in low and high dose	Neuron density; caspase-3, & TUNEL positive cells	Morphological changes & apoptosis	↓	ALA ↓ morphological changes & apoptosis vs control in the cortex & hippocampus; ALA ↓ oxidative stress vs control @ 48hr post-injury; benefit limited to cortex; greater benefits associated with higher dosage
		GPx, SOD, and MDA levels	Oxidative stress	↓	
Wei et al. (2015)	α-lipoic acid (ALA) delivered via gavage	Caspase-3, Cytochrome C, Bax expression; Nissl body staining, TUNEL positive cells	Morphological changes & apoptosis	↓	ALA ↓ morphological changes & apoptosis, ↓ oxidative stress, & ↓ edema vs control @ 48hr post-injury
		MDA & GPx activity	Oxidative stress	↓	
		Brain water content	Blood brain barrier integrity	↑	
Cheng et al. (2016)	(-)-epicatechin supplementation delivered via gavage in low, moderate, high dose	Fluoro-jade, Nissl body & propidium iodide staining; TUNEL positive cells	Morphological changes & apoptosis	↓	(-)-epicatechin ↓ morphological changes & apoptosis, ↓ oxidative stress, & ↓ edema @ pid 3; ↓ myelin damage vs control @ pid 28; greater benefits associated with higher dosage within acute phase; dosing not compared for all outcomes
		Brain water content	Blood brain barrier integrity	↑	
		MPO, HO-1, oxidized hydroethidine, Perls staining	Oxidative stress	↓	
		MBP, Luxol fast blue staining; MMP-2 & MMP-9 activity	Cell membrane homeostasis	↑	
Ji et al. (2017)	Astaxanthin diluted in olive oil	lesion volume, cell density (mm^3)	Morphological changes & apoptosis	↓	Astaxanthin ↓ lesion volume, ↑ cell density & ↑ markers of plasticity,

	delivered via gavage	Synapsin, GAP43, SYP, BDNF	Plasticity, neurotransmission, & neurogenesis	↑	neurotransmission, & neurogenesis astaxanthin vs control @ pid 7
Jiang et al. (2017)	Catechin supplementation delivered via gavage	Brain infarction volume	Morphological changes & apoptosis	↓	Catechin ↓ infarction volume, ↓ blood brain barrier disruption, ↓ immune response vs control @ 24hr post-injury; greater benefit associated with higher dosages
		Brain water content, Evans blue extravasation, occludin, ZO-1	Blood brain barrier integrity	↑	
		IL-1β, iNOS, IL-6, arginase	Immune response	↓	
Krishna et al. (2019)	5% Blueberry enriched diet	BDNF, CREB, & CaMKII	Plasticity, neurotransmission, & neurogenesis	↑	Blueberry enriched diet ↓ oxidative stress vs control; blueberry ↑ markers of plasticity vs control @ ~ pid 22; these ↑ were associated with improved behavioural outcomes
		4-HNE	Oxidative stress	↓	
Branched chain amino acids					
Cole et al. (2010)	Tap water containing valine, leucine, and isoleucine; or tap water containing phenylalanine	BCAA concentrations, net synaptic efficacy, Glutamate, GABA, BCATc, BCATm, BCKD, GAD, GDH, AAT1, AAT2	Plasticity, neurotransmission, & neurogenesis	↑	LIV BCAAs ↑ net synaptic efficacy vs control @ pid 7; TBI ↓ BCAA transanimation and enzymes responsible for glutamate metabolism
Lim et al. (2013)	Tap water containing valine, leucine, and isoleucine	Orexin activated neurons	Plasticity, neurotransmission, & neurogenesis	↑	BCAAs ↑ orexin activated neurons vs control @ pid 28, restoring regulation of arousal and wakefulness
Elliott et al. (2018)	Tap water containing valine, leucine, and isoleucine	Glutamate and GABA labeling	Plasticity, neurotransmission, & neurogenesis	↑	BCAA ↑ excitatory signalling in presynaptic terminals of hypothalamus & ↓ excitatory signalling in presynaptic terminals of cortex vs control @ pid 7; no difference in inhibitory signalling in hypothalamus or cortex
Creatine					
Saraiva et al. (2012)	Creatine supplementation delivered via gavage	TBARS levels, protein carbonylation	Oxidative stress	↓	Creatine ↓ oxidative stress vs control @ pid 4 & 8
		Na+ -K+ ATPase activity	Cellular energy imbalance	NC	
Gerbatin et al. (2019)	Creatine supplementation delivered via gavage	Cell loss	Morphological changes & apoptosis	↓	↓ cell loss within hippocampus & ↑ inhibitory action creatine vs control @ pid 35
		GABAergic inhibitory neurons, GAD, GAD67, & [3H]flunitrazepam binding	Plasticity, neurotransmission, & neurogenesis	↑	

Fasting & caloric restriction					
Davis et al. (2008)	Fasting	Tissue sparing	Morphological changes & apoptosis	MIX	24hr fast ↑ tissue sparing & ↓ oxidative stress vs control @ 24hr post moderate TBI; no differences in tissue sparing 24hr fast post severe TBI; nor 48hr fast @ 48 hr post moderate or severe TBI
		Ca^2+ load & mitochondrial complex function	Cellular energy imbalance	↓	
		ROS production, protein carbonyls, & lipid peroxidation	Oxidative stress	↓	
Liu et al. (2017)	Caloric restriction	Nissl bodies	Morphological changes & apoptosis	↓	Caloric restriction ↑ autophagy, ↓ glial activation, ↑ cell density compared to control & high energy group @ pid 36
		GFAP positive cells	Glial activation	↓	
		mTOR, LC3B & Beclin-1 positive cells	Immune response	↑	
Rubovitch et al. (2019)	Intermittent fasting or caloric restriction	SIRT1 expression	Cellular energy imbalance	MIX	Caloric restriction & intermittent fasting ↑ energy metabolism vs control @ pid 30 in cortex; only fasting ↑ energy metabolism vs control in hippocampus @ pid 30
Ketogenic diet					
Prins et al. (2005)	Ketogenic diet	fluoro-jade staining, contusion volume, & neuronal preservation	Morphological changes & apoptosis	MIX	Ketogenic diet ↓ cellular degeneration vs control @ 6 & 24hr post-injury; & ↓ contusion volume vs control @ pid 7, benefits were specific to the pre-adolescent and adolescent animals
		plasma glucose, lactate, & βOHB levels	Cellular energy imbalance	MIX	
Hu et al. (2009a)	Ketogenic diet	TUNEL positive cells, caspase-3 positive cells, cytosolic cytochrome c release, & mitochondrial cytochrome c release	Morphological changes & apoptosis	↓	Ketogenic diet ↓ apoptosis, ↓ mitochondrial dysfunction, & ↓ edema vs control @ 72hr post-injury
		plasma glucose & βOHB levels	Cellular energy imbalance	↓	
		brain water content	Blood brain barrier integrity	↑	
Hu et al. (2009b)	Ketogenic diet	TUNEL positive cells, Bax mRNA, & Bcl-2 mRNA	Morphological changes & apoptosis	↓	Ketogenic diet ↓ apoptosis & ↓ edema vs control @ 72hr post-injury
		plasma βOHB levels	Cellular energy imbalance	↓	
		brain water content	Blood brain barrier integrity	↑	
Prins et al. (2009)	Ketogenic diet	contusion volume	Morphological changes & apoptosis	MIX	Ketogenic diet ↓ contusion volume & ↓ cerebral metabolic rates of glucose in ipsilateral

		cerebral metabolic rates for glucose; plasma glucose, lactate, & βOHB levels	Cellular energy imbalance	MIX	cortex, hippocampus, & thalamus @ pid 1, 3, & 7, benefits limited to younger animals
Schwartz kroin et al. (2010)	Ketogenic diet	cell counts; dorsal hippocampal volume	Morphological changes & apoptosis	NC	No difference cell loss ketogenic diet vs control @ pid 63; ketogenic diet ↓ amount of astrogliosis and microgliosis vs control @ pid 63
		hippocampal astrogliosis & microgliosis	Glial activation	↓	
Deng-Bryant et al. (2011)	Ketogenic diet	plasma glucose, lactate, βOHB levels; nuclear magnetic resonance spectroscopy to quantify metabolites within brain: ATP, creatine, phosphocreatine, GABA, glutamate, glutamine, choline, taurine, glycine, myo-inositol, aspartate, alanine, NAA, lactate, PEtn, PChol, GroPEtn, GroP-Chol, & NAD	Cellular energy imbalance	MIX	Mixed results of ketogenic diet on energy metabolites vs control; results were age and time post-injury specific; broadly, ketogenic diet ↓ metabolic deficits in younger animals vs control
Greco et al. (2016)	Ketogenic diet	3NT, 4-HNE, SOD1, SOD2, & NQO1	Oxidative stress	MIX	Ketogenic diet ↓ oxidative stress vs control. Benefits varied between 6-24 hr post-injury, and were limited to younger animals
		mitochondrial complex function	Cellular energy imbalance	MIX	
Zhang et al (2018)	Ketogenic diet	plasma βOHB levels; NAA:creatine ratio & choline:creatine ratio	Cellular energy imbalance	MIX	Mixed results of ketogenic diet on energy metabolism vs control @ pid 7; no difference in edema between all groups; ↑ autophagy ketogenic diet vs control @ pid 7
		Brain edema	Blood brain barrier integrity	NC	
		Beclin-1 expression	Immune response	↑	
Multi-supplement					
Wu et al. (2014)	Curcumin enriched diet, DHA enriched diet, or curcumin + DHA enriched diet	BDNF & p-TrkB	Plasticity, neurotransmission, & neurogenesis	↑	DHA & curcumin (in isolation and combination) ↓ oxidative stress, ↑ cell membrane homeostasis, & ↑ markers of plasticity vs control @ pid 14; combination of DHA & curcumin elicited greater benefits than either in isolation
		4-HNE & 4-HHE	Oxidative stress	↓	
		FADS2, 17β-HSD4, DHA content, n-3 DPA content, & serum cholesterol	Cell membrane homeostasis	↑	
Thau-Zuchman	Fortasyn Connect multi-supplement enriched diet	Lesion size	Morphological changes & apoptosis	↓	Multi-supplement ↓ morphological changes & apoptosis, ↑ cell

et al. (2019)		Iba-1, TSPO, GFAP, APC positive cells, & NogoA levels	Glial activation	↓	membrane homeostasis, ↓ glial activation, & ↑ markers of plasticity & neurogenesis vs control @ pid 70
		BrdU positive cells, DCX positive cells, synaptophysin, & β-APP levels	Plasticity, neurotransmission, & neurogenesis	↑	
		Plasma AA, EPA & DHA levels; MBP, phosphatidylcholine & phosphatidylethanolamine levels	Cell membrane homeostasis	↑	
Polyunsaturated fatty acids					
Bailes & Mills (2010)	Low or high DHA enriched diet	Caspase-3 positive axons	Morphological changes & apoptosis	↓	Both low and high dose of DHA ↓ apoptosis, ↑ cell membrane homeostasis, & ↓ number of injured axons vs control @ pid 30; high dose DHA had greater benefit on membrane homeostasis than low dose
		Serum DHA & EPA levels; AA:EPA ratio	Cell membrane homeostasis	↑	
		β-APP positive axons	Plasticity, neurotransmission, & neurogenesis	↑	
Shin & Dixon (2011)	Fish oil supplementation delivered via gavage	Dopamine release	Plasticity, neurotransmission, & neurogenesis	↑	Fish oil ↑ dopamine release vs control @ pid 7
Mills et al. (2011)	Low or high fish oil (EPA + DHA) supplementation delivered via gavage	Axons positive for active caspase-3 & cytochrome c	Morphological changes & apoptosis	MIX	Mixed results for influence of fish oil on apoptosis vs control @ pid 30; fish oil ↑ cell membrane homeostasis, & ↓ axonal damage vs control @ pid 30; no apparent differences between fish oil dosages
		AA:EPA ratio	Cell membrane homeostasis	↑	
		APP-positive axons; axons positive for active RMO-14	Plasticity, neurotransmission, & neurogenesis	↑	
Wu et al. (2011)	DHA enriched diet	4-HNE, SOD, & SIR2 levels	Oxidative stress	↓	DHA ↓ oxidative stress & ↑ cell membrane homeostasis vs control @ ~ pid 7-12; DHA ↑ markers of plasticity to sham levels @ ~ pid 7-12
		Brain DHA content & iPLA2 levels	Cell membrane homeostasis	↑	
		BDNF, CAMKII, Syn-1, CREB, & STX-3 levels	Plasticity, neurotransmission, & neurogenesis	↑	
Schober et al. (2016)	DHA enriched diet	TNF-α, IL-1B, CCL2, IL-6, IL-10, IL-2	Immune response	MIX	DHA ↑ tissue sparing @ pid 3 & 50, ↓ edema @ pid 12, & ↓ oxidative stress @ pid 1 within the cortex vs control; mixed results for MRI measures @ pid 12 & 28; mixed immune response DHA vs control @ pid 1 & 2
		NOx	Oxidative stress	↓	
		Tissue sparing & contusion volume	Morphological changes & apoptosis	↓	
		Edema	Blood brain barrier integrity	↑	
		fractional anisotropy, radial diffusivity, axial diffusivity, & tract volume measured by MRI	Plasticity, neurotransmission, & neurogenesis	MIX	

Su et al. (2016)	Moderate (1:5) or high (1:1) ω-3/ω-6 ratio PUFA oil delivered via gavage	Evans blue extravasation, edema; occludin & MFSD2A levels	Blood brain barrier integrity	↑	High dose of ω-3 fatty acids ↓ oxidative stress & ↓ blood brain barrier disruption @ pid 5 vs "western diet" control; No benefit moderate dose of ω-3 fatty acids vs "western diet" control @ pid 5
		4-HNE	Oxidative stress	↓	
Zhu et al. (2017)	Low or high DHA supplementation delivered via gavage	gross morphology; Bcl-2, Bax, & caspase-3 expression	Morphological changes & apoptosis	↓	Low & high dose of DHA ↓ morphological changes & apoptosis @ pid 15 vs control; high dose ↓ markers of apoptosis closer to sham levels than low dose
Traditional eastern medicine					
Sharma et al. (2010)	Curcumin enriched diet	4-HNE levels	Oxidative stress	↓	Curcumin ↓ oxidative stress, ↑ cell membrane homeostasis, & ↑ markers of plasticity, neurotransmission, & neurogenesis vs control @ pid 15; these benefits were associated with improved behavioural outcomes
		iPLA2 & FATPs levels	Cell membrane homeostasis	↑	
		STX-3, GAP-43, & NR2B levels	Plasticity, neurotransmission, & neurogenesis	↑	
Kumar et al. (2014)	Panax ginseng suspended in CMC delivered via oral route in low, moderate, and high dosage	MDA, GSH, nitrite, catalase, & SOD levels	Oxidative stress	↓	Moderate & high dose of panax ginseng ↓ oxidative stress, ↓ impaired neurotransmission, & ↓ immune response within cortex & hippocampus vs control @ pid 28; no difference between low dose of panax ginseng vs control for outcomes
		Acetylcholinesterase levels	Plasticity, neurotransmission, & neurogenesis	↑	
		TNF-α & IL-6 levels	Immune response	↓	
Zhao et al. (2014)	Modified Shengyu decoction (MSD) supplementation delivered via gavage in low, moderate, and high dosage	water content	Blood brain barrier integrity	MIX	Both moderate and high dose of MSD ↓ cell loss in cortex & hippocampus @ pid 14; moderate dose MSD ↓ edema vs control @ pid 1, & ↓ glial activation @ pid 5 in cortex & hippocampus; mixed results of moderate dose MSD on immune response vs control @ pid 3; no doses led to a difference in contusion volume vs control @ pid 14
		Contusion volume & neuronal loss	Morphological changes & apoptosis	MIX	
		IL-1β, IL-6, TNF-α, & IL-10 levels	Immune response	MIX	
		GFAP & Iba-1 positive cells	Glial activation	MIX	

Wang et al. (2016a)	Hydroxysafflor yellow A (HYSA) supplementation delivered via gavage in low and high dosage	MDA, SOD, catalase levels; & GSH:GSSG ratio	Oxidative stress	↓	Both low and high dose HSYA ↓ oxidative stress vs control @ 6, 12, & 24hr post-injury; high dose appeared to have greater benefit than low dose
Wang et al. (2016b)	Rhizoma drynariae (RZD) supplementation delivered via gavage	lesion volume	Morphological changes & apoptosis	↓	RZD ↓ lesion volume @ pid 3 vs control; & mixed results for immune response vs control
		plasma IL-6 & IL-10 levels	Immune response	MIX	
Xing et al. (2016)	Xuefu Zhuyu decoction (XFZY) supplementation delivered via gavage in low and high dosage	AKT	Morphological changes & apoptosis	MIX	Low dose of XFZY ↓ phosphorylation of markers within the PI3K/AKT/mTOR signaling pathway, thereby collectively ↓ immune response & neuroinflammation vs control @ pid 1, 3, 7, & 14; Low dose XFZY ↑ cell membrane homeostasis vs control @ pid 1, 3, 7, & 14; mixed results for the high dose of XFZY vs control, overall low dose associated with better outcomes vs control
		mTOR	Cellular energy imbalance	MIX	
		p70S6K	Plasticity, neurotransmission, & neurogenesis	MIX	
		AA levels	Cell membrane homeostasis	MIX	
		TNF-α & IL-1β levels	Immune response	↓	
Xu et al. (2017)	Rhubarb (low, moderate, high) or rhein (single dose) supplementation delivered via gavage	SOD, catalase, MDA levels; GSH:GSSH ratio	Oxidative stress	↓	Rhubarb and rhein ↓ oxidative stress vs control @ 8, 16, 24hr post-injury; greater benefits associated with higher dosages; similar benefit observed between high dose of rhubarb and dose of rhein
Özevren et al. (2018)	Ganoderma lucidum supplementation delivered via gavage	MDA, GSH, & MPO levels	Oxidative stress	↓	Ganoderma lucidum ↓ oxidative stress, ↓ blood brain barrier permeability, ↑ neurogenesis, & ↑ immune response vs control @ pid 7; no difference in apoptosis ganoderma lucidum vs control @ pid 7
		brain water content & blood brain barrier permeability	Blood brain barrier integrity	↑	
		CD68	Immune response	↑	
		Bcl-2	Morphological changes & apoptosis	NC	
		VEGF	Plasticity, neurotransmission, & neurogenesis	↑	
Wang et al. (2018)	Berberine supplementation delivered via gavage	Bax, cytochrome c, NF-κB, & p38 mAPK expression	Morphological changes & apoptosis	↓	Berberine ↓ apoptosis, ↓ immune response, & ↑ angiogenesis vs control @ pid 28
		VEGF expression	Plasticity, neurotransmission, & neurogenesis	↑	

		IL-1β, TNF-α, MCP-1, & ATF-2 expression	Immune response	↓	
Xie et al. (2018)	Astragali Radix and Radix Angelica Sinensis (AR-RAS) herbal formula supplementation delivered via gavage	NogoA expression	Glial activation	↓	AR-RAS ↓ glial activation vs control @ pid 14

Note: MIX - mixed results for biomarkers, NC - no change of biomarkers, ↓ - reduction of biomarkers, ↑ - increase of biomarkers.

Discussion

State and limitations of the evidence

The purpose of this review was to consolidate available evidence for post-TBI NUTs and their associated effects on NP outcomes in both animal and human studies. The aim was to identify NUTs that demonstrated the most plausible "over-the-counter" potential to reduce NP dysfunction (at a cellular level) after TBI in a way that may reduce downstream deficits experienced by clinical patients. Our review revealed that no studies have evaluated NP outcomes post-TBI in response to a NUT in humans. Several post-TBI NUTs were investigated for efficacy on clinical outcomes only, since these studies did not provide insight into how the NUTs interacted with pathophysiological mechanisms of TBI they are beyond the scope of this review [264-273]. Therefore, knowledge of how NUTs may promote favourable NP outcomes after TBI relies entirely on pre-clinical evidence. Animal studies provide valuable insight into the molecular underpinnings and safety of emerging interventions [274]. However, methodological inconsistencies between animal studies and clinical investigations often lead to failed translation [258, 274, 275]. A main review finding was the number of methodological caveats (and their relation to the biofidelity and validity of the evidence) that need to be considered before interpreting the translational potential and salient findings of interventions reviewed. Biofidelity refers to "concordance between specific features of a given animal model and the human disease or condition being modeled" and "goes hand in hand with confirmation of experimental results across different animal models with clinical findings in humans (i.e., validity)" ([16] pg 8). Aspects of study design including characteristics of animals used, method of TBI induction, intervention parameters, and measurement of outcomes in many of the included studies threaten biofidelity.

Animal characteristics

Males have typically accounted for a higher proportion of recorded TBIs than females in clinical practice, however, females have typically experienced worse symptom burden and longer recovery timelines than males [172, 176, 276]. Only one study within our review included both male and female animals – but no sex-related results were presented [248]. This male bias has been extensively reported across multiple

disciplines and is particularly prevalent within neuroscience [277]. Exclusion of female rodents was often justified by the assumption that female rodents demonstrate higher variability than males due to the estrous cycle [277]. However, a 2014 review consolidated data from 293 articles and concluded that females were not more variable than males across a range of physiological, morphological, and behavioural measures [277]. There is a need to ensure inclusion of females in future pre-clinical and clinical investigations to understand if sex-differences exist and to increase the likelihood of translation and generalizability.

Neurophysiological outcomes across multiple maturational stages in response to a post-TBI NUT were only evaluated in four studies, all of which administered a ketogenic diet [148, 222, 232, 233]. The beneficial effect associated with ketogenic diet was limited to younger animals. Evidence suggests maturational differences in NP consequences and recovery post-TBI [50]. Therefore, interactions would likely be shared between maturational stage and influence of NUTs on NP outcomes after TBI. Expanded knowledge of such interactions would contribute to identification of nutritional candidates likely to translate to clinical practice.

All the interventions investigated within this review were administered to rodents, and no investigations evaluated the benefit of the intervention across multiple species. Concordance of results across more than one animal species has been shown to increase the likelihood of translation to humans in pharmaceutical toxicity studies [278]. While financial and logistical challenges may make adopting a multi-species methodology difficult, such efforts would inform the design of future RCTs that would potentially provide information that may benefit TBI patients sooner.

TBI induction

In most real-world TBIs the head moves freely and results in a mild closed head injury ([19, 279] for reviews). Most included studies performed craniotomy to deliver a traumatic force directly to exposed dura. Freedom of head movement was prevented or unclear during TBI modeling in many studies. In a clinical setting, patients rarely require anesthetic following TBI, yet for ethical reasons, nearly all studies anesthetised animals while TBI was modeled. Anesthetic has been reported to effect behavioural and NP outcomes following TBI and may produce artefacts that complicate interpretation of study findings ([16, 274] for reviews). Therefore, differences in the type and level of anesthetics delivered between laboratories during TBI induction require consideration when interpreting study outcomes. Since these factors are inconsistent with clinical mTBI (representing 95% of all TBIs) there is a need to adopt next-generation TBI modeling techniques that more accurately replicate the injury conditions experienced by the bulk of clinical patients [274, 279]. Pre-clinical studies evaluating the effect of a NUT following blast-related TBIs, commonly seen during military service, are needed.

Most of the evidence consolidated within this review has been gained from animal studies replicating conditions of moderate to severe TBI. While these animal models aim to serve as surrogates for clinical presentations of TBI, differences in criteria used to ascertain TBI severity in animal studies (i.e., cortical deformation or velocity) versus clinical practice (Glasgow Coma Score and neuroimaging findings) must be considered when considering potential translation of study findings. Given the differing severity of pathophysiology across the TBI spectrum, it remains to be seen whether an intervention that shows promise within one severity of TBI severity is beneficial in more/less severe cases [280].

Intervention parameters and outcome measurements

Animals were either fed ad libitum or gavaged to deliver the NUT in studies within this review. Animals fed ad libitum may be representative of humans prescribed a supplement or altered diet as an intervention following TBI; however, these methods of delivery may lead to high variability in the amount of nutrient/compound ingested across subjects. Additionally, adherence may be an issue in clinical populations if the prescribed diet is vastly different than the patient's habitual diet. On the other hand, gavage allows for precise dosing but is invasive and involves bypassing the early stages of digestion and absorption. Delivery of a supplement orally would be more representative of what would be viable for clinical patients with mTBI, while gavage replicates tube feeding that may be necessary in humans following moderate or severe TBI. Future research should investigate if differences in post-TBI outcomes exist if the same NUT is fed ad libitum or delivered via gavage.

Evidence from pharmaceutical studies show that discontinuity between timing and mode of drug administration in animal and clinical studies contribute to failed translation [203, 281]. These shortcomings may be due to our limited understanding of physiological and pathological temporal differences between humans and rodents [281]. For example, the lifespan of a rat is highly accelerated to that of a human; with one rat day equating to ~27 human days, and ~13.7 rat days being comparable to one human year [281]. Based on these figures initiating a NUT 24 hours post-injury in a rat may be representative of ~21 days post-injury in humans. Measuring outcomes at seven days post-TBI in rats could possibly be indicative of outcomes at six months for humans. Research is needed to elucidate the possible temporal discordance between rodent and human TBI pathophysiology to increase the likelihood of intervention translation from the lab to clinical practice. Future studies should consider delivering a NUT in separate groups of animals to compare NP outcomes under ideal conditions (i.e., immediately post-injury) and under delayed conditions that are clinically representative.

A distinct advantage of animal studies is the ability to collect invasive outcome measures that would be unethical and impractical to acquire in humans. However, a strong case has been presented that "pre-clinical researchers should also prioritize incorporating methods/outcomes that are applicable in both the experimental and clinical settings" ([274] p 404). Most studies in this review involved biochemical assays on

slices of brain tissue post-sacrifice to quantify NP biomarkers. While this provided an accurate post-mortem measurement of outcomes within the brain, it may not be representative of the amount of circulating biomarkers while the animal was still alive. Incorporation of blood biomarkers and neuroimaging in animal studies would expand understanding of the relationships shared between biomarkers measured pre- and post-sacrifice. Incorporation of biomarkers in animal studies would allow comparisons and cross-validation against the growing number of biomarkers that can be measured in human clinical populations [274].

Taken together, the examples in this section illustrate methodological factors that limit the biofidelity of the available evidence for NUTs to improve NP outcomes post-TBI. These factors need to be carefully considered when interpreting study results. Researchers must find ways to account for financial and logistical constraints in order to improve the biofidelity and validity of investigations in a manner that will benefit clinical patients with TBI.

Risk of bias

There is difficulty with assessing RoB in systematic reviews of animal studies due to poor methodological reporting [216, 258, 275]. Studies within this review were often missing (or did not clearly communicate) key methodological details, including: blinding procedures, severity of TBI modeled, mortality rate following TBI, age of animals used, sample size, number of animals used for each outcome measure, and whether animals were randomised for outcome measure assessment [282, 283]. As a result, determination of RoB was limited without these key methodological details (Table 4.2 and Table 4.3). In some cases, authors of the original studies may have assumed that the reader had prior knowledge of commonly used methodologies in TBI animal studies, and thus these details weren't explicitly stated. For example, random selection of housing may not be possible in a TBI study because uninjured control animals may overpower the TBI animals in competition for food. To overcome this issue animals belonging to the same study groups may have been housed together. To enhance methodological transparency, it is recommended that future studies incorporate a figure/diagram that clearly depicts key aspects of study design as shown in some studies [149, 150, 227, 235, 239, 245, 249].

The quality of results reported was low in many studies where only simple figures with p-values were published. Values or descriptive statistics for outcomes were rarely provided in tabular format or within text, preventing meta-analysis to evaluate the size and meaningfulness of effects attributed to the delivered NUT. However, all studies reported a positive benefit of the NUT administered on NP outcomes compared to control. The absence of negative findings may be indicative of potential publication bias, possibly skewing the NUT associated benefits reported. Registration of animal studies to prevent publication bias, innovation of TBI models to improve biofidelity, thorough and transparent reporting of methodologies and results, and clinically relevant intervention timing and delivery are the focus of ongoing initiatives [16, 203, 216, 279, 282-285].

Implications for clinical TBI patients

Unfortunately, due to limitations of the evidence, this review could not definitively recommend a single NUT as the primary "over-the-counter" candidate for clinical patients post-TBI. Further research should focus on anti-oxidant compounds (ALA, curcumin, phenols), BCAAs (combined leucine, isoleucine, valine), and ω-3 PUFAs (specifically DHA) which appear to be the most promising supplementation candidates that might facilitate improved clinical outcomes for TBI patients in the future.

Following TBI, patients often endure an array of functional and neurobehavioural deficits which seemingly develop as consequences of cellular damage and dysfunction triggered by the secondary injury phase of TBI [42, 61]. Functional consequences of TBI often include mood instability, impaired cognition and memory, confusion, intolerance to cognitive and physical loading, vestibulo-ocular deficiencies, and sleep disturbances [28, 33, 61, 98, 138, 286]. Cellular disruptions impair neurological networks and subsequently manifest in symptom complaints and deficits at the experiential and social levels [61]. Approximately 50% of patients experience sleep-wake disturbances after TBI [286]. Excessive glutamate release post-TBI creates an imbalance in the glutamate/GABA pool and contributes to impaired neurotransmission which in turn dysregulates networks responsible for restorative sleep [61]. Without restorative sleep the patient may experience impaired executive function, mood instability, and high levels of stress that further compound sleep-wake disruption [61]. In this regard, pre-clinical evidence suggests patients with post-TBI sleep-wake disorders may experience a positive benefit from BCAA supplementation via improved balance of glutamate/GABA pools within orexin neurons [219, 221, 228].

Ionic dysregulation and subsequent mitochondrial dysfunction are hallmarks of TBI that contribute to oxidative stress [42, 287]. Excessive free radical production can trigger apoptosis and lipid peroxidation of cell membranes [42, 288, 289]. Moderate and severe TBI can elicit considerable amounts of neuronal death severely impairing neuroconnectivity and transmission [42, 61]. Across the TBI spectrum, mitochondrial dysfunction and impaired cell membrane homeostasis within neuronal, axonal, and glial cells can exacerbate neurotransmission impairment. Biomechanical forces deforming brain cells during TBI can damage axons and increase BBB permeability, both of which can be further aggravated by unregulated free radicals [290, 291]. Compromised tight junctions between endothelial cells within the BBB results in cerebral edema further compromising neurotransmission [61, 292]. Globally impaired neurotransmission can affect TBI patients through diminished sensorimotor integration, executive function, and working memory while manifesting clinically as mood instability, cognitive fatigue, and inability to complete activities of daily living [61]. Introduction of anti-oxidants such as ALA, curcumin, or phenols early after moderate or severe TBI may promote tissue sparing and combat oxidative stress to blunt the overall cellular damage and downstream impairments experienced by the patient [149, 230, 251, 253-255]. ω-3 PUFAs have demonstrated the same anti-apoptotic and anti-oxidant benefits as anti-oxidant compounds

but with additional advantages [214, 293]. DHA is a major constituent of cell membranes within the central nervous system [294]. At a cellular level, delivery of DHA post-TBI may proactively combat oxidative stress while providing the necessary building blocks to repair phospholipid bilayers damaged by lipid peroxidation. Pre-clinical evidence suggests that administration of DHA after TBI upregulates brain-derived neurotrophic factor (BDNF) expression. Expression of BDNF plays a crucial role in the regulation of neuroplasticity and neurogenesis so that injured cells/networks can repair and/or reorganise themselves [295, 296]. Upregulation of BDNF by DHA supplementation (or other means) creates an internal environment that promotes recovery at the cellular and network level that may further alleviate functional deficits and symptom burden for patients. A pre-clinical study demonstrated that combining curcumin with DHA led to an additive benefit that exceeded the benefits observed following administration of either compound in isolation [243]. There is a need to better understand how constituents of multi-supplement interact with outcomes.

Outside of the neurotrauma literature, reviews of NUTs to improve outcomes following musculoskeletal injuries and soft-tissue wounds have concluded that evidence supporting a single intervention in humans remains elusive [297-299]. These reviews recommended a well-balanced diet of whole foods that are minimally processed and abstaining from alcohol consumption [297, 298]. Supplementation is likely only appropriate if the individual's habitual diet is deficient in a given nutrient or compound [297, 298]. Physiological responses to injury such as inflammation and apoptosis are important biological processes for recovery under normal conditions and complete elimination of these mechanisms would be unfavourable [298]. These general recommendations appear appropriate for patients with TBI in the interim while the biofidelity and validity of NUTs for TBI continue to be elucidated.

Future directions

Modification of lifestyle factors, such as diet and exercise, are considered safe for investigation in human subjects given contraindications are considered and accounted for. This presents a unique opportunity in the case of NUTs for TBI to potentially conduct pre-clinical and clinical studies in parallel to accelerate the identification of strategies that might benefit clinical patients. Such study designs have begun to be adopted in other areas of translational research and serve as an innovative approach to account for the innate ethical and logistical limitations of both clinical and pre-clinical study designs [300]. Although studies evaluating NUTs have been conducted in humans with TBI, none were included in this review due to a lack of NP outcome measures. Future clinical investigations of post-TBI NUTs should make efforts to include NP measures within their outcome batteries. Due to the difficulties of running RCTs, it is imperative to include NP measures alongside clinical/behavioural measures in order to maximise the data acquired during a given collection. Inclusion of NP measures in human trials would improve our understanding of why interventions evaluated in RCTs fail or succeed, while providing cross-validation of biomarkers acquired in pre-clinical

studies. Preliminary evidence suggests a positive effect of delivering exercise and DHA in combination after TBI on NP outcomes [301]. Given exercise has become a pillar of TBI rehabilitation, future investigation into the relationship of exercise and NUTs is warranted. Finally, pre-clinical studies investigating diets/supplements that may be detrimental for post-TBI NP outcomes would be highly informative.

Conclusions

Our objective was to identify NUTs introduced post-TBI demonstrating potential to improve NP outcomes after TBI. Available evidence regarding the efficacy of NUTs delivered to attenuate NP outcomes secondary to TBI was from 43 pre-clinical animal studies utilising 2,897 rodents. No studies evaluated the effect of a NUT on NP outcomes of TBI in humans. The overall reporting of methods and results of included pre-clinical studies was poor, limiting the biofidelity of evidence. Decisive recommendations about which NUTs most effectively combat secondary injury following TBI in a manner that would benefit clinical patients could not be made. Initial evidence from animal studies provides a platform from which future investigations can be launched to determine which aspects of nutrition (particularly anti-oxidant compounds, BCAAs, and ω-3 PUFAs) could be manipulated to promote better recovery outcomes across the TBI spectrum for humans. With TBI complexity and heterogeneity of outcomes, innovative and collaborative efforts are needed for the identification of interventions to reduce the global health burden of this injury.

Personal development as a researcher resulting from Chapter 4

- How to conduct a systematic review and critically appraise evidence.
- Thorough understanding of the strengths and weaknesses of animal studies.
- Deeper knowledge of TBI pathophysiology, and biomarkers that provide insight into different cellular pathways.
- Greater insight into why so many promising findings from animal studies do not translate to humans.

Link between Section 2 Part A (Chapter 4) and Section 2 Part B (Chapters 5-8)

Chapter 4 summarised the strengths and weaknesses of the literature on nutritional interventions for TBI, providing a platform to improve the biofidelity of future animal studies and to begin to explore the feasibility of conducting clinical trials to determine if nutritional interventions can complement and improve clinical practice. None of the studies conducted to date have evaluated how nutritional interventions influence neurophysiological outcomes in TBI patients. This is likely due to costs, logistics, invasiveness, and ethical considerations of measuring neurophysiological outcomes in humans. Since current clinical practice is highly reliant on clinical training/experience and honest symptom reporting there is a need to identify objective, accurate, and affordable measures that perform under clinically realistic conditions. From my own clinical experience working with SOBI patients, conversations with practicing clinicians, and gaps in the current evidence there are three main areas of the clinical management of SOBI that would benefit identifying more objective assessment methods including: 1) diagnostic and prognostic decision making; 2) accurate classification of SOBI subgroups that have different underlying impairments responsible for symptomology; and 3) understanding why some patients recover faster than others. Advances in our understanding of the neurophysiological provide targets for novel interventions, and for new methods of assessing SOBI patients. Due to the heterogeneity of SOBI symptom reports and recovery outcomes, it is likely that different neurophysiological outcome measures will be necessary to address each of these clinical needs. Therefore Section 2 Part B is comprised of four chapters, each evaluating the translational potential of a neurophysiological approach to address the needs of clinicians who work with SOBI patients. The studies within Section 2 Part B were conducted with two specific objectives in mind. First, to explore the translational potential of several neurophysiological approaches to promote more accurate clinical management of SOBI. Second, to evaluate the potential of these approaches to serve as neurophysiological outcome measures to evaluate the feasibility and effectiveness of nutritional interventions to improve SOBI recovery outcomes. Chapter 5 evaluates the clinical utility of a somatosensory testing device called a Brain Gauge (produced by Cortical Metrics) to assist SOBI diagnosis and management decisions. Marketing for the Brain Gauge suggests it can be used to monitor and track recovery post-SOBI and to guide return-to-play decisions. No available published studies have evaluated the utility of the assessments delivered by the Brain Gauge under ecologically valid conditions.

Chapter 5: Preliminary evidence for the clinical utility of tactile somatosensory assessments of sport-related mTBI.

This chapter comprises the following paper submitted to *Sports Medicine – Open:* **McGeown, J. P.**, Hume, P. A., Kara, S., King, D., & Theadom, A. Preliminary evidence for the clinical utility of tactile somatosensory assessments of sport-related mTBI.

Author contribution

McGeown, J. P. 80%, Hume, P. A. 5%, Kara, S. 5%, King, D. 5%, Theadom, A. 5%

Overview

Objectives: To evaluate clinical utility of tactile somatosensory assessments to assist clinicians in diagnosing sport-related mild traumatic brain injury (SR-mTBI), classifying recovery trajectory based on performance at initial clinical assessment, and determining if neurophysiological recovery coincided with clinical recovery. **Research design:** Prospective cohort study with normative controls. **Methods:** SR-mTBI patients (n=79) completed the SCAT-5 symptom scale and Cortical Metrics Brain Gauge somatosensory assessments (BG-SA; TOJ – temporal order judgement; TOJc – TOJ with confounding condition; DUR – duration discrimination) at admission and discharge (n= 45/79). To assist SR-mTBI diagnosis on admission BG-SA performance was used in logistic regression to discriminate cases belonging to the SR-mTBI sample or a healthy reference sample (pooled BG-SA data for healthy participants in previous studies). Decision trees evaluated how accurately BG-SA performance classified SR-mTBI recovery trajectories. **Results:** BG-SA TOJ, TOJc, and DUR poorly discriminated between cases belonging to the SR-mTBI sample or a healthy reference sample (0.54-0.70 AUC, 47.46-64.71 PPV, 48.48-61.11 NPV). The BG-SA evaluated did not accurately classify SR-mTBI recovery trajectories (>14-days resolution 48%, ≤14-days resolution 54%, lost to referral/follow-up 45%). Mann-Whitney U tests revealed differences in BG-SA TOJc performance between SR-mTBI participants and the healthy reference sample at initial clinical assessment and at clinical recovery (p<0.05). **Conclusions:** BG-SA TOJ, TOJc, and DUR appear to have limited clinical utility to assist clinicians with diagnosing SR-mTBI or predicting recovery trajectories under ecologically valid conditions. Neurophysiological abnormalities persisted beyond clinical recovery given abnormal BG-SA TOJc performance observed when SR-mTBI patients achieved clinical recovery.

Introduction

Mild traumatic brain injuries (mTBIs) sustained during sport or physical activity represent an estimated 20-25% of all traumatic brain injuries [10, 302]. mTBI is commonly described as an invisible injury because structural abnormalities are not detected post-mTBI using standard neuroimaging techniques [18, 19]. The invisible nature of mTBI requires that diagnosis to be made via clinical examination where self-reported symptoms are one of the key indicators used by clinicians [39, 43]. Reliance on self-reported symptoms can be problematic because of the non-specific nature of mTBI symptoms and delayed symptom onset in some patients [43, 82-86]. In the case of sport-related mTBI (SR-mTBI) some athletes underreport when they have sustained an mTBI and/or minimise related symptomology [4-7]. Taken together, these limitations highlight the need for objective measures that can assist clinical decision making when symptom reports may be untrustworthy.

Many widely used tools to assist clinicians in the assessment of SR-mTBI require or recommend pre-season baseline testing to use as a reference for subsequent evaluations [76]. Comprehensive baseline testing may be possible at the elite and professional levels of sport, but logistical constraints limit the feasibility of this approach for most athletes engaged in the recreational or amateur sporting environment. Recent evidence questions the clinical utility of commonly implemented SR-mTBI assessment tools even if a baseline reference is available due to sub-optimal test-retest reliability [76]. Objective measurement techniques including advanced functional imaging, transcranial magnetic stimulation, and electroencephalography have identified abnormalities in functional connectivity post-mTBI [134, 135, 303-313] which appear to underlie a wide array of symptoms observed clinically due to altered neurotransmission and information processing [61]. Whilst valuable insights are gained utilising advanced techniques, logistical constraints such as accessibility, cost, ease of use, and time to administer again limit the likelihood of their widespread integration into the clinical management of mTBI. To advance current best-practice mTBI management, the identification of objective, affordable, quick, and easy to administer neurophysiological tools demonstrating discriminative or predictive capacity without the need for a baseline reference is required.

Sensorimotor processing represents one domain of functional connectivity that can be impaired following mTBI. Tactile somatosensory assessments (SA) take advantage of the highly organised structure of the somatosensory cortex providing one means of evaluating sensorimotor processing in individuals with neurological conditions, including those with mTBI [121-123, 133-137]. The computer mouse shaped Cortical Metrics Brain Gauge is a portable, quick, and easy to administer tool able to evaluate tactile somatosensory function. Mechanoreceptors in the fingertips of the non-dominant hand detect light vibrations delivered by the Brain Gauge, transmitting resultant sensory information to corresponding areas of the contra-lateral somatosensory cortex. Here the sensory stimulus is processed before being relayed through commissures, such as the corpus callosum, to the motor cortex in the opposite hemisphere. A

motor response is coordinated in the form of using the dominant hand to indicate an answer to a question about the stimulus [314]. Studies have indicated that aspects of the Brain Gauge SA (BG-SA) protocol can detect differences between individuals diagnosed with mTBI when compared to non-injured controls [122, 123, 135]. However, to date, no studies have evaluated the feasibility and utility of SA via the Brain Gauge to provide clinicians with objective information that might assist diagnostic and management decisions for SR-mTBI patients.

Study purpose and research questions

The purpose of this investigation was to evaluate the clinical utility of tactile BG-SA to assist clinicians when making SR-mTBI diagnosis and management decisions. Specifically, this study aimed to address four questions:

1. Can performance on tactile BG-SA during initial clinical assessment accurately discriminate between patients with SR-mTBI and a healthy reference sample;
2. Can performance on tactile BG-SA during initial clinical assessment alone, or in combination with symptom burden accurately classify recovery trajectory;
3. Do SR-mTBI patients perform differently on BG-SA at initial clinical assessment and/or clinical recovery compared to a healthy reference sample; and
4. Does BG-SA performance in SR-mTBI patients change between initial clinical assessment (symptomatic) and clinical discharge (asymptomatic)?

Methods

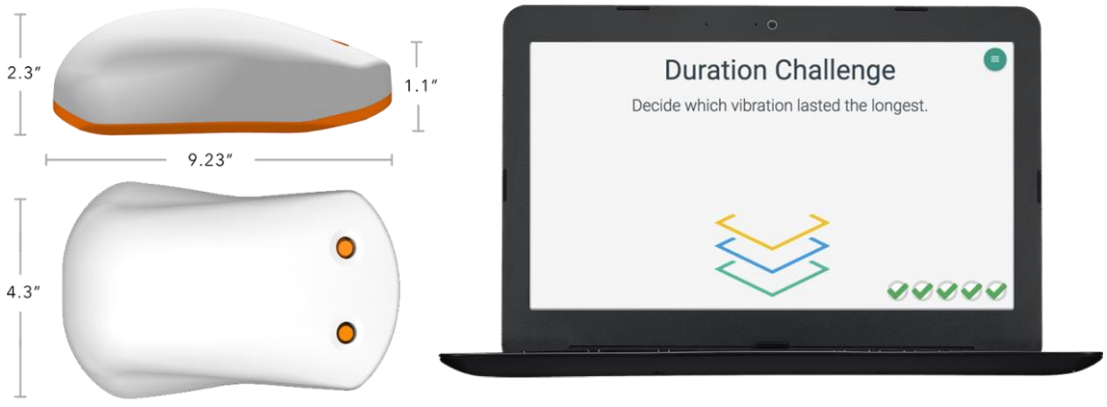


Figure 5.1 The 'Brain Gauge' (A) two-digit vibro-tactile stimulation handheld device (Brain Gauge. Cortical Metrics, Chapel Hill, NC, USA www.corticalmetrics.com) and (B) example of visual cueing test screen.

Research design

A prospective cohort design enabled evaluation of the clinical utility of BG-SA to provide objective information to assist clinicians when diagnosing SR-mTBI, predicting recovery outcomes based on initial

assessment performance, and determining if neurophysiological recovery coincides with clinical recovery. Data collection took place at a single dedicated SR-mTBI clinic between April 2019 and July 2019.

Participants

A total of 79 consenting patients diagnosed with SR-mTBI by a sport and exercise medicine physician during their initial clinical assessment were recruited for the study. The sample was inclusive for age and sex. BG-SA data collected during initial clinical assessment was available for all 79 participants. Follow-up BG-SA data were collected from 45/79 (57%) participants once they met clinical recovery criteria. Follow-up data were not available for the remaining participants because they were referred to a different service (4/79), lost to follow-up (12/79), or declined to complete their second BG-SA (18/79).

Procedures

Ethical conduct

Institutional (AUTEC 18/374) and health and disability committee (HDEC 18/NTA/108) ethical approvals were obtained, and this study was conducted according to the ethical standards of the Declaration of Helsinki. Participants provided written consent (participant assent and parental consent was acquired for participants <16 years old) to having their data used for research and publication. All participant data were de-identified prior to extraction/data analysis to ensure confidentiality.

Clinical management and definitions of recovery

Patients received usual clinical care as per a previously described service protocol which is briefly summarised in this paper. Additional details about the criteria/assessments used to identify SR-mTBI and treatments participants received have been published [315, 316].

As part of routine care, participants completed the Sports Concussion Assessment Tool (SCAT-5) symptom scale at the beginning of each visit, followed by a consultation with the supervising physician. During the initial clinical assessment, the physician completed a thorough history and physical examination. Participants received treatment in line with international recommendations in the form of education, written guidance, and individualised management to target the underlying causes of their signs and symptoms. Participants were scheduled for follow-up assessments every 7-14 days to evaluate their progress and, if necessary, to modify their treatment plan.

Before departing the initial assessment, participants completed tactile BG-SA requiring ~10 minutes. To control for environmental noise during BG-SA, participants underwent testing in a private area of the clinic free from visual distractions while wearing noise cancelling headphones. Once participants achieved all clinical recovery criteria, they began a graduated return-to-play protocol and, before departing the clinic, completed the BG-SA battery for a second time.

Participants in this study were deemed to be clinically recovered once they achieved recovery criteria: (1) asymptomatic (defined as SSS ≤ 5 for males and ≤ 6 for females [83]); (2) demonstrated exercise tolerance, and (3) any abnormalities identified during the initial physical examination had resolved [315]. For this study neurophysiological recovery was defined as participants demonstrating BG-SA performance similar to that reported in studies evaluating BG-SA performance in non-injured healthy controls.

Instrumentation

SCAT-5 symptom burden

Participants completed the SCAT-5 [73] symptom scale during all appointments to quantify subjective SR-mTBI symptom reports. Participants were queried about 22 symptoms commonly related to SR-mTBI and asked to rank each symptom on a Likert scale from 0 (no symptom) to 6 (severe symptom). A Positive Symptom Total (PST; number of symptoms reported out of 22), and a Symptom Severity Score (SSS; sum of severity reported across the 22 symptoms out of 132) were composite scores derived from the symptom scale to represent the SCAT-5 symptom burden.

Brain Gauge somatosensory assessment

The Cortical Metrics BG-SA protocol [121-123, 134, 135, 317] was adapted to reduce administration time from the typical ~20-25 minutes to ~10 minutes to preserve clinical flow. Participants placed their non-dominant hand on the BG with the tips of D2 (index finger) and D3 (middle finger) aligned with two 5 mm probes that delivered a vibrotactile stimulus. A 2-alternative forced choice testing paradigm was employed wherein participants responded to the stimulus, via computer interface, with a mouse in their dominant hand by clicking on whether the left (D3) or right (D2) stimulus came first or lasted longer.

Somatosensory processing was quantified utilising three vibrotactile tasks: 1) temporal order judgement (TOJ); 2) temporal order judgement in the presence of a confounding stimulus (TOJc); and 3) duration discrimination (DUR). To assess TOJ two sequential vibrotactile pulses were delivered randomly to D2 and D3 and the participant was queried as to which digit was stimulated first. TOJc employed the same testing procedure as TOJ with the addition of a 25 Hz concurrent stimulus that lasted the length of each trial. Previous research [122, 133, 137] has indicated that the concurrent stimulus during TOJc leads to worse performance than TOJ in healthy individuals, whereas this performance drop is not observed in samples of individuals known to have a neurological condition. DUR was assessed by delivering sequential stimulus of different durations randomly to D2 and D3 and the participant had to discriminate which of the two stimuli lasted longer. Before each test began, participants had to correctly respond to three training trials (with feedback) to ensure they understood the testing protocol. Then twenty trials, without feedback, were completed for each testing component. If the participant responded correctly to a given TOJ or TOJc test trial the inter-stimulus interval of the following trial was reduced to increase difficulty. Conversely, the inter-stimulus interval was increased if the participant responded incorrectly to make the following TOJ or

TOJc test trial easier. The duration of test stimulus was decreased following correct responses to DUR test trials and increased after incorrect responses. Somatosensory processing for each task was measured in milliseconds (ms) and is presented as the average of the three best trials to which the participant responded correctly.

Healthy reference sample

To understand how a patient performs on a given outcome measure, healthcare practitioners commonly consult literature describing how healthy samples perform on the same measure. Therefore, for research questions 1 and 3, we implemented a novel approach where comparisons for the patient cohort were made against simulated healthy distributions generated using pooled BG-SA data (TOJ, TOJc, and DUR performance) for healthy participants extracted from studies [122, 133, 135, 318] (see Table 5.1).

Table 5.1 BG-SA and SCAT-5 symptom burden data pooled to create healthy reference samples.

Study	Participants	Age (years)	TOJ	TOJc	DUR	SSS
Lovell 2006	Healthy young women n = 355 Healthy young men n = 1391	~13-24[◊]	-	-	-	4 [0-78][b] 2 [0-56][b]
Nguyen 2013	Healthy adults n = 19	46.4 ± 2.4[a#]	25.1 ± 13.9 ms[a†]	84.7 ± 58.4 ms[a†]	65.0 ± 33.1 ms[a†□]	
Iverson 2015	Female high school athletes n = 14,668 Male high school athletes n = 17,290	15.5 ± 1.3[a]	-	-	-	3 [0;9][c] 1 [0;6][c]
Jones 2016	Healthy adults n = 16 (6 females)	23.0 ± 5.2[a]	48.8 ± 15.2 ms[a†]	-	-	-
Tommerdahl 2016	Healthy college athletes n = 58	20.1 ± 1.2[a*]	36.4 ± 21.3 ms[a†]	95.2 ± 32.7 ms[a†]	64.6 ± 28.2 ms[a†]	-
Pearce 2019	Healthy adults n = 20 (4 females)	37.7 ± 8.0[a]	23.8 ± 10.1 ms[a‡]	-	48.7 ± 19.1 ms[a‡]	-
Radoi 2020	Healthy community volunteers n = 60 (22 females)	36.2 ± 13.9[a]	-	-	-	4 [0-31][b]
Healthy reference pooled sample size			n = 113	n = 77	n = 101	n = 33,764
Healthy reference pooled results			34.0 ± 17.0 ms[a]	92.6 ± 40.4 ms[a]	61.5 ± 27.9 ms[a]	3 (2-4)[d]
Healthy reference adjusted pooled results			31.3 [21.9; 43.3][c]	87.4 [65.1; 115.0][c]	56.9 [40.9; 75.8][c]	-

Notes: [a] Mean ± SD, [b] Median [Min-Max], [c] Median [IQR], [d] Median (95% CI), [◊] age inferred as not explicitly reported, [#] controls were age and gender matched to patients with migraine in this study, age details for controls were not reported; * age was reported for n = 89 participants, data were presented for n = 58; □ n = 23 for this measure; ‡ study reported confidence intervals which were transformed into standard deviation; † study reported standard error which was transformed into standard deviation.

The means and standard errors of somatosensory performance for healthy participants were presented in three studies [122, 133, 318]. A fourth study [135] presented healthy data as means and 95% confidence intervals. This information was extracted from each article, then standard errors and confidence intervals were subsequently transformed into standard deviations (see Table 5.1). The resultant standard deviations and accompanying means were pooled to provide an estimate of how healthy individuals perform on the BG-SA TOJ, TOJc, and DUR tasks based on previous research. Transformation of standard errors/confidence intervals to standard deviations and pooling of results followed the methods described in the 2011 Cochrane Handbook for Systematic Reviews of Interventions [319].

The reporting of means in previous studies suggests that performance on BG-SA are normally distributed in healthy individuals. However, previous reports highlight that measures of sensorimotor function (i.e., reaction/response times) were commonly positively skewed [320-322]. A previous study comparing baseline BG-SA performance to that observed within 7 days of SR-mTBI presented histograms demonstrating positively skewed distributions both pre- and post-injury, although measures of central tendency were not reported [123]. Positive skew was also observed for the BG-SA data obtained from participants with SR-mTBI in our clinical cohort. Therefore, the distribution of BG-SA performance in healthy populations is likely positively skewed and only contains positive values.

Healthy reference samples for TOJ, TOJc, and DUR were randomly simulated based on a gamma distribution with shape ($mean^2/standard\ deviation^2$) and rate $1/(standard\ deviation^2/mean)$ parameters based on the pooled means and standard deviations from previous studies (see Table 5.1). Gamma distributions are characterised by positive skew and values greater than zero. Simulation of healthy reference samples for each BG-SA variable was accomplished using the '*rgamma'* function in the 'stats' R package. Sizes of the healthy reference samples were dependent on the research question being evaluated. For research question 1 a random gamma distribution was generated for TOJ, TOJc, and DUR with a sample size of 79 to simulate a healthy comparison group for each variable with the same number of observations as those collected from SR-mTBI patients at initial assessment. Performance on TOJ, TOJc, and DUR was acquired at initial assessment as well as clinical discharge from 45 participants who sustained SR-mTBI, so the sample size of random gamma distributions generated for research question 3 was also 45. Since the central tendency and dispersion of skewed distributions are more appropriately described using medians and interquartile ranges, the procedure described above was used to simulate 100 samples of 45 observations for TOJ, TOJc, and DUR, respectively, to approximate the pooled median and average interquartile range for each variable. These adjusted pooled values are presented in Table 5.1.

Whilst the purpose of this study was to evaluate the clinical utility of BG-SA, clinical best practice utilises subjective symptom reports as one indicator to determine when a SR-mTBI patient has achieved clinical recovery [39, 43]. However, at clinical recovery a SR-mTBI patient may not be completely symptom free

(PST = 0, SSS = 0), due to the non-specific nature of mTBI-like symptoms. This has been shown in several studies investigating mTBI-like symptom reports in healthy individuals [83-86]. Data from these studies were also pooled as a reference (see Table 5.1). For consistency, symptom endorsement data were only pooled from studies reporting medians and interquartile/minimum-maximum ranges [323] because symptom reports also appear to be positively skewed in healthy individuals, and once an SR-mTBI patient achieves clinical recovery [85, 86, 316, 324].

Data analyses

Data distribution and non-parametric statistical techniques

Exploratory data analysis revealed skewed distributions containing outliers for both clinical (age, days until initial assessment, days until asymptomatic, PST, and SSS) and somatosensory variables (TOJ, TOJc, and DUR; Shapiro-Wilks $p \leq 0.001$ for each variable). Since the purpose of this investigation was to determine the clinical utility of BG-SA in a real-world clinical environment, outliers were not removed from the data, as these cases are representative of SR-mTBI patients that would present clinically. Due to the shape of the distributions, as well as heterogeneity of variances/covariances between sub-groups, non-parametric statistical techniques were implemented for each research question. Kruskal-Wallis and Mann-Whitney U tests were used to assess between group differences for continuous variables.

Logistic regressions

Logistic regressions enabled evaluation of TOJ, TOJc, or DUR accuracy to discriminate between SR-mTBI versus healthy reference samples for each variable. A single random gamma distribution was generated for TOJ, TOJc, and DUR to serve as a healthy reference sample (n = 79 to match the full SR-mTBI sample size). Due to a modest sample size, leave-one-out-cross-validation (LOOCV) was undertaken to evaluate the model on n-1 participants, and to test the model's capacity to correctly identify the class of the left-out case. This process was repeated for each participant and accuracy metrics including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), percent correctly classified, area under the curve (AUC) and receiver operating characteristic (ROC) were derived for each somatosensory test. This approach is representative of how well a model trained on the current data would perform at classifying a new patient presenting with an SR-mTBI. Training and cross-validation of logistic regressions was performed using the 'caret' and 'pROC' R packages.

Decision trees

Recursive partitioning in the form of a classification tree was utilised to explore if TOJ, TOJc, DUR and/or intra-subject difference between TOJ and TOJc alone, or in combination with PST and/or SSS, at initial assessment were indicators of participant recovery trajectory. A classification tree approach does not rely on the assumptions of normality and homogeneity of covariance, allows for classification into >2 groups, and presents a statistical representation of how medical professionals rule in/out factors that might

identify the cause of a condition [325]. Classification trees were trained and tested using 'rpart' in the R package to determine which variables could correctly classify participants who became asymptomatic in >14-days, ≤14-days, or were lost to follow-up/referral. In some cases, participants became asymptomatic in ≤14-days but due to scheduling limitations, were not seen for a follow-up visit until beyond 14-days post-injury. In this case, if the participant self-reported becoming asymptomatic at day 11 post-injury then 11 days until asymptomatic was recorded.

LOOCV was used to tune and evaluate the classification trees. Tree pruning took place during cross-validation to reduce the likelihood of overfitting to the training data. Ten complexity parameters were evaluated as part of cross-validation to penalise the tree if an additional partition or inclusion of another variable did not enhance the model predictive accuracy as measured by cross-validated error. The final decision tree was determined by the complexity parameter that produced the simplest model (least number of partitions) that demonstrated the greatest classification accuracy. The sensitivity, specificity, balanced accuracy, and overall accuracy were calculated based on the predictions made by the final model for each iteration of LOOCV.

Between- and within-group comparisons

Mann-Whitney U tests were used to compare if differences in somatosensory performance existed between SR-mTBI and healthy reference samples (see Table 5.1). The points of reference were when symptomatic at initial assessment and/or when clinically recovered at discharge. A random sample was simulated for each comparison using the process described in Section 2.5 and a Mann-Whitney U test between the reference samples and SR-mTBI data was performed. To reduce the risk of Type 1 error that could occur because of the distribution of a single random sample this process was repeated 100 times and the distributions of the 100-resultant *p*-values are visualised using boxplots for TOJ, TOJc, and DUR at each variable and timepoint. The percentage of comparisons that yielded a p-value <0.05 are reported to indicate the likelihood that observed differences in BG-SA performance between SR-mTBI participants and the healthy reference samples are due to chance.

Changes in somatosensory performance and symptom endorsement for the 45 participants when symptomatic at initial assessment, and clinically recovered at discharge, were tested using the Wilcoxon Signed-Rank test. Due to variability of clinical data, a priori α was set to 0.05 for comparisons. Statistical analyses and visualisations were performed using Python v3.6.10 and RStudio v1.1.456.

Results

Descriptive statistics grouped by recovery trajectory are summarised in Table 5.2 (top panel shows data for the 79 participants who completed the BG-SA at initial assessment; bottom panel shows data for the 45

participants tested at both initial and discharge). Distributions of continuous variables were highly skewed therefore descriptive results are presented as Median [IQR].

Demographic and clinical information

Females accounted for 25% of the total sample (n = 79) and 50% of participants were between 16 and 23 years of age. Playing rugby union or rugby league was the inciting factor of SR-mTBI for 68% of the participants.

Participants with >14-days symptom resolution (11.0 [6.0, 17.0]) and those who were lost to referral/follow-up (11.0 [6.0, 14.0]) took longer to present for their initial assessment than those with ≤14-days resolution (6.5 [4.0, 10.0]). Participants lost to referral/follow-up reported the highest level of symptom burden at initial assessment (PST: 16.0 [10.5, 18.0]; SSS: 36.0 [23.5, 48.3]) followed by >14-days resolution (PST: 14.0 [7.0, 17.0]; SSS: 25.0 [13.0, 47.0]) and ≤14-days resolution (PST: 6.0 [3.3, 7.0]; SSS: 8.0 [5.0, 14.3]). Participants who experienced >14-days resolution required a median 30.0 [20.0, 45.0] days to become asymptomatic compared 10.5 [6.0, 12.0] for those in the ≤14-days group.

Demographic and clinical data for the 45 participants with data from both initial assessment and discharge followed the same trends as described above and are presented in Table 5.2.

Discriminative utility of somatosensory assessments

Univariable logistic regression and ROC analysis were performed using TOJ, TOJc, and DUR, respectively, to evaluate how accurately any one of these BG-SA can discriminate participants with SR-mTBI when compared with a healthy reference sample simulated based on published results (see Table 5.2). TOJc demonstrated the best discriminative performance by correctly classifying 63% of cases to the correct group with an AUC of 0.70, 64.71 PPV, and 61.11 NPV (see Figure 5.2). Limited discriminative accuracy was observed for TOJ (48%, 0.54 AUC, 47.46 PPV, 48.48 NPV) and DUR (56%, 0.61 AUC, 56.92 PPV, 54.84 NPV).

Prognostic utility of somatosensory assessments

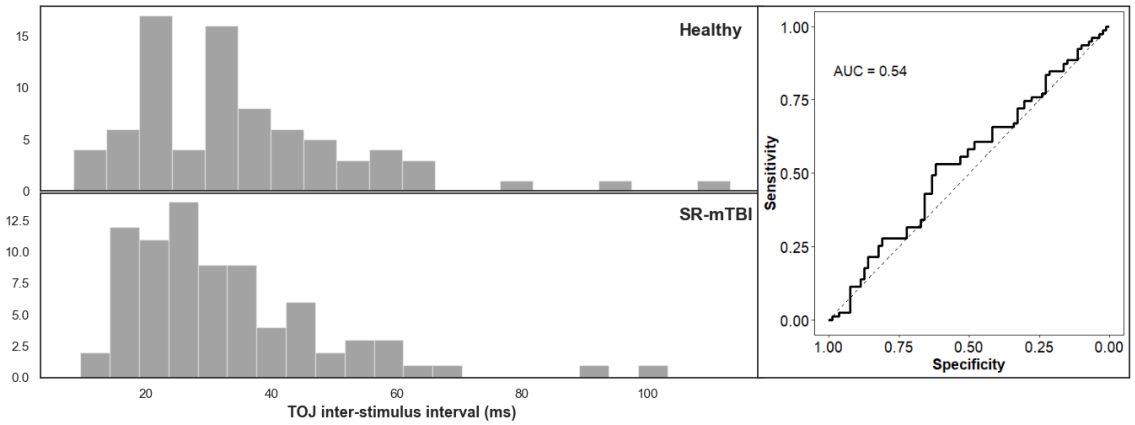
Two classification trees were developed using LOOCV to determine the prognostic capacity of BG-SA alone or in combination with symptom reports to correctly assign >14-days resolution, ≤14-days resolution, or lost to referral/follow-up recovery trajectory membership to the 79 SR-mTBI participants (see Table 5.3). The first decision tree was developed using BG-SA alone. The final somatosensory decision tree used TOJ ≥50 ms, TOJc ≥68 ms, and TOJc <47 ms as decision thresholds and demonstrated 48%, 54%, and 45% balanced accuracy results for >14-days resolution, ≤14-days resolution, or lost to referral/follow-up, respectively. The second tree was developed using BG-SA and symptom reports to see if greater three group classification accuracy could be obtained (see Table 5.3). The final combination decision tree used SSS ≥20 as the lone decision threshold resulting in better balanced accuracy for classifying >14-days

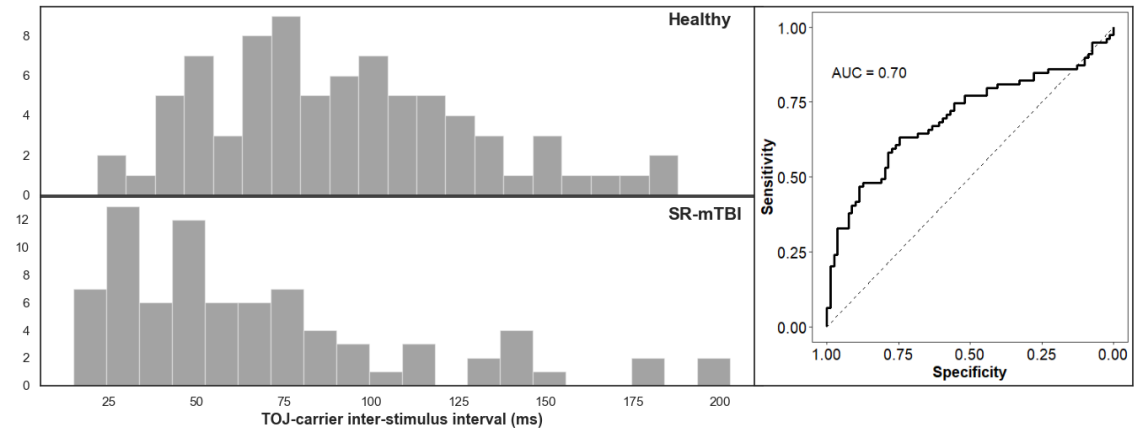
resolution (63%), ≤14-days resolution (79%), or lost to referral/follow-up (50%) recovery trajectory membership.

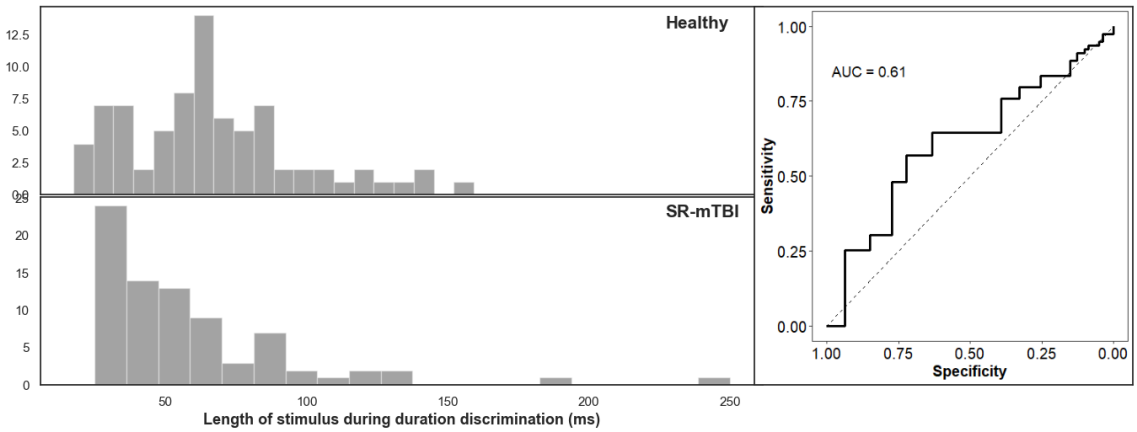
Table 5.2 Descriptive statistics grouped by recovery trajectory for SR-mTBI patients assessed using BG-SA at initial clinical assessment only (n=79) and at initial and discharge assessments (n=45).

Descriptive statistics for patients assessed with BG-SA at initial SR-mTBI assessment only				
	Total (n=79)	Resolution ≤14-days (n=22)	Resolution >14-days (n=41)	Lost to follow-up (n=16)
Sex[a]				
Male	60 (76%)	21.0 (95%)	28 (68%)	11.0 (69%)
Female	19 (24%)	1.0 (5%)	13 (32%)	5.0 (31%)
Sport[a]				
Rugby codes	54 (68%)	20 (91%)	26 (63%)	8 (50%)
Other	15 (19%)	1 (5%)	10 (24%)	4 (25%)
Football	10 (13%)	1 (5%)	5 (12%)	4 (25%)
Age[b]	19.0 [16.0; 23.0]	19.0 [16.0; 23.0]	19.0 [15.0; 23.0]	20.5 [16.8; 30.8]
Days until initial assessment[b]	10.0 [5.5; 14.5]	6.5 [4.0; 10.0]*	11.0 [6.0; 17.0]	11.0 [6.0; 14.0]
Days until asymptomatic[b]	20.0 [12.0; 34.5]	10.5 [6.0; 12.0]◊	30.0 [20.0; 45.0]	-
Initial PST[b]	11.0 [6.0; 17.0]	6.0 [3.25; 7.0] *†	14.0 [7.0; 17.0] ‡	16.0 [10.5; 18.0]
Initial symptom severity[b]	20.0 [8.5; 42.0]	8.0 [5.0; 14.3]*†	25.0 [13.0; 47.0]‡	36.0 [23.5; 48.3]
TOJ performance (ms)[b]	29.0 [20.3; 40.2]	26.5 [17.1; 32.9]	29.0 [20.5; 40.6]	32.7 [22.5; 53.8]
TOJc performance (ms) [b]	59.9 [34.1; 84.4]	57.8 [40.7; 77.5]	63.9 [32.0; 86.2]	54.9 [42.2; 84.1]
DUR performance (ms)[b]	50.0 [29.2; 66.7]	50.0 [41.7; 72.9]	50.0 [25.0; 66.7]	45.8 [25.0; 72.9]
Descriptive statistics for participants assessed with BG-SA at initial and discharge SR-mTBI assessments				
	Total (n=45)	Resolution ≤14-days (n=15)	Resolution >14-days (n=30)	
Sex[a]				
Male	35 (78%)	14.0 (93%)	21 (70%)	
Female	10 (22%)	1.0 (7%)	9 (30%)	
Sport[a]				
Rugby codes	33 (73%)	13 (87%)	20 (66%)	
Other	8 (18%)	1 (7%)	7 (23%)	
Football	4 (9%)	1 (7%)	3 (10%)	
Age[b]	19.0 [16.0; 23.0]	19.0 [15.5; 23.0]	19.5 [16.0; 24.0]	
Days until initial assessment[b]	10.0 [5.0; 12.0]	10.0 [4.0; 10.0] ◊	11.0 [6.0; 16.5]	
Days until asymptomatic[b]	20.0 [12.0; 32.0]	10.0 [5.0; 12.0]◊	27.5 [20.0; 42.5]	
Initial PST[b]	7.0 [5.0; 14.0]	5.0 [2.5; 7.0] ◊	11.0 [6.25; 17.0]	
Discharge PST[b]	1.0 [0.0; 2.0]	0.0 [0.0; 1.0] ◊	1.0 [0.25; 3.0]	
Initial symptom severity[b]	15.0 [7.0; 29.0]	7.0 [3.0; 11.0] ◊	22.0 [10.5; 44.5]	
Discharge symptom severity[b]	1.0 [0.0; 2.0]	0.0 [0.0; 1.0] ◊	1.0 [0.25; 4.0]	
TOJ performance (ms)[b]	27.2 [18.2; 36.8]	24.7 [15.7; 31.9]◊	28.3 [20.2; 40.4]	
TOJc performance (ms) [b]	55.6 [34.9; 82.5]	51.3 [42.3; 60.5]◊	66.1 [32.7; 85.3]	
DUR performance (ms)[b]	50.0 [25.0; 66.7]	41.7 [29.2; 54.2] ◊	50.0 [29.2; 66.7]	

Notes: [a] Frequency (%), [b] Median [25th percentile; 75th percentile], *Dunn's post-hoc comparison of Kruskal-Wallis tests $p < 0.05$ ≤14-days vs >14 days resolution , †Dunn's post-hoc comparison of Kruskal-Wallis tests $p < 0.05$ ≤14-days vs lost to follow-up, ‡Dunn's post-hoc comparison of Kruskal-Wallis tests >14 days resolution vs lost to follow-up, ◊ Mann-Whitney U $p < 0.05$ for ≤14-days vs >14 days resolution







	Healthy Median [IQR]†	SR-mTBI Median [IQR]	Sensitivity	Specificity	Correctly classified, %	PPV	NPV	AUC	95% CI
TOJ	31.3 [21.9; 43.3]	29.0 [20.23; 40.2]	0.35	0.61	48	47.46	48.48	0.54	0.45-0.63
TOJc	87.4 [65.1; 115.0]	59.9 [34.1; 84.4]	0.56	0.70	63	64.71	61.11	0.70	0.61-0.78
DUR	56.9 [40.9; 75.8]	50.0 [29.2; 66.7]	0.47	0.65	56	56.92	54.84	0.61	0.52-0.70

Note: † Median IQR for healthy reference group in this figure is from a single randomly simulated skewed distribution based on the process described in the Healthy reference sample section of the Methods. OR – Odds ratio, PPV – Positive predictive value, NPV – Negative predictive value, AUC – Area under the curve.

Figure 5.2 Discriminative performance of TOJ, TOJc and DUR.

Table 5.3 Prognostic utility of BG-SA to classify patient recovery trajectory (n=79).

	Variables included and decision thresholds	Sensitivity	Specificity	Balanced Accuracy	Overall accuracy	95% CI
Three group classification using Brain Gauge performance only						
>14-days resolution	TOJ ≥50 ms	0.83	0.13	48%		
≤14-days resolution	TOJc ≥68 ms	0.14	0.95	54%	47%	36-58%
Lost to follow-up	TOJc <47 ms	0	1	45%		
Three group classification using Brain Gauge performance combined with clinical variables						
>14-days resolution		0.68	0.58	63%		
≤14-days resolution	SSS ≥20	0.86	0.72	79%	60%	48-70%
Lost to follow-up		0	1	50%		

SR-mTBI somatosensory performance at initial assessment and discharge

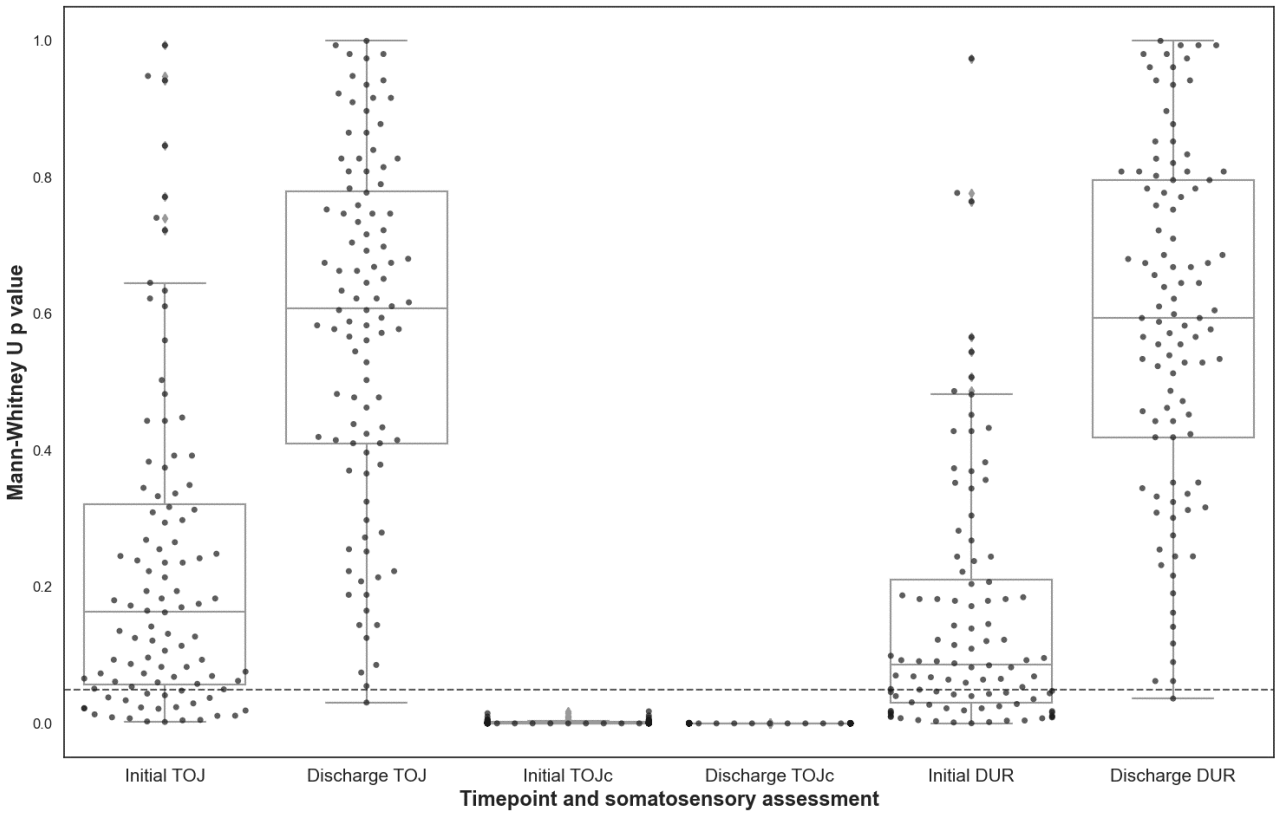
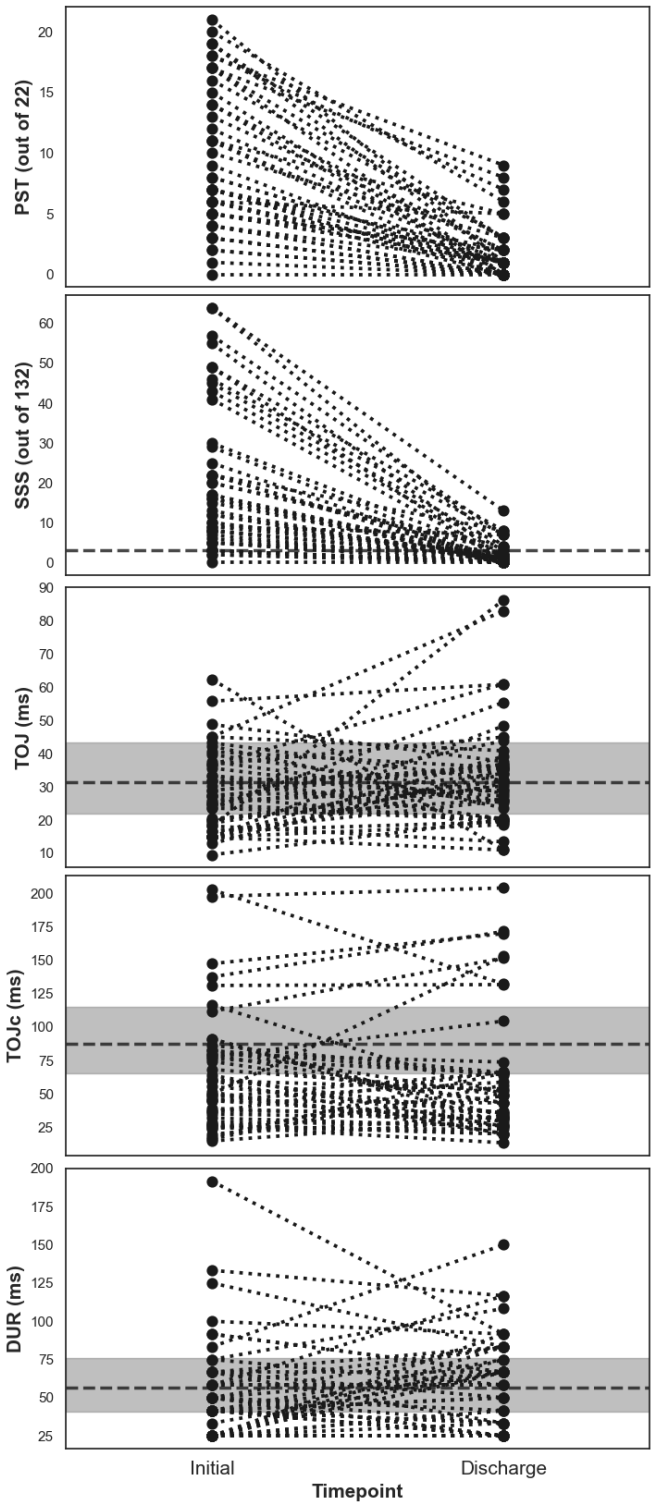


Figure 5.3 Results of comparing SR-mTBI TOJ, TOJc, and DUR performance against 100 simulated healthy reference samples. Datapoints represent resultant p-values of each experiment. The dashed line represents p = 0.05.

Between-group differences in TOJc performance (consistent with $p<0.05$) were observed in 100% of the comparisons between SR-mTBI participants and simulated healthy reference samples both at initial assessment and discharge (see Figure 5.3). Statistically significant differences in TOJ and DUR performance were observed in 23% and 37% of comparisons at initial assessment, respectively. At clinical discharge 1%

of TOJ and DUR comparisons between participants with SR-mTBI and healthy reference samples yielded a *p*-value <0.05 (see Figure 5.3).



Note: Dashed black lines represent the pooled median performance in healthy individuals shown in Table 5.1. Grey bands represent the average IQR from 100 simulations of healthy distributions shown in Table 5.1.

Figure 5.4 Initial assessment versus discharge BG-SA performance (TOJ, TOJc, DUR) and SCAT-5 symptom burden (SSS and PST).

Change in somatosensory performance and symptom burden across timepoints

Figure 5.4 visualises changes in somatosensory performance and symptom endorsement from initial clinical assessment to discharge. There were no within-group changes in BG-SA between initial assessment and clinical discharge when participants were clinically recovered at discharge (TOJ p=0.074, TOJc p=0.170, DUR p=0.234). A considerable drop was seen between timepoints for PST (p<0.001) and SSS (p<0.001).

Discussion

The purpose of this study was to evaluate the clinical utility of BG-SA to provide objective information to assist clinicians when diagnosing SR-mTBI, to predict recovery outcomes based on initial assessment performance, and to determine if neurophysiological recovery coincides with clinical recovery. While previous studies have demonstrated aspects of BG-SA can identify differences or discriminate between individuals with known neurological conditions and controls; none have evaluated the prognostic capability of BG-SA, nor evaluated BG-SA performance once participants met clinical recovery criteria. To our knowledge this is the first study to evaluate BG-SA in an ecologically valid setting and to independently assess BG-SA outside the group responsible for its development and commercialisation.

Marginal utility of somatosensory assessments to assist diagnostic decisions

SR-mTBI diagnosis must be made by a physician based on a comprehensive clinical examination, but previous research suggests BG-SA may provide physicians with additional objective information to guide their evaluation and potentially assist when establishing SR-mTBI diagnosis. Such information would be particularly useful if there is delayed symptom onset or when non-compliant patients appear to be underreporting their symptomology [4-7]. Favorov et al. evaluated whether BG-SA could be used to discriminate between pre-season baseline performance versus performance within one week of SR-mTBI and reported that reaction time variability yielded the greatest accuracy (0.91 AUC), followed by amplitude discrimination (0.83 AUC), DUR (0.78 AUC), mean reaction time (0.69 AUC), and TOJ (0.53 AUC) [123]. Results for TOJc were not available in that study and BG-SA reaction time variability was not evaluated in the present study because of a lack of studies reporting how healthy controls perform on this measure, preventing pooling and simulation. Our findings suggest limited discriminative accuracy of the BG-SA TOJ, TOJc, and DUR to assist clinicians faced with the challenge of diagnosing SR-mTBI when patients present for initial assessment ~1-1.5 weeks after the suspected injury. Our results indicated that only TOJc demonstrated marginal discriminative accuracy of BG-SA assessed (0.70 AUC, 64.71 PPV, 61.11 NPV). The low to moderate PPVs and NPVs observed for TOJ, TOJc, and DUR suggest that these measures would offer little assistance to clinicians working under similar clinical conditions as those in this study when establishing SR-mTBI diagnosis.

Differences in outcomes between the current study and previous reports may be explained by the conditions under which BG-SA were performed. Participants in Favorov et al.'s study appeared to be assessed under highly controlled/laboratory conditions which permitted the collection of pre-injury baseline data, assessment of a greater number of BG-SA tasks, and earlier post-injury evaluation [123]. Our study was embedded within a busy SR-mTBI clinic which meant the number of BG-SA tasks had to be limited to preserve clinical flow. Participants were members of the public presenting with SR-mTBI from a variety of sports, levels of competition, and geographic areas meaning baseline testing was not possible and comparison to previously published data from healthy individuals was necessary. Participants underwent initial clinical assessment a median of 10 days post-injury because of the realities of clinical scheduling constraints. While previous reports suggest promising potential for BG-SA to assist diagnostic decisions there is a lack of ecological validity. For these reasons, our findings are likely more representative of how TOJ, TOJc, and DUR would perform at discriminating between healthy individuals and those who have suffered SR-mTBI under conditions which many clinicians operate. It is possible that a greatly reduced BG-SA protocol (<5 minutes) may be useful in high volume environments where patients present on the day of injury such as emergency departments and walk-in clinics, but research into this potential application is needed.

Limited prognostic utility of somatosensory assessments

While a screening tool that can accurately discriminate between cases with or without a given condition is certainly useful, an optimal tool would pair discriminative and prognostic capabilities. In the case of SR-mTBI, early and accurate prediction of recovery trajectories would possibly reduce the need for patients likely to recover quickly to attend follow-up appointments, subsequently, keeping limited follow-up appointments available for the patients who require them most. Our analysis evaluated whether BG-SA performance at initial clinical assessment (regardless of days until initial assessment) could classify participants who would become asymptomatic in ≤14-days, >14-days, or those who were lost to follow-up/referral. Early identification of those lost to referral/follow-up would be particularly useful to identify patients requiring referral to a different service early on, or to begin to understand why some patients do not present for follow-up. Our data suggest limited utility of BG-SA to classify participants accurately into their respective resolution trajectory group. The lack of classification accuracy can likely be explained by the overlap in BG-SA performance across the three groups at initial clinical assessment (see Table 5.2).

Neurophysiological abnormalities still present at clinical discharge

A growing body of literature highlights that some individuals with a history of mTBI present with persistent neurophysiological abnormalities for weeks, months, and years post-injury when compared to healthy controls [308, 311, 326-329]. Several studies have reported that these abnormalities still exist once an mTBI patient becomes asymptomatic and/or meets clinical recovery criteria [132, 140, 330-333]. It appears that

TOJ and DUR lack sensitivity to detect group-level performance differences between individuals who have recently suffered SR-mTBI and performance consistent with healthy individuals, as shown by the lack of difference in performance when participants were symptomatic at initial assessment (see Figure 5.3 and Figure 5.4). In contrast, TOJc performance was impaired both at initial assessment ~10-days post-injury and remained impaired at clinical discharge in individuals with SR-mTBI (see Figure 5.3 and Figure 5.4). Our findings support the notion that clinical recovery precedes complete neurophysiological recovery based on the dissimilarity of BG-SA TOJc performance between SR-mTBI participants at clinical discharge and healthy reference values.

The impact of these persistent neurophysiological abnormalities beyond clinical recovery remains to be understood. This phenomenon may be analogous to managing an athlete after a fracture. The athlete may report being pain free during clinical examination, while X-ray images may suggest the athlete requires more time for fracture union before returning to play. In this scenario the objective information gained using X-ray may assist the clinician in refining an individualised management plan that reduces the risk of the athlete returning to play when they may be susceptible to reinjury. Future longitudinal cohort studies are needed to quantify BG-SA post-mTBI, through clinical recovery, and the weeks/months after clinical recovery to determine the utility of BG-SA as an objective means to track neurophysiological recovery in a clinically useful manner. Overall, reports of lingering neurophysiological disruption across multiple independent studies utilising a variety of objective outcome measures raises the question of whether definitions and expectations of recovery post-mTBI require further revision.

Limitations

This study was embedded in a busy SR-mTBI clinic that required several compromises to preserve clinical flow and ecological validity of study findings. These compromises led to limitations that should be accounted for when considering the findings of this investigation. A shortened version of the BG-SA protocol had to be implemented because the complete battery required too much time and burden on behalf of the participants. It is possible that the BG-SA components omitted from the current study may have yielded greater discriminative or prognostic accuracy than TOJ, TOJc, or DUR. In practice SR-mTBI patients commonly do not present for clinical evaluation until days after sustaining the injury because of delays in seeking medical treatment and/or due to clinical scheduling constraints [203]. Collection of BG-SA data on the date of injury may have been more favourable to understand the immediate consequences of SR-mTBI and how performance changes between onset of injury and initial clinical assessment. Collection of BG-SA data on the day of injury may potentially have enabled development of a better prognostic model.

The current investigation only evaluated the clinical utility of BG-SA in relation to SCAT-5 symptom burden because resolution of symptoms was a main criteria used to determine clinical recovery. It is possible that

performance on BG-SA may have related to specific symptoms or predicted outcomes for other aspects of the SCAT-5 such as cognitive and balance assessments. It is worth noting that the cognitive and balance assessments within the SCAT-5 lack clinical utility to detect meaningful differences at the time which most participants presented for initial clinical assessment in this study [87, 88, 316]. Nevertheless, more independent research into the reliability and validity of BG-SA under clinical conditions, and their relation to clinical outcome measures are needed before integrating the use of BG-SA into clinical practice.

We adopted a novel approach to pool and simulate healthy reference data to replicate clinical decision-making conditions. Assumptions were made during the simulation of healthy reference samples used for research questions 1 and 3; particularly the skewness of healthy BG-SA performance and the use of gamma distributions during simulations. There may be issues with representativeness of the pooled healthy reference data due to the relatively low sample size. A more traditional approach would have included the recruitment of a healthy control group but issues with representativeness can also be a common limitation of such designs due to matching issues. Since the BG-SA is a standardised and computerised testing protocol we argue that pooling of healthy data from multiple independent studies may have provided a more representative comparison group than a convenience sample of healthy individuals. Given the preliminary nature of this investigation and the conservative findings presented these assumptions and limitations seem justifiable. Our modest sample size included unbalanced resolution trajectory subgroups which may have prevented the development of an accurate prognostic model. Furthermore, due to sample size it was not possible to perform further subgroup analysis based on age, sex, sport, or predominant symptom cluster [75]. Acquisition of BG-SA requires sustained attention and screen time exposure. Disruptions in attention during BG-SA and/or intolerance to screens due to SR-mTBI may have influenced testing.

Conclusions

Our findings suggest that the discriminative and prognostic utility of BG-SA TOJ, TOJc, and DUR to assist diagnostic decision making and to predict recovery trajectory under ecologically valid conditions appears limited. Abnormal BG-SA TOJc performance was observed when participants with SR-mTBI met clinical recovery criteria. This finding adds to a growing body of literature reporting that clinical recovery is not necessarily indicative of complete neurophysiological recovery. Given the realities of time and budget constraints in clinical practice, our findings do not provide sufficient justification to recommend the allocation of time and resources to acquire BG-SA TOJ, TOJc, or DUR to assist clinical management of SR-mTBI patients at this time. Further expansion and replication of these preliminary findings would determine whether BG-SA is a tool that clinicians could integrate into regular practice.

Personal development as a researcher resulting from Chapter 5

- How to pool previously published data and simulate reference samples for statistical comparisons.
- How to deploy decision trees and leave-one-out-cross-validation in RStudio.
- Importance of independent research.
- Importance of reporting the most appropriate measure of central tendency for a given distribution.
- How study design directly influences study findings and how this can hinder translation of findings into clinical practice.

Link between Chapters 5 and 6

Previous reports suggested Brain Gauge somatosensory assessments could be a useful tool to aid diagnostic and prognostic decision making for clinicians working with SOBI patients. To our knowledge Chapter 5 presents the first study to evaluate components of the Brain Gauge testing protocol in a real-world clinical environment and is the first independent assessment of this protocol outside the group responsible for its development and commercialisation. In the context of this thesis, it was hoped that Brain Gauge somatosensory assessments would offer prognostic value equal to or greater than the model presented in Chapter 3. Had such results been observed it would have provided evidence for a more objective means of identifying patients with greater likelihood to experience longer recovery trajectories without the need to rely on symptom reports. Such a finding would have valuable clinical applications, particularly in environments where clinicians with experience managing SOBI are lacking. Additionally, an accurate prognostic model using BG-SA would have provided a more objective method to identify SOBI patients who might benefit from a nutritional intervention. The results presented in Chapter 5 suggested limited accuracy and clinical value of an abbreviated battery of Brain Gauge somatosensory assessments when administered under ecologically valid conditions. These findings highlighted the importance of independent research into products that have been commercialised and marketed towards clinicians seeking tools to assist them manage SOBI. In Chapter 6 the potential to objectively classify different SOBI-related PSCs using wearable sensor data acquired during treadmill stress testing and analysed using machine learning algorithms was explored. In Chapter 3 we highlighted that physiological PSC was most prevalent within in our cohort followed by vestibulo-ocular PSC. Given that findings in Chapters 2 and 3 both indicated that vestibulo-ocular symptomology were associated with worse outcomes in patients with a history of SOBI, Chapter 6 specifically focused on developing a machine learning model that could accurately classify these two clinical groups.

Chapter 6: A deep learning approach to classifying sport-originated brain injury subgroups using wearable sensor data acquired during exercise stress testing: A pilot study.

This chapter comprises the following paper to be submitted to *Artificial Intelligence in Medicine:* **McGeown, J. P.**, Hume, P. A., Theadom, A., Kara, S., & Russell, B. A deep learning approach to classifying sport-originated brain injury subgroups using wearable sensor data acquired during exercise stress testing: A pilot study.

Author contribution

McGeown, J. P. 80%, Hume, P. A. 5%, Theadom, A. 5%, Kara, S. 5%, Russell, B. 5%

Overview

Aim: To investigate whether a deep learning approach could accurately classify between SOBI patients with physiological predominant symptom cluster (PSC) versus vestibulo-ocular PSC using wearable sensor data collected during the Buffalo Concussion Treadmill Test (BCTT). **Methods:** A cross-sectional design enabled evaluation of a deep learning model trained with electrocardiography (ECG) and accelerometry (ACC) data to classify patients with physiological PSC versus vestibulo-ocular PSC. Three temporal slices of the ECG and ACC signals were defined to standardise data inputs into the model. These slices included the first 60 seconds of testing, the latest common time for all subjects (third minute), and the final 60 seconds before the BCTT was ended. **Results:** Hyperparameter tuning revealed the best classification occurred with a window size of 256 and an overlap of 128 between windows for the first- or third-minute slices of the BCTT data. Using a k-folds approach to implement leave-one-out-cross-validation the convolutional neural network correctly classified 3 of 5 (60%) vestibulo-ocular cases and 11 of 12 (92%) physiological cases using ECG and ACC data from the third minute of each participants BCTT. The same classification accuracy was observed using only ACC data from the third minute. **Conclusion:** Our results provide proof of concept that incorporation of wearable sensors during BCTT and machine learning techniques have potential to assist decision making for clinicians working with SOBI patients. With further research the addition of wearable sensors during clinical tests like the BCTT, combined with deep learning models, may have utility to assist management decisions for SOBI patients.

Introduction

Traumatic brain injuries (TBIs) occur as a consequence of exposure to sudden physical trauma from external forces resulting in deformation and damage of neuronal and vascular tissues within the brain [21]. An estimated 95% of TBI patients have suffered mild TBI determined by a Glasgow Coma Scale score ≥13, absence of skull fracture or positive neuroimaging, and associated with symptoms resulting from impaired neurological function [20, 22, 24, 27]. Approximately 20% of TBIs are a result of a sport/physical activity related mechanism of injury, with 98% of these sport-related mTBI considered to be mild TBI [10]. Given the various terms used for brain injures resulting from sport participation such as sport-related concussion and sport-related mTBI, and the Centers for Disease Control and Prevention recommended single use of mTBI over concussion [3], yet needing a word easily said and understood by the public (e.g., strains, sprains, and SOBI), the term sport-originated brain injury (SOBI) is used in this paper [11].

Until recently, recommendations for the management of SOBI followed a one-size-fits-all model, where all patients received the same care regardless of their specific symptom burden [93]. It was thought that 85-90% of these SOBI patients would experience spontaneous clinical recovery and resolution of symptoms within 10-14 days post-injury; thus a "wait and see" approach was adopted. Access to proactive interventions was consequently limited [43]. However, updated reports from large epidemiological studies of the general population have indicated that nearly half of mTBI/SOBI patients experienced prolonged symptom burden beyond this window [10, 315]. Although injury mechanisms of SOBI may be similar across patients, it is becoming increasingly clear that patients cannot be treated as one homogenous group as several predominant symptom clusters (PSCs) have been identified, each requiring specific and individualised treatment plans [75, 96, 98, 161, 334, 335]. Criteria have been developed to identify different PSCs to determine whether a predominantly physiological, vestibulo-ocular, or cervicogenic origin appears to contribute to unresolved symptoms at ≥21 days post-injury [75]. The criteria to determine the PSC utilise the patient's clinical history and examination along with results of a provocative exercise test, commonly referred to as the Buffalo Concussion Treadmill Test (BCTT) [75, 89-91]. A framework has been provided to help understand the pathophysiology responsible for each PSC, and how to approach prescribing individualised exercise-based interventions to address and resolve these underlying mechanisms [75, 98].

It is hypothesised that symptoms consistent with physiological PSC are manifestations caused by an uncoupling of the autonomic nervous system and cardiovascular system [12, 61, 75, 124]. Previous laboratory research has identified differences in autonomic regulation during exercise, but not at rest, between athletes who recently sustained a SOBI, and healthy controls as measured by heart rate variability using three-lead electrocardiography (ECG) [12, 124]. Conversely, symptoms that characterise vestibulo-ocular PSC are thought to be due to underlying issues with sensorimotor integration and vestibular function [61, 75, 138, 139]. Static balance and gait differences between healthy controls and participants with SOBI

have been found using force plates and accelerometers [127, 128, 140]. Individuals with vestibulo-ocular PSC may display greater impairment in balance/gait than those with physiological PSC, and vice versa for autonomic regulation. Presently, even when the BCTT is used, the process of identifying PSCs relies on athlete's honest symptom reporting which is an issue when managing athletes with SOBI [4-7], and the training/experience of the supervising clinician [336-338]. Previous observations with laboratory grade equipment highlight that technological methods may be sensitive to impairments not detected by clinical tests [12, 124, 127, 128, 140]. However, restricted accessibility to laboratory grade equipment in clinical environments limits the translation of these findings.

Development of wearable sensors validated against laboratory grade equipment offer an opportunity to transition research from the lab to the real-world [339]. Integration of the BCTT within clinical practice presents an opportunity to use a wearable sensor to collect ECG and accelerometry (ACC) data under ecologically valid conditions. The addition of objective measures to this testing protocol might assist accurate PSC classification when symptom reporting may be untrustworthy and/or when clinicians have limited experience working with mTBI/SOBI patients. Machine learning models are an increasingly popular approach for analysing time series data like that collected by ECG and accelerometry [339-343]. There is potential for machine learning algorithms, specifically deep learning techniques, to support medical decision making by automatically detecting the most important features related to patient outcomes i.e., high versus low risk for a given condition [340, 344]. The capability of a deep learning model trained using time series signals from ECG (as measure of autonomic control) and accelerometry during gait (as a surrogate of sensorimotor integration) to classify PSC subgroups in mTBI/SOBI patients has not been previously explored. Therefore, the aim of this pilot study was to investigate whether a deep learning approach could accurately classify SOBI patients with physiological PSC versus vestibulo-ocular PSC using wearable sensor data collected during the BCTT. A second question investigates the hypothesised connection between the two conditions and two data types.

Methods

Study design and participants

A cross-sectional design was adopted to evaluate the accuracy of a deep learning model trained with ECG and ACC data to classify patients with physiological PSC versus vestibulo-ocular PSC. Data collection took place at a single dedicated SOBI clinic between April 2019 and July 2019. A total of 43 consenting patients diagnosed with mTBI by a sport and exercise medicine physician completed a BCTT during the data collection period for this study. The sample was inclusive for age and sex. Institutional (AUTEC 18/374) and health and disability committee (HDEC 18/NTA/108) ethical approvals were obtained, and this study was conducted according to the ethical standards of the Declaration of Helsinki. Participants provided written consent (child assent and parental consent for participants <16 years old) for their data to be used for

research and publication. All participant data were de-identified prior to extraction/data analysis to ensure confidentiality.

Procedures

Timing of BCTT

Participants received SOBI management as part of usual clinical care, the details about this clinical pathway have been previously described [315]. Clinical scheduling constraints meant that not all patients completed a BCTT. Testing was completed if requested by the supervising physician to confirm suspected PSC based on patient history and clinical examination and/or to inform an individualised treatment plan. The appointment during which the BCTT was administered depended on the proximity of the participant's initial clinical assessment to the date of injury. Efforts were made to administer a BCTT approximately two weeks post-injury to aid in developing a targeted and individualised exercise treatment plan for participants with unresolved symptoms based on their PSC. Participants presenting for initial clinical assessment ≥10 days post-injury completed a BCTT on the day of their first visit if requested by the physician. Treadmill testing was performed during the first follow-up appointment if participants initially presented within 10 days of their injury and had not reported improvement in their symptoms between the first and second visit.

Treadmill testing protocol

The BCTT protocol is a safe and reliable method to assist patient prognosis, PSC identification, and management decisions for SOBI patients with unresolved symptoms [75, 89-91, 345]. Contraindications including inability to exercise because of orthopedic injury or increased cardiorespiratory risk were considered when deciding whether a participant completed a BCTT [90]. Pre-testing symptom burden was established by participants rating their current symptom burden on a 1-10 scale (1 = minimal/no symptoms; 10 = severe symptoms), and specific symptoms were recorded [90].

Participants walked on a treadmill at 5 km/h at 0% incline for one minute then the incline was increased to 2% and a further 1% every minute thereafter with the speed remaining at 5 km/h [90]. Participants were asked to walk normally, without resting their hands on the rails of the treadmill. After every minute of the protocol, participants were asked to report any changes in their symptom burden and rating of perceived exertion (scale of 0-10; 10 representing maximal effort) [346]. Heart rate was monitored and recorded at the end of each minute. Testing proceeded in this way until either participants experienced symptom exacerbation (increase of +3 from their initial reports) or volitional fatigue (rating of perceived exertion ≥9) [90]. If the participant did not experience symptom exacerbation or fatigue by the time the maximum incline of 15% was reached, speed was increased by 1 km/h while maintaining the 15% incline to a maximum testing duration of 20 minutes. All BCTTs were delivered by a clinician and/or researcher with training in exercise physiology.

Expert labelling

The physicians with training and experience managing SOBI athletes identified the PSC using published criteria [75]. Given that physiological and vestibulo-ocular PSCs were the most prevalent in our previous research [316] combined with the novel nature of the current study, we elected to focus specifically on these two groups. Data from 26 participants (18 physiological PSC; 8 vestibulo-ocular PSC) who completed a BCTT were eligible for the current study. For analysis purposes, vestibulo-ocular PSC was considered the positive class since it is less prevalent and a predictor of worse recovery outcomes.

Instrumentation

During the BCTT heart rate was recorded using a Zephyr BioHarness (Medtronic, Dublin, Ireland) that contained three-lead ECG and tri-axial ACC (x-axis = vertical, y = lateral, z-axis = sagittal). The BioHarness has been shown to be a valid and reliable tool for physiological monitoring in field-based applications with military, first responders, and athletes [142, 347, 348]. Data were transmitted in real-time via Bluetooth to a smartphone application (IoTool sensor platform from SenLab). Raw data were stored within local memory and downloaded after recording. The BioHarness was worn the same way as a typical heart rate monitor where the electrodes were lightly dampened with cold water and the strap was placed snugly around the torso at the level of the xiphoid process. Two BioHarness of different sizes were used for all testing based on subject chest size (sizes XS-M and M-XL). Preservation of clinical flow and ecological validity of findings was a priority of this study. Once a consistent heart rate registered on the smartphone application, normal procedures for explaining and administering the BCTT were initiated without further adjustment of the BioHarness. All BCTTs were performed on a Life Fitness Engage 95T treadmill (Hamilton, New Zealand).

Deep learning pipeline

While there are several deep learning methods, previous findings indicate a convolutional neural network (CNN) trained with ECG or ACC time series data can accurately classify patients with/without cardiac dysfunction [343] and different human activities [339, 341, 342], respectively. Notable advantages of CNNs include computational efficiency and little to no manual feature engineering [341, 349]. For these reasons, a CNN appeared to be a good starting point to explore if deep learning could automatically identify features in ECG and/or ACC signals that differentiate between SOBI PSCs. Figure 6.1 shows the data pipeline from patient identification through the experiments executed in this study. All data pre-processing and deep learning models were completed in Python (v3.8.5) using the Sklearn (v0.23.2), Tensorflow (v2.3.1), and Keras (v2.4.0) packages.

Dataset preparation and pre-processing

The BioHarness sampled ECG and ACC signals at 250 Hz and 100 Hz, respectively. Prior to analysis ACC time series data were time aligned, labelled and upsampled using linear interpolation to match the sampling

frequency of ECG data. After upsampling, three temporal slices of the ECG and ACC signals were defined to standardise data inputs to the CNN across participants due to the symptom-dependent variation in BCTT duration. These slices included the first 60 seconds of testing, the third minute, and the final 60 seconds before the BCTT was ended. The first minute was chosen to explore if different PSCs could be classified using sensor data collected early in the protocol without the need to push the patient to symptom exacerbation, whereas the final 60 seconds should coincide with the onset of symptom exacerbation or volitional fatigue. The third minute slice was selected as the latest common time for all subjects because the shortest BCTT collected for this study was four minutes. The third minute provided a standardised timepoint to evaluate all participants while under physiological load (5 km/h, 4% incline), but prior to symptom exacerbation or fatigue. When administering the BCTT the speed of the treadmill gradually increased to 5 km/h, and the first minute of testing began once the treadmill was up to speed and the participant settled into steady state walking. The beginning of the testing was identified in each participant's time series file by plotting their ACC data and manually identifying the timestamp when the participant settled into a repeatable gait pattern defined by 5+ consecutive gait cycles with similar waveform profiles (see Figure 6.2 panel B).

The first minute slice was the next 60 seconds from the manually defined point, and the third minute was from 180 to 240 seconds. A similar approach was used to determine the final 60 second slice. The time series ACC plots were manually inspected to identify when there was a sudden change in the gait pattern representing when the participant stepped onto the rails of the treadmill or decreased the speed or stopped the treadmill (Figure 6.2 panel C). The 60 seconds of signal leading up to the timestamp of this disruption in gait served as the final slice of data to be explored.

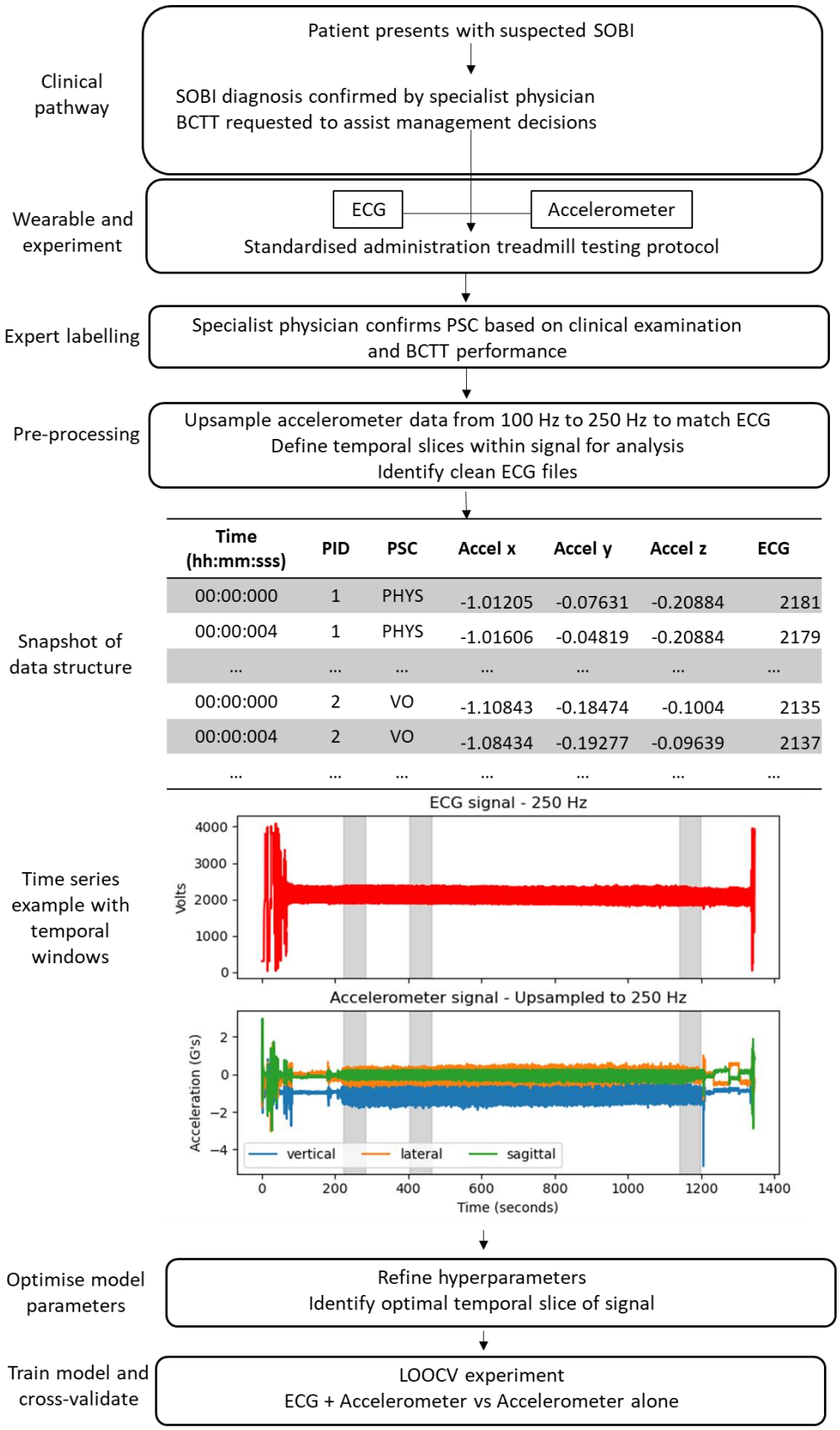


Figure 6.1. Data pipeline from SOBI to deep learning model.

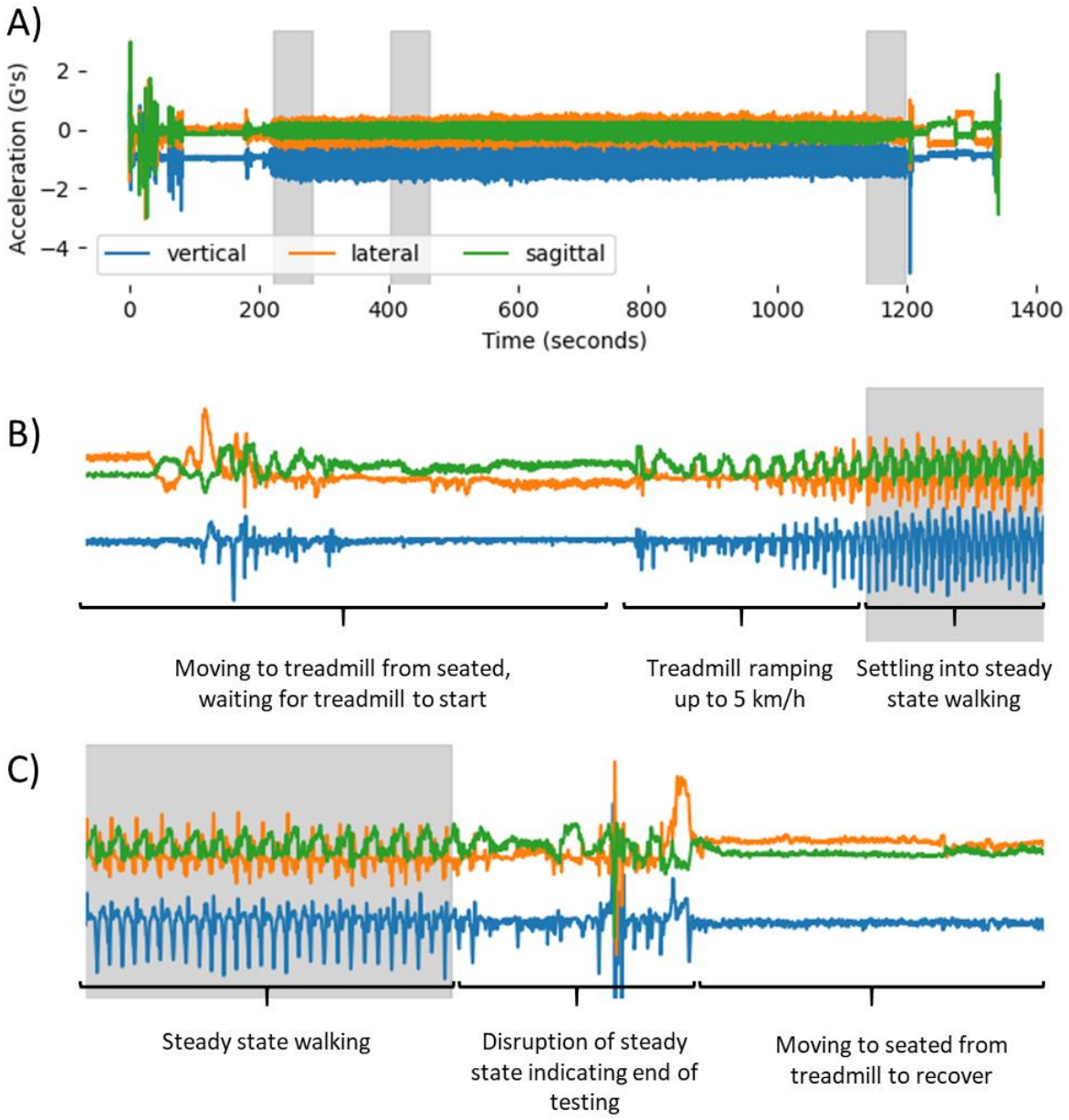


Figure 6.2 Process of how temporal slices were determined. A) Example of complete signal from time BioHarness was turned on until turned off with temporal slices shown in grey. B) How ACC waveform were used to determine start of first minute. C) How ACC waveforms were used to determine end of BCTT.

ECG files were visually inspected to assess if a clean ECG signal with distinct and repeatable QRS complexes had been recorded within the temporal slices of interest. Clean ECG time series were observed in 17/26 participant files (12 physiological PSC; 5 vestibulo-ocular PSC). The remaining nine files were deemed unfit for further analysis because they contained large amounts of noise making it difficult to discern the QRS pattern. Noise was likely due to poor coupling between the ECG nodes and the skin due to quickly deploying the BioHarness to prioritise clinical flow. An example of the columns included in the final dataset is provided in Figure 6.1.

Time series data were segmented for training and cross-validation of CNN models, where n samples were divided into windows of W samples wide with S overlap producing D rows of data (Equation 1) [339].

$$D = 1 + \frac{n-W}{S} \quad (1)$$

Data were transformed into an array (*D, W, F*) with *D* rows, width of *W* samples, and *F* features (*F* = 4 for ECG + ACC (x,y,z); *F* = 3 for ACC (x,y,z) only) so they were compatible with the CNN [339]. A separate one-dimensional array contained PSC labels corresponding to each row within the array of signal data based on which participant the data belonged to.

Deep learning model

The CNN consisted of four separate 1D convolutional networks for each axis of the ACC signal (x,y,z) and ECG signal (see Figure 6.3 for CNN topology) combined into a single classifier output stage. Each channel used two convolutional 1D layers and a ReLu activation function with filter and kernel sizes of 64 and 3, respectively. The convolutional layers were followed by a drop out layer of 50% for generalizability and a max pool layer with pool size 0.5. A flatten layer was used to combine the separate channels, followed by a dense layer with ReLu activation. The output weights from the previous layers were transformed into probabilities for each class using a SoftMax activation function. Learning used an epoch of 50 and batch size of 100. Classification metrics including accuracy, Cohen's kappa, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to evaluate the CNN's performance when classifying windows with either physiological labels versus vestibulo-ocular labels.

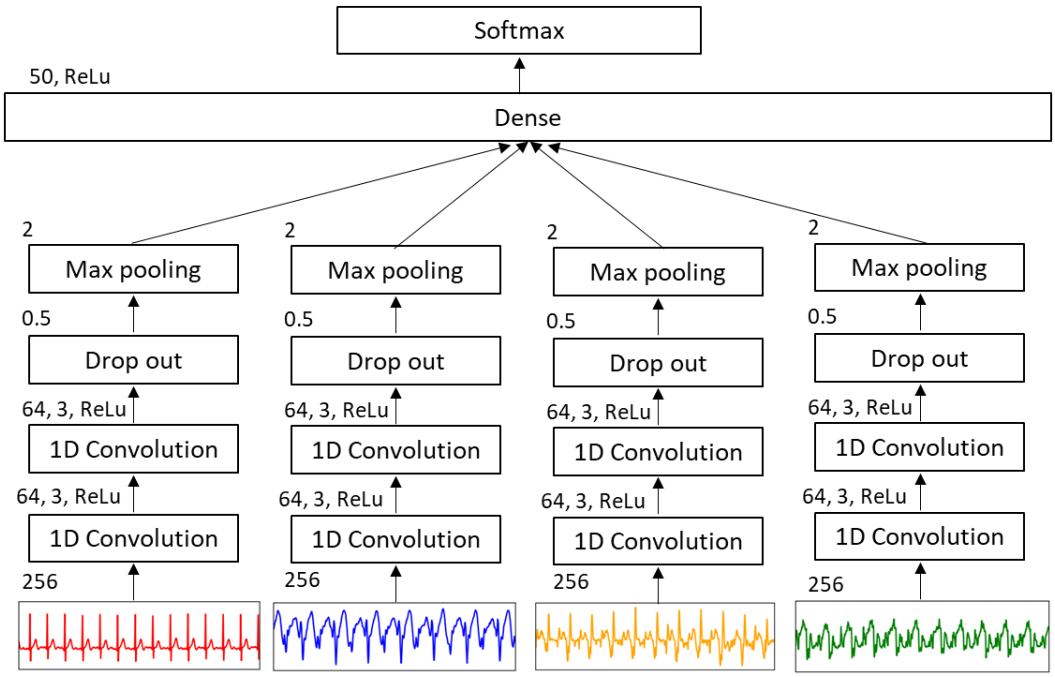


Figure 6.3 Structure of CNN

Hyperparameter tuning

Window size tuning was performed to optimise PSC classification accuracy. Windows ranging in size from 16, 32, 64, 128, and 256 with a 50% overlap between windows were evaluated. For each set of hyperparameters, a randomised train test split of 0.66/0.33 was selected using Sklearn in Python. During hyperparameter tuning, classification metrics were calculated based on the performance of the CNN to correctly classify the label associated with each window in the test set, irrespective of which participant the window came from. Priority was placed on the hyperparameters that maximised Cohen's kappa and PPV due to the imbalance in PSC classes. This process was applied for each temporal slice (first minute, third minute, final minute) to cross-reference optimal hyperparameters within each slice and to evaluate if differences in classification metrics were evident.

LOOCV Experiment

Once the optimum hyperparameters were determined the following experiment was conducted to explore whether the CNN could correctly classify a given participant's PSC. Due to a limited sample size, a leave-one-out-cross-validation (LOOCV) methodology was implemented to train a CNN on data from n-1 participants, followed by testing the model's capacity to correctly identify the PSC of the left-out case using their sensor data. This process was repeated for each participant and allows exploration of how well a model trained on the current data might perform at classifying physiological versus vestibulo-ocular PSC following a BCTT in a new SOBI patient.

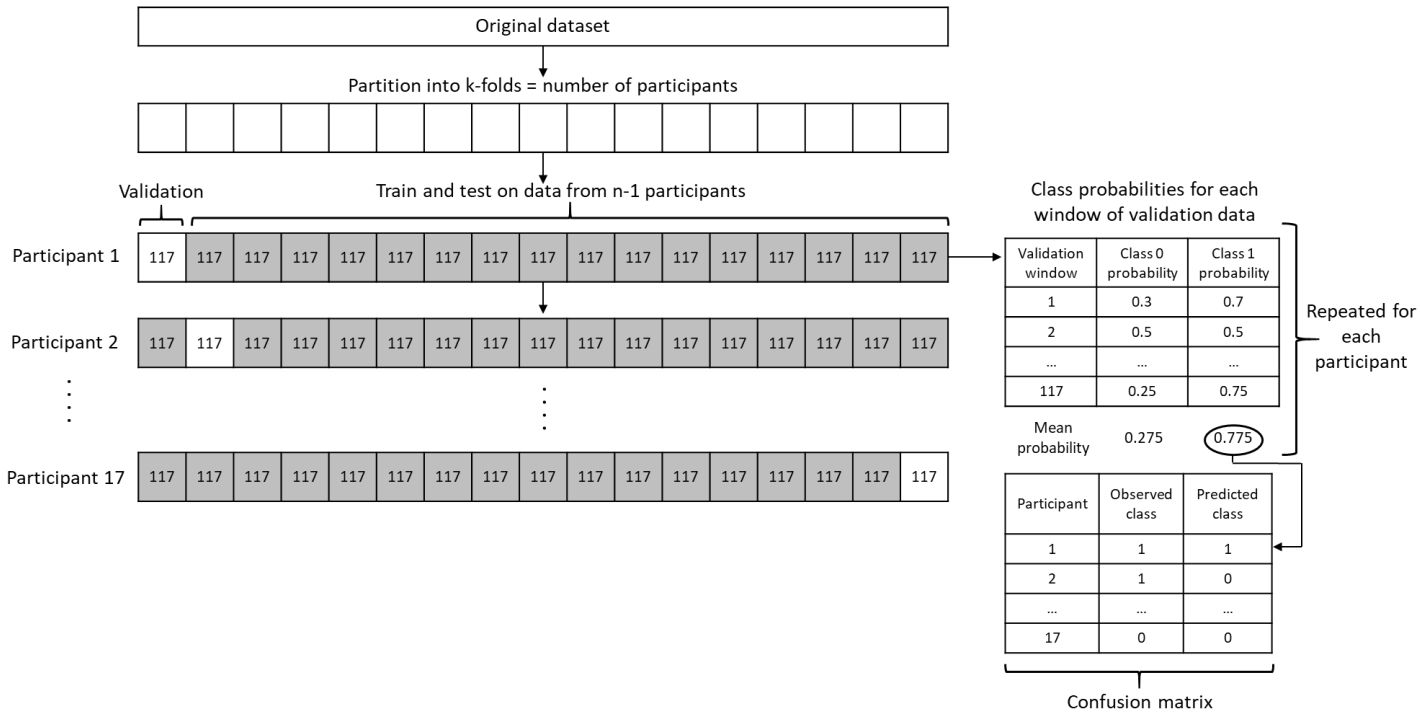


Figure 6.4 Diagram of how LOOCV was implemented to evaluate potential utility of a CNN to predict the class of 'new' sensor data collected during a BCTT.

Use of time series data in this study meant that 15,000 rows (250 Hz x 60 seconds) corresponded to each participant per temporal slice. To effectively accomplish LOOCV for each participant, Sklearn's stratified k-folds method was used, wherein all data were split into 17 equal folds of 15,000 rows of data without shuffling. The first iteration held out rows 0-14,999 from participant 1 for LOOCV and trained on rows 15,000-254,999 (participants 2-17), the second iteration held out rows 15,000-29,999 from participant 2 while training on 0-14,999 and 30,000-254,999 (participants 1, 3-17), and so on. The CNN was trained on k-1 folds using a randomised train test split of 0.66/0.33. A probability for each class was calculated corresponding to each of the *D* windows in the validation fold and these probabilities were stored in an array. For a given participant, some windows may have had a higher probability of belonging to the physiological PSC class, while the rest indicated vestibulo-ocular PSC was more probable. Therefore, the mean probability across the *D* windows was calculated for each class and the class with the greater mean probability was recorded as the predicted class for the participant to which the windows belonged. The predicted class was saved after each iteration. Once all iterations were complete a confusion matrix was produced from which sensitivity, specificity, PPV, NPV, and area under the curve (AUC) of the receiver operating characteristic (ROC) curve were calculated. This process is visualised in the diagram presented in Figure 6.4.

This methodology was first used to explore the potential of a CNN trained with both ECG and ACC time series data to accurately classify the PSC of the 17 participants with clean ECG data. Since noisy ECG data led to the exclusion of nearly 1/3 of eligible participants (9/26), this experiment was repeated using only ACC data to see the level of classification accuracy that might be obtained without the need to collect ECG data.

Results

Demographics

Of the 17 participants whose data were used to evaluate whether a deep learning approach could accurately classify SOBI patients with physiological PSC versus vestibulo-ocular PSC using wearable sensor data collected during the BCTT, 11 were male (8 physiological PSC; 3 vestibulo-ocular PSC) and 6 were female (4 physiological PSC; 2 vestibulo-ocular PSC) with a median age of 21.0 [IQR: 16.0; 28.0] years. These participants completed a BCTT with a median of 17.0 [IQR: 13.0; 18.5] days post-injury, and with a median BCTT duration (mm:ss) of 14:12 [IQR: 9:12; 17:55).

Hyperparameters and temporal slices

Results of hyperparameter tuning are shown in Table 6.1. The best classification performance of windows corresponding to physiological PSC versus vestibulo-ocular PSC occurred with a window size of 256 and an overlap of 128 between windows, as determined by Cohen's Kappa and PPV. Similar levels of accuracy were

observed using these hyperparameters using data from both the first- and third-minute slices. This meant that for each participant their 60 seconds of data (sampled at 250 Hz) fed into the CNN was separated into D = 117 windows for the LOOCV experiments.

Table 6.1 Hyperparameter tuning results from each temporal slice.

	Window size	Overlap	Accuracy	Kappa	Sensitivity	Specificity	PPV	NPV
First minute	16	8	0.90	0.76	0.84	0.92	0.82	0.93
	32	16	0.93	0.83	0.92	0.93	0.84	0.97
	64	32	0.95	0.88	0.93	0.96	0.9	0.97
	128	64	0.97	0.92	0.92	0.98	0.96	0.97
	256	128	0.98	0.96	0.98	0.99	0.97	0.99
Third minute	16	8	0.89	0.72	0.7	0.97	0.91	0.88
	32	16	0.93	0.83	0.88	0.95	0.88	0.95
	64	32	0.96	0.90	0.92	0.98	0.94	0.96
	128	64	0.95	0.88	0.87	0.99	0.96	0.94
	256	128	0.97	0.94	0.96	0.98	0.95	0.98
Final minute	16	8	0.88	0.69	0.7	0.95	0.86	0.88
	32	16	0.93	0.82	0.8	0.98	0.94	0.92
	64	32	0.96	0.91	0.95	0.97	0.92	0.98
	128	64	0.96	0.90	0.91	0.97	0.94	0.96
	256	128	0.94	0.86	0.92	0.95	0.88	0.97

Note: Shaded row indicates window size and overlap with best performance for each temporal slice.

LOOCV Experiment

Using a k-folds approach to implement LOOCV the CNN correctly classified 3 of 5 (60%) vestibulo-ocular cases and 10 of 12 (83%) physiological cases using ECG and ACC data from the first minute of each participants BCTT. Similar performance was observed when using ECG and ACC data from the third minute of the treadmill protocol with 3 of 5 (60%) vestibulo-ocular and 11 of 12 (92%) physiological cases correctly classified. The final minute performed worse than chance classifying 0 of 5 vestibulo-ocular cases and 8 of 12 (67%) physiological cases. Comparable classification accuracy was seen for each temporal slice when ECG data from these participants were omitted and CNNs were trained using only ACC data. See Figure 6.5 for receiver operating characteristic curves and related accuracy metrics for each condition, and Table 6.2 for individual results.

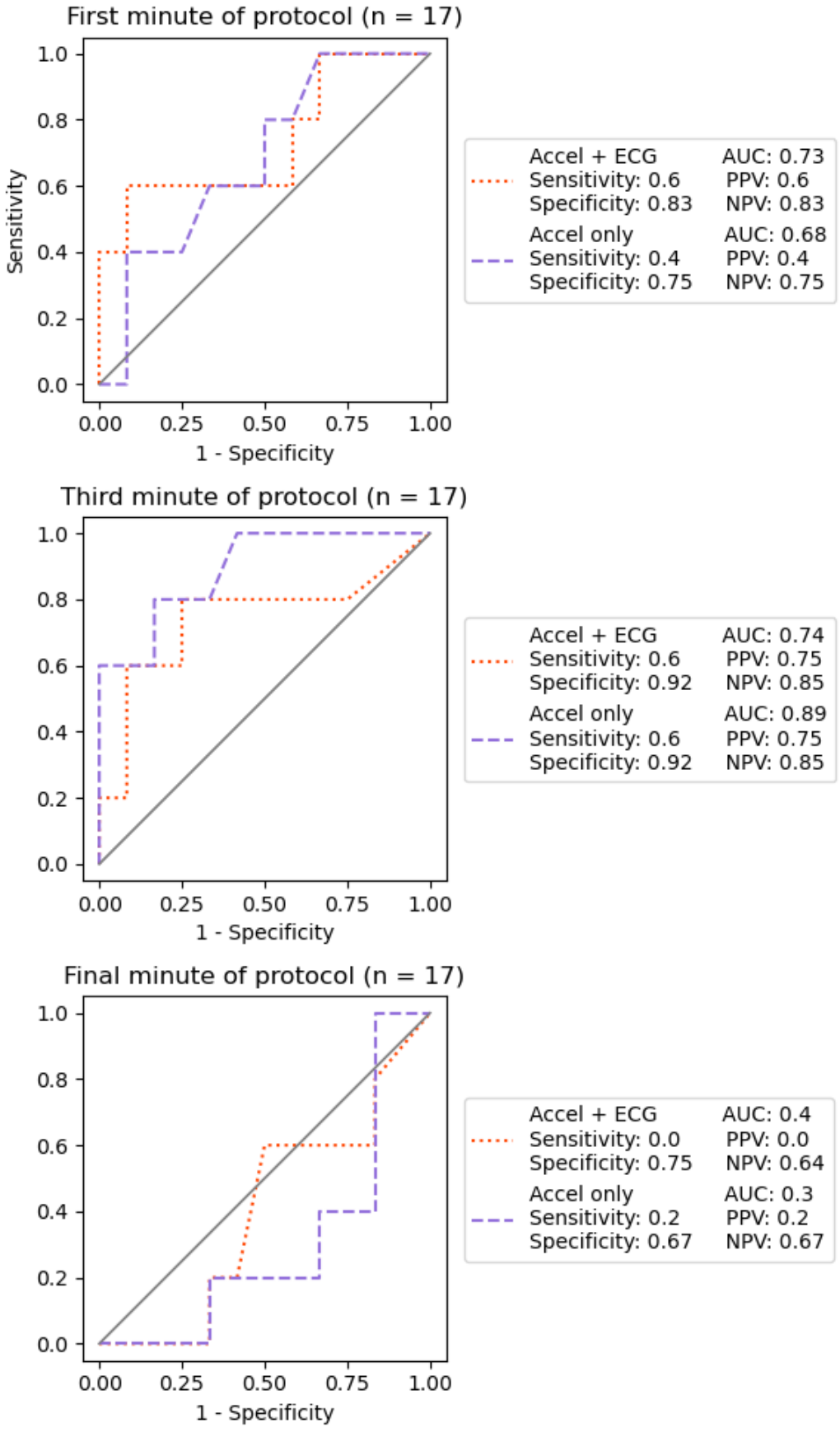


Figure 6.5 LOOCV classification performance of the CNN using ACC data with and without ECG data from each temporal slice.

Table 6.2 Predicted class probabilities for each participant and temporal slice using LOOCV.

Accelerometer + ECG																			
Participant	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	# of VO Correct	# of Phys correct
PSC	V	V	P	V	P	P	V	P	P	P	P	V	P	P	P	P	P		
First minute	.01	.01	.29	.84	.00	.09	.65	.39	.62	.00	.49	.93	.04	.22	.54	.00	.00	3/5	10/12
Third minute	.00	.56	.03	.85	.00	.10	.30	.73	.17	.00	.35	.63	.23	.39	.11	.02	.00	3/5	11/12
Final minute	.02	.00	.19	.19	.00	.26	.19	.80	.15	.16	.83	.46	.16	.51	.50	.18	.00	0/5	8/12
Accelerometer Only																			
Participant	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	# of VO Correct	# of Phys correct
PSC	V	V	P	V	P	P	V	P	P	P	P	V	P	P	P	P	P		
First minute	.01	.08	.30	.89	.00	.06	.36	.36	.22	.00	.91	.70	.01	.52	.54	.00	.00	2/5	9/12
Third minute	.05	.95	.03	.79	.00	.18	.38	.44	.04	.01	.61	.85	.01	.33	.05	.02	.00	3/5	11/12
Final minute	.07	.01	.21	.07	.00	.10	.11	.88	.34	.08	.88	.51	.41	.28	.54	.87	.00	1/5	8/12

Note: Values presented are predicted probability of left out participant belonging to vestibulo-ocular class. Predicted probabilities >0.5 indicated vestibulo-ocular was more likely and <0.5 physiological was more likely. Shaded cells indicate correct predictions.

Discussion

To our knowledge, this is the first study to explore whether a deep learning approach could accurately classify PSCs in SOBI patients using sensor data collected during BCTTs. Utilising a LOOCV approach, moderate levels of physiological versus vestibulo-ocular PSC classification accuracy were observed when a CNN was trained using ACC and ECG data acquired during a BCTT. The third minute demonstrated the best classification performance based on area under the ROC curve and total correct scores 14/17 for ACC + ECG and 14/17 for ACC only, while the final minute performed worse than chance. The poor performance in the final minute were likely because BCTT duration varied for participants based on symptom exacerbation/fatigue, thus the demands experienced by each participant within this temporal slice were non-standardised. The third minute performed slightly better at classification than the first minute which may be explained by greater physical load generating more symptomatic behaviour. Overall, these findings suggest that with further research the addition of wearable sensors during clinical tests like the BCTT, combined with deep learning models, may have clinical utility to assist clinicians when classifying PSCs in SOBI patients.

Future directions

It is possible that overly-optimistic findings were observed because of the low sample size, imbalanced classes, and cross-validation approach used. These results are proof of concept that further work to determine generalisability of this approach appears justifiable. Three key considerations should be accounted for in future investigations.

First, when optimal coupling between the BioHarness and the skin was achieved (as determined by clean ECG signal) a CNN trained with only ACC data demonstrated similar levels of classification accuracy as a CNN trained using a both ECG and ACC data. Some studies have shown reliable and valid measurement of static balance in neurologically compromised participants using ACCs and gyroscopes within smartphones [350, 351]. If future research extends our findings that ACC is sensitive to both physiological and vestibular-ocular groups, then it could make clinical implementation of a wearable sensor during treadmill testing easier and more affordable as a smartphone application could be developed to predict the most probable PSC based on data recorded using the internal ACC.

Second, due to the novelty of this study we opted to focus on SOBI patients with the two PSCs (physiological and vestibulo-ocular) that were most prevalent in our previous research [316]. Theoretically, distinct differences in the degree of autonomic uncoupling and impaired sensorimotor integration could be present between these two clinical subgroups, and such differences would allow accurate classification of these groups as suggested by the results of this study. However, in clinical practice, an important subgroup are the patients whose symptoms and clinical examination suggest both physiological and vestibulo-ocular

PSCs are present. These patients with mixed PSC require multi-modal treatment plans to restore autonomic function and sensorimotor integration. While the results of the current study are encouraging, they do not account for this important subgroup. To have true clinical utility, an optimal model would be capable of separately classifying physiological, vestibulo-ocular, and mixed PSC with a high degree of accuracy. If such a model could be developed using wearable sensor data and expert labelling of this third class, it would provide a more objective tool for clinicians to utilise when establishing individualised treatment plans for their SOBI patients.

Third, an advantage of deep learning models such as CNN is that they automatically detect features in the data that maximise classification accuracy and may not be apparent to a human analyst. This significantly reduces the time and expertise required to develop the model compared to machine learning techniques that rely on manually engineered features [344]. Such an approach was appropriate for the current study since our aim was to evaluate if a model trained using wearable sensor data collected during the BCTT *can* classify different PSCs in SOBI patients [340]. This approach does not allow inferences to be made as to *how* the model differentiated between groups leading to deep learning techniques like CNNs to be commonly described as a 'black box' [340]. Better classification was observed using a combination of ACC + ECG data as well as ACC data alone from the third minute versus the first minute. With the current approach inferences about why the third minute outperformed the first minute cannot be made. Using the deep learning approach may give insight that allows future work using more conventional statistical techniques and manual feature engineering could allow inferences that may lead to useful clinical applications. For example, identification of key features could be used to assess the effectiveness of interventions targeting underlying impairments related with each PSC by re-administering a BCTT at follow-up appointments to assess changes from one timepoint to the next. The findings of this study are the first to showcase that combining wearable sensors and machine learning algorithms under ecologically valid conditions has exciting potential to advance the clinical management of SOBI.

Limitations

Sample size was a limitation of this pilot study as only 17 participants had good ECG and ACC data. The most plausible explanation for the signal quality issue was suboptimal coupling between the BioHarness and the skin. While deploying a BioHarness is very similar to a commercial heart rate monitor, it appears that additional time needs to be dedicated to ensure optimal coupling and resulting data quality.

The decision to use the k-folds method of implementing LOOCV was due to limited sample size. A more common machine learning approach would have been to randomly assign 66% of participants (and their corresponding time series data) for training the model and to test the model performance on the remaining participants. Utilising a LOOCV approach was an appropriate compromise since our aim was to assess the

potential clinical utility of how a model trained on the current data might perform if a new patient completed a BCTT wearing a BioHarness, while making the most of the limited data available. There was an imbalance in the number of participants with physiological (n = 12) and vestibular (n = 5) PSC in this pilot study. While this class imbalance is suboptimal for analysis, as it may contribute to overly optimistic classification accuracy, it is clinically representative of our previous findings. Vestibulo-ocular PSC was considered the positive class in this study since it is less common and a predictor of worse recovery outcomes. However, physiological PSC was only labeled as the negative class since most participants belonged to this class. Even if results were biased towards identifying physiological PSC this could still be viewed as clinically meaningful as it could assist management decisions. To account for this class imbalance results depicted in Figure 6.5 could have been better visualised using a Precision/Recall curve rather than a ROC curve [352]. Because of the aim of this study, we chose to present ROC curves, since both classes are clinically meaningful and ROC curves are more familiar to clinicians [353]. To address this compromise we also reported PPV and NPV, as PPV is associated with Precision/Recall [352], and these terms are commonly used in medicine [354]. Overall, recruitment of a greater sample would account for these limitations, and efforts to do so appear justified based on our findings. Finally, these preliminary findings are from a sample of athletes who sustained SOBI, the potential for these findings to be applicable to individuals who sustain mTBI due to non-sport-related causes is unknown.

Conclusion

This is the first study to explore whether a deep learning approach could accurately classify PSCs in SOBI patients using sensor data collected during BCTTs. Our results provide proof of concept that incorporation of wearable sensors during BCTT and machine learning techniques have potential to assist decision making for clinicians working with SOBI patients.

Personal development as a researcher resulting from Chapter 6

- First experience working with accelerometer and ECG data.
- How to time align and match frequencies of signals sampled at different rates.
- Introduction to advanced machine learning.
- Understanding advantages and disadvantages of deep learning algorithms.
- How to tune hyperparameters for a convolutional neural network.
- How to deploy K-folds cross-validation on time series data to accomplish leave-one-out-cross-validation.
- How to accomplish all of the above using Python libraries.

Link between Chapters 6 and 7

The preliminary findings presented in Chapter 6 provide proof of concept that applying machine learning algorithms to wearable sensor data collected during BCTT could support the accurate prescription of individualised treatment plans by assisting clinicians identify a given SOBI patient's PSC. Use of wearable sensors and machine learning algorithms might particularly support clinicians who have not received training on how to identify PSCs and cannot refer their SOBI patients to a specialist clinic due to geographical or socioeconomic barriers. Instead, the supervising clinician might be able to simply administer a BCTT while the patient wears a sensor like the BioHarness, the data could be run through a classifier, and then the patient could be referred to a local healthcare practitioner with the skillset (i.e., vestibular physiotherapist, ophthalmologist, exercise physiologist, etc.) to address their specific needs. Improved classification of SOBI subgroups would also aid the investigation of novel interventions to improve recovery outcomes; as some subgroups may respond differently to treatment than others, the effectiveness of a novel treatment may not be apparent if SOBI patients are analysed as one homogenous group. Of course, further research, streamlining of data pipelines, and a large database of sensor data acquired during BCTT an labelled with different PSCs will be necessary for this to become a reality. With a potential future solution to help clinicians classify subgroups of SOBI patients identified, the next step in this thesis was to explore the translational potential of BDNF, measured non-invasively via saliva as a biomarker of neuroplasticity, to provide clinicians with insight towards why some SOBI patients recover faster than others. But first, Chapter 7 presents an experimental pilot study with healthy participants to evaluate whether intra-individual comparisons in salivary-BDNF concentrations can be made when timing of sampling and delays in storage cannot be standardised as would be the case in most outpatient clinical environments.

Chapter 7: Shortcomings of saliva as a practical means to measure intra-individual variation of BDNF – A pilot study.

This chapter comprises the following paper submitted to *Scandinavian Journal of Clinical and Laboratory Investigation:* **McGeown, J. P.**, Hume, P. A., Quarrie, K.L., Theadom, A., & Dulson, D.K. Shortcomings of saliva as a practical means to measure intra-individual variation of BDNF – A pilot study.

Author contribution

McGeown, J. P. 80%, Hume, P. A. 5%, Quarrie, K.L. 5%, Theadom, A. 5%, Dulson, D.K. 5%.

Overview

Brain derived neurotrophic factor (BDNF) plays a major regulatory role in neuroplasticity, making it a biomarker of interest for researchers investigating the pathophysiology of neurodegenerative and neuropsychiatric conditions. While typically measured via blood samples, BDNF can also be quantified non-invasively via saliva which potentially provides an easier means of quantifying BDNF in real-world clinical settings. In addition to being non-invasive, saliva would have to demonstrate practical advantages for clinical research to overtake blood as the biological sample of choice for measuring BDNF protein concentrations. The aim of this pilot study was to develop methodologies and provide preliminary evidence regarding how timing of sampling and delays in storage influence intra-individual salivary-BDNF protein concentrations. Ten healthy volunteers (28.67± 2.18 years old, and 19.33± 1.5 years of education, five females) provided saliva samples via passive drool at two-hour intervals between 09:00h and 17:00h (to mimic clinical operation hours) for a total of five samples each. All samples were processed and stored within one hour of collection, with a subset of samples kept on ice for several hours to evaluate sample stability under delayed storage conditions. An optimised ELISA procedure was used to quantify salivary-BDNF. No consistent trends in salivary-BDNF concentrations were observed in relation to time of sampling or sample stability as evident in the high degree of variation within and between participants. These results are most likely explained by the risk of sampling error when trying to measure proteins via passive drool. This pilot study does not provide evidence to justify using saliva as a valid and practical means of evaluating intra-individual variation in BDNF concentrations. These results should not discourage future research into the potential clinical applications of BDNF as a biomarker to advance understanding of neurological conditions; rather our findings highlight that the best method to measure BDNF in ecologically valid environments remains elusive.

Introduction

Brain-derived neurotrophic factor (BDNF) is a neuropeptide belonging to the neurotrophin family that collectively play major regulatory roles in neurogenesis, proliferation, neural function, and neuroplasticity [129, 143]. BDNF also contributes to the formation and consolidation of memories by promoting long-term potentiation in response to activity-dependent stimulation [129]. Expression of BDNF has been observed both in humans and animals within the amygdala, cerebellum, cerebral cortex and hippocampus; with the highest expression present in the latter region explaining BDNF's contribution to memory formation [129]. The known functions of BDNF have garnered great attention from researchers investigating the pathophysiology of neurodegenerative and neuropsychiatric conditions (see [129] for a recent comprehensive review). Abnormalities in circulating BDNF concentrations have been observed in a diverse collection of neurological conditions, examples of which include: Alzheimer's disease [129, 355], depression [356, 357], schizophrenia [358], bi-polar disorder [359], autism [360], and traumatic brain injury [65]. Expanded knowledge regarding the presentation of BDNF in these conditions has led to the identification of intervention strategies to upregulate BDNF in an effort to promote neurogenesis, neuroplasticity, and improved memory performance. Intervention strategies utilising environmental enrichment and exercise programs have demonstrated improved BDNF levels associated with better performance on functional outcome measures in both animal and human studies [129]. This growing body of evidence highlights BDNF as a promising biomarker to not only understand pathophysiological characteristics of neurological conditions at a group level, but also as a potential means to track intra-individual changes throughout the recovery/treatment process for neurologically compromised patients.

Previous studies have commonly measured BDNF in humans under laboratory conditions using plasma or serum collected via venepuncture [129, 361, 362]. Animal studies suggest these peripheral measures relate to some degree with tissue levels of BDNF within the brain, although the strength of this relationship is inconsistent across studies [363, 364]. Investigations on human participants have provided valuable insights regarding the role BDNF plays in neurological function but acquisition of blood by this invasive method requires a trained phlebotomist, a sterile environment, and specialist equipment. Moreover, participants are commonly required to fast overnight before blood draws, repeated measures sampling typically has to take place at the same time of day, and samples are processed and stored or analysed immediately after collection [365, 366]. These logistical constraints, coupled with the prevalent aversion to needles within the general population [367], increases the difficulty of conducting experiments to understand whether measurement of BDNF concentrations may translate to real-world clinical settings.

BDNF protein concentrations can also be measured in saliva collected by passive drool [368, 369], and has been used in several previous investigations [370-374]. The relationship between salivary-BDNF concentrations and levels of BDNF within brain tissue does not appear to have been studied. The

correlation between salivary-BDNF and serum/plasma-BDNF has been previously investigated although the results are mixed with studies reporting weak relationships in healthy samples [369, 373, 375], while a moderate relationship has been observed in individuals who suffered mild traumatic brain injury [373]. Given that functional outcomes in neurological conditions appear to relate to blood-based measures of BDNF despite the unclear relationship between these measures and levels of BDNF within the brain; investigating whether salivary-BDNF could be a method of assessing these relationships is justifiable. This non-invasive method could be a means to quantify BDNF more easily in populations of interest presenting for clinical assessment. But to overtake blood as the biological sample of choice for measuring BDNF concentrations, in addition to being non-invasive, saliva sampling would need to demonstrate practical advantages for clinical research. For example, sampling BDNF in functioning clinical environments would be more manageable, and more useful, if saliva samples could provide a valid and reliable measurement of BDNF without the need to strictly control for potential confounding factors (i.e., time of sample collection or when the subject last ate). Many clinics do not have an onsite laboratory where samples can be processed for long-term storage immediately after collection. Some analytes measurable in saliva remain stable at room temperature for weeks or months whereas others degrade rapidly without the addition of protease inhibitors followed by immediate storage [376, 377]. Because some clinics are not equipped to collect BDNF via blood samples, investigations evaluating the relationship between BDNF and various neurological conditions could take place in a greater number of clinics if salivary-BDNF were to remain stable when kept on ice for several hours until it can be transported for storage at an off-site laboratory. If saliva were to be a valid and practical means of measuring individual BDNF changes in neurological populations then limited variation, or at least consistent trends in intra-individual variation, in salivary-BDNF would be required to accurately compare concentrations observed at one timepoint to another. To date, no studies have investigated intra-individual variation in salivary-BDNF concentrations when sampling takes place at different times of day during clinical operation hours. Additionally, previous research has not evaluated whether levels of salivary-BDNF remain stable if not immediately stored after collection.

Aim

The aim of this pilot study was to develop methodologies and provide preliminary evidence regarding how timing of sampling and delays in storage influence intra-individual salivary-BDNF protein concentrations. This study investigated intra-individual salivary-BDNF concentrations when:

- samples are collected at different times on the same day within clinical operation hours (09:00h, 11:00h, 13:00h, 15:00h, 17:00h);
- samples are collected when fasted versus after eating;
- there is delay in sample processing and storage (10 hr, 5 hr, 3 hr).

If limited or similar trends in intra-individual variation are observed between these factors and salivary-BDNF concentrations it would provide encouraging evidence towards potentially translating the use of

salivary-BDNF as a practical biomarker to advance clinical practice and improve individualised care in the future.

Methods

Research design

To evaluate how timing of sampling and delays in storage influence salivary-BDNF protein concentrations two experiments were conducted as part of this pilot study. Institutional ethical approval (AUTEC 18/437) was obtained, and this study was conducted according to the ethical standards of the Declaration of Helsinki. Participants provided written consent to having their data used for research and publication. Figure 7.1 provides a diagram depicting the conditions under which samples were collected and processed for each experiment.

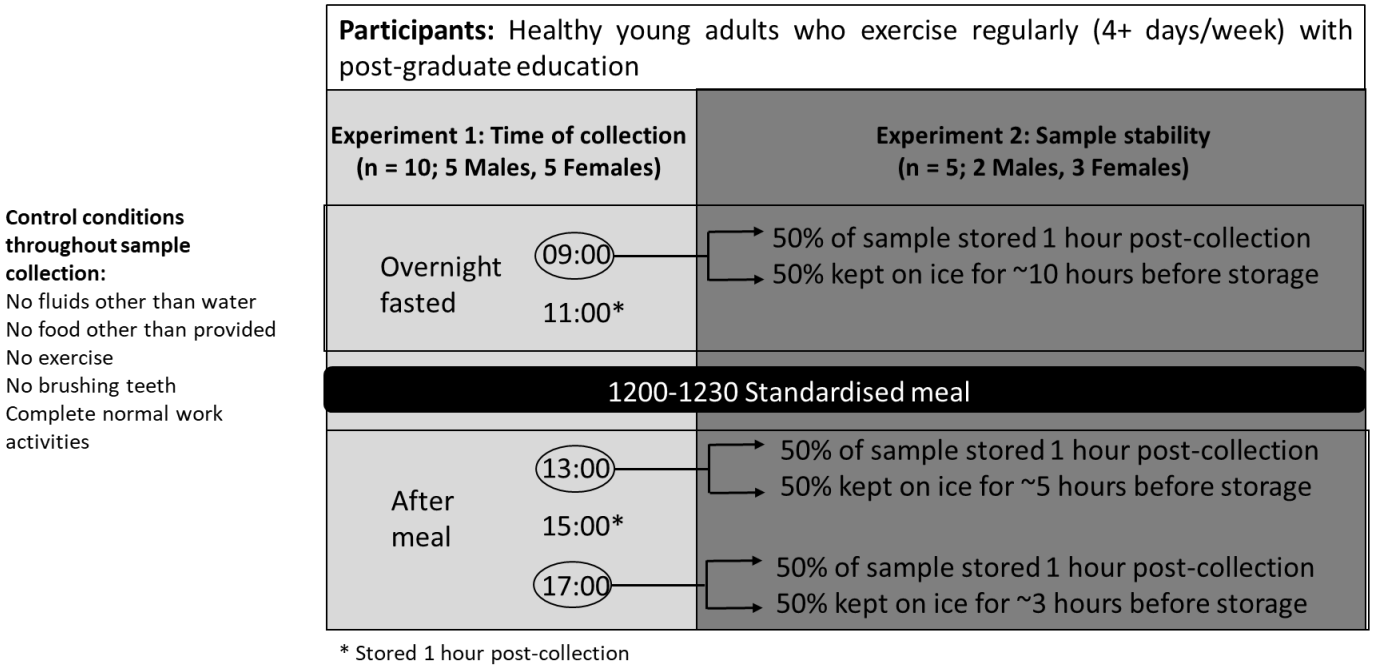


Figure 7.1 Diagram depicting study design for Experiment 1 and Experiment 2.

Experiment 1 was designed primarily to assess how the time of day at which saliva samples were collected contributes to intra-individual variation in salivary-BDNF concentrations. Since the aim of this pilot study was to evaluate the practicality of using saliva to measure BDNF in clinical settings, samples were collected at two-hour intervals between 09:00h and 17:00h to represent clinical operation hours. Experiment 1 also assessed the influence of feeding on salivary-BDNF concentrations by collecting samples while overnight fasted, then providing a standardised meal, followed by further sampling. All samples for Experiment 1 were stored as soon as possible after collection and processing. Experiment 2 evaluated the stability of salivary-BDNF when immediate processing and storage is not possible, by using a portion of the sample from experiment 1. A subset of samples collected in Experiment 1 were kept on ice for several hours to

replicate scenarios where samples are collected over the course of a clinical day and then transported to a laboratory for processing and storage. Great care was taken during all handling and processing of samples to ensure that samples were not accidentally mishandled so that samples for Experiment 1 ended up in Experiment 2 and vice versa.

Participants

Participants in this study were a convenience sample of healthy young adults who are completing/or have completed post-graduate education. The average age and years of education for participants in this study were 28.67± 2.18 and 19.33± 1.5 years, respectively. All participants reported regularly engaging (4+ days/week) in moderate to intense physical activity/exercise. Ten participants (five males, five females) participated in Experiment 1. Half of the participants in Experiment 1 were able to provide enough saliva so that half the sample could be stored immediately, while the other half was left on ice for Experiment 2. Budgeting constraints determined the amount of consumable materials that could be purchased and subsequently limited sample sizes for each experiment.

Procedures

Participants were instructed to fast from 22:00h the night before participation, and to brush their teeth no later than 07:00h on the day of data collection as toothpaste/mouthwash may disrupt quantification of salivary-BDNF [365, 366]. Participants arrived on the day of data collection overnight fasted and remained fasted for the first three hours of data collection. Participants received a standardised meal for lunch on the day of collection between 12:00h and 12:30h. The meal provided was the only food participants consumed during data collection, they were able to self-select the number of portions they ate to ensure satisfactory satiation to make it through the day. Additionally, participants only drank water throughout data collection and abstained from all other beverages (i.e., juice, milk, coffee, tea, caffeinated drinks, etc.). Participants did not exercise prior to or during data collection as acute exercise influences BDNF concentrations [112, 378].

Before providing each saliva sample, participants were asked to take a small sip of water, to swish this water around their mouth for a few seconds, and then to swallow the water. This sip + swish technique was implemented to clear any food debris that may have been in the mouth [376]. Saliva samples were collected via passive drool. Participants were asked to produce the sample with their head slightly tilted forward, whilst keeping eyes open and limiting orofacial movement to a minimum. Once enough saliva had pooled in the mouth, a small plastic straw and gravity was used to collect the sample into microcentrifuge eppendorfs. Participants were asked to provide 1 ml of saliva in 1.5 ml, however, some participants had difficulty producing this much saliva, so the minimum sample required for analysis was 0.5 ml. A total of five saliva samples were collected from each participant in this fashion. After each sample was collected it

was placed on ice until processing and storage. Between saliva sampling timepoints participants were free to complete regular desk/laboratory-based work activities.

Sample processing and analysis

After each sampling timepoint, saliva samples were immediately transported on ice to the laboratory for pre-storage processing. Samples from participants who were able to provide 1 ml of saliva were separated into two eppendorfs using a pastette. For Experiment 2, one eppendorf was kept on ice for several hours (as described in Figure 7.1) to evaluate sample stability under delayed storage conditions. All remaining samples were centrifuged at 4000 rpm for 20 minutes at 4^{o}C. Following centrifugation 250 µl aliquots were pipetted into new eppendorfs, and protease inhibitor cocktail (Sigma-Aldrich) was added to a final dilution of 1:100. Samples were briefly vortexed to ensure mixing of protease inhibitor, and then stored at -80^{o}C until batch processing once all samples were collected from all participants. The same process was repeated for the samples kept on ice for Experiment 2 until the end of the day of data collection. A detailed plate map was strictly followed throughout all steps of the ELISA to prevent mishandling of samples. All samples for each participant, including samples used for Experiment 1 and 2, were run on the same plate.

Previous studies have raised concerns about the validity of BDNF measurement with both saliva and serum using commercial kits [362, 369, 370]. Therefore the optimised sandwich ELISA technique developed and described by Mandel et al. [369] to detect BDNF in saliva was utilised. 100 µl of monoclonal mouse anti-human BDNF (Merck-Millipore), diluted to 1 µg/ml in filter-sterilized phosphate buffered saline (PBS) at a pH of 7.4 was added to a 96-well microtiter plate (Nunc Maxisorp) and left to incubate at 4^{o}C overnight. The following day, samples were removed from -80^{o}C to thaw on ice. The plate was manually washed three times with tri-buffered saline (TBS) plus 5% tween (TBST), soaking for one minute between each wash. Next, the plate was blocked with 300 µl of 3% bovine serum albumin (BSA) in 0.05% PBST for 2.5 hours at room temperature. During the blocking period the thawed samples were spun in a microcentrifuge for two minutes at 13,400 rpm and then 200 µl of supernatant was moved to a new eppendorf. These samples were acidified to 3.0 pH for 20 minutes, after which samples were re-neutralized. For batch processing 3.1 µl and 3.6 µl of 1M HCl and 1M NaOH were used for the acidification and neutralization steps, respectively. Once re-neutralized, 16 µl of the sample was diluted in 1% BSA buffer in PBST to a ratio on 1:16. Concentrations of BDNF detected within samples were compared to standards, ranging from 15.63 to 500 pg/ml using a full-length homodimeric recombinant BDNF (Peprotech), diluted in the same buffer as the samples. The original protocol by Mandel et al. diluted samples to a 1:4 ratio, however, during piloting many of our samples were well above the top standard indicating further dilution was required. Once blocking was complete the plate was washed five more times, and 100 µl of each sample/standard was added to the plate in duplicate. The plate was incubated for one hour at room temperature on a plate shaker, and then moved to 4^{o}C overnight. The next morning the plate was washed five times, then 100 µl of

poly-clonal chicken anti-human BDNF (2.5 µg/ml; Promega) was added to each well and left at room temperature for 2.5 hours. Afterwards, the plate was again washed five times followed by the addition of 100 µl of anti-chicken IgY-HRP (1 µg/ml; Promega) to the wells and one hour of room temperature incubation. Following this last incubation, the plate was washed a final five times, after which 100 µl of room temperature TMB One solution (Promega) was added to each well for 10 minutes. Afterwards, 100 µl 1M HCl was added to stop the reaction, and the assay was read at 450 nanometres (nm). The amount of BDNF (pg/ml) was calculated using a linear regression fitted to the standards of each plate [369]. R-squared values of 0.94 and 0.92 were obtained from standard curves for the two plates analysed and the mean coefficient of variance for all samples was 0.10.

Exploratory analysis

Given the preliminary nature of this study, data were explored to evaluate if similar trends in intra-individual variation of salivary-BDNF concentrations were observed between participants for Experiments 1 and 2. Salivary-BDNF concentrations (pg/ml) were reported for each participant and within-participant coefficient of variance (CV) was calculated for both experiments. Individual results for Experiment 1 were visualised using point plots and the overall group trend was visually evaluated by fitting a trendline with 95% confidence intervals using a locally estimated scatterplot smoothing (LOESS) technique. For Experiment 2, the percent change between samples processed and stored under delayed conditions was calculated against the same samples when stored within an hour of collection.

Results

All participants reported compliance regarding overnight fasting, tooth brushing, and abstaining from exercise as described in above in the procedures section. No participants reported smoking or consuming any alcohol the night prior to data collection. Participants indicated 7 hours and 17 minutes± 54 minutes of sleep the night before participation and woke an average of 124± 52 minutes before providing their first sample. Raw data for all participants is provided in Table 7.1 accompanied by the within-participant mean, standard deviation and CV for Experiment 1, and the percent change CV presented for participants who provided enough saliva to be included in Experiment 2. Of the 50 samples collected from all participants for Experiment 1, salivary-BDNF concentrations were above the limit of detection (LOD) for the ELISA protocol for 43 (86%) samples. Five of the seven samples below the LOD were all from one participant, thus data for this participant was not included in Figure 7.2 and Figure 7.3.

Table 7.1 Within-participant results and descriptive statistics for Experiments 1 and 2.

Sampling timepoints - Experiment 1									Storage delays and sample stability - Experiment 2			
ID	**900**	**1100**	**1300**	**1500**	**1700**	**Mean**	**SD**	**CV**	**10 hr (% change)**	**5 hr (% change)**	**3 hr (% change)**	**% change CV**
1	8110	4881	4851	10014	7797	7131	2235	0.31	-	-	-	-
2	9885	5323	2403	6745	8286	6529	2867	0.44	-	-	-	-
3	LOD	LOD	LOD	LOD	LOD	-	-	-	-	-	-	-
4	14414	5103	10123	11863	10376	10376	3405	0.33	-	-	-	-
5	LOD	6310	8340	6909	7053	7153	854	0.12	-	-	-	-
6	5017	3607	3546	1725	3910	3561	1185	0.33	5807 (16)	4763 (34)	4301 (10)	0.62
7	712	3374	1563	12549	5615	4727	4780	1.01	LOD (-100)	LOD (-100)	507 (-91)	0.05
8	9426	9411	7792	10858	8473	9192	1157	0.13	9851 (5)	9113 (17)	9815 (16)	0.53
9	6665	2869	1191	235	LOD	2740	2834	1.03	2909 (-56)	2159 (81)	LOD (0)	8.27
10	5089	3730	11969	5558	6764	6622	3180	0.48	5541 (9)	3885 (-68)	6558 (-3)	2.00

Note: Shaded cells represent corresponding values when a given sample was stored within one hour for Experiment 1 versus a subset of the same sample used for Experiment 2 that was kept on ice for the indicated amount of time before storage. Units are pg/ml.

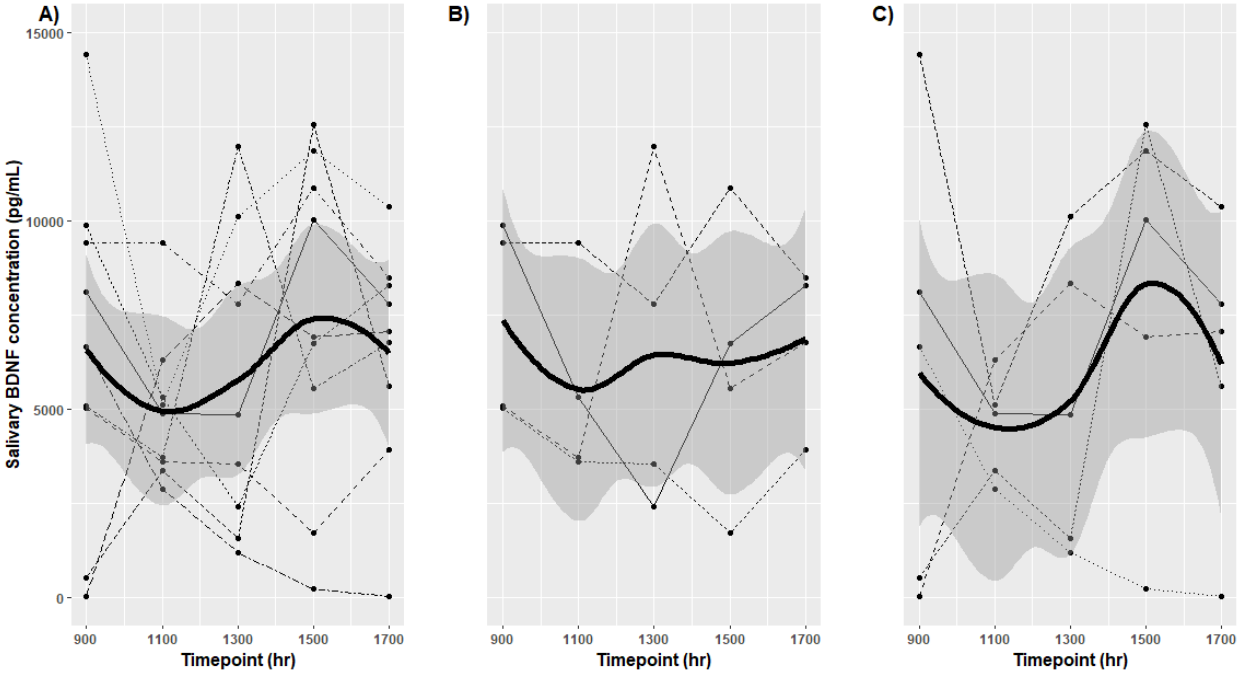


Figure 7.2 A) Intra-individual changes in salivary-BDNF concentrations sampled at different time points for all participants (n = 9) with a LOESS overlay; B) Males only; C) Females only.

Experiment 1 – Timing of sample collection

A considerable amount of intra-individual variation was observed in salivary-BDNF concentrations when sampled at two-hour intervals between 09:00h and 17:00h as shown by the large standard deviations (minimum: 854 pg/ml; maximum: 4780 pg/ml) and CVs (minimum: 0.12; maximum: 1.03) across participants in Table 7.1. At a group level, the LOESS plot in Figure 7.2 (panel A) appears to indicate that salivary-BDNF levels measured while overnight fasted are higher earlier in the morning and fall as the morning goes on; and that concentrations increase in the mid-afternoon after feeding but then return to levels similar to those observed at 09:00h by 17:00h. However, the large confidence bands around the LOESS plot paired with the point plots for each participant indicate no consistent inter-individual trend in intra-individual changes in salivary-BDNF between the hours of 09:00h and 17:00h. Similarly, the individual results do not show a consistent trend in salivary-BDNF changes after participants consumed a standardised meal between 12:00-12:30h. The inconsistency in salivary-BDNF concentrations collected at different times on the same day remained when males and females were evaluated separately (Figure 7.2 panel B and C, respectively).

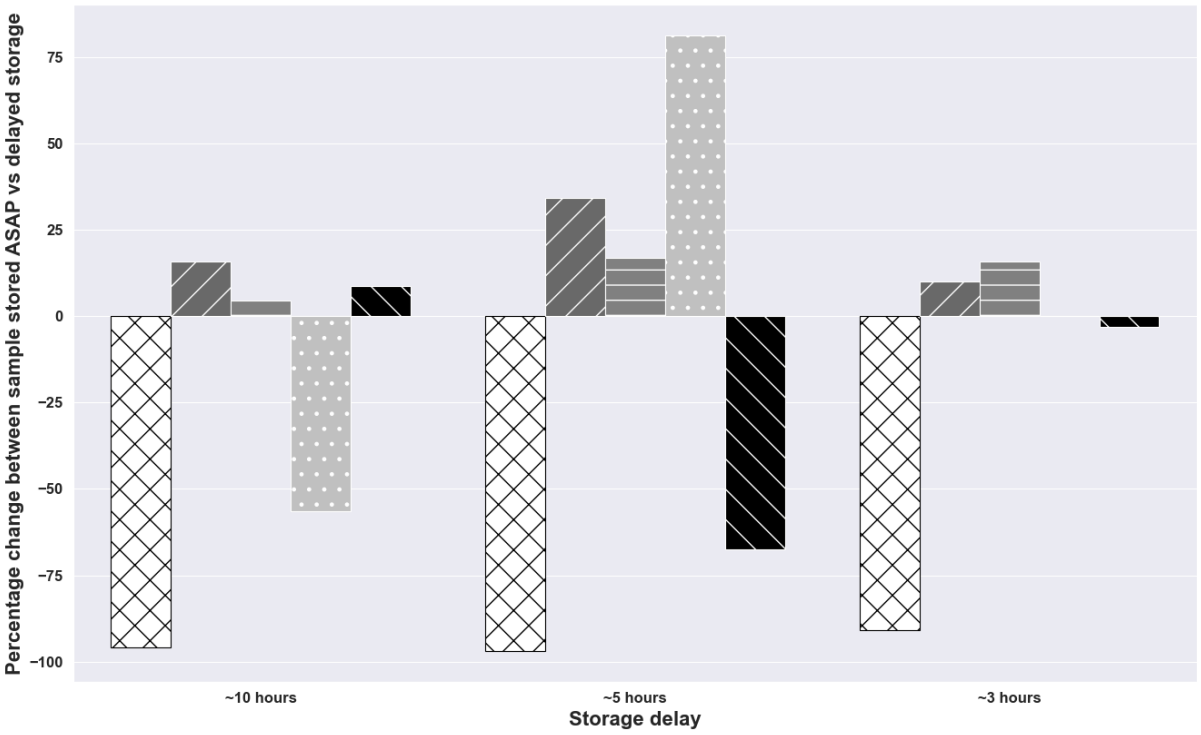


Figure 7.3 Percent change between samples stored 1-hour post-collection and samples kept on ice for several hours before storage for each participant in Experiment 2.

Experiment 2 – Sample stability

Experiment 2 yielded similar results to Experiment 1, with a large amount of intra-individual variation in salivary-BDNF stability shown using CVs in Table 7.1 and visually in Figure 7.3. There appeared to be no consistent trend in stability of salivary-BDNF under different storage conditions between the five participants in Experiment 2. One participant saw a sharp degradation in salivary-BDNF at all delayed storage timepoints, with both decreases and increases in BDNF observed for some participants depending on timepoint, and others showing modest increases in concentrations after delayed storage.

Discussion

The aim of this pilot study was to present methodologies and preliminary findings regarding trends in intra-individual salivary-BDNF variations from healthy participants to understand if saliva (collected via passive drool) may be a practical biological sample for the measurement of BDNF in clinical populations. Although the sample size was low, our results do not indicate any consistent trends between participants in regard to intra-individual salivary-BDNF variation related to time of sampling or sample stability.

These findings from healthy participants suggest that measurement of BDNF protein concentrations via saliva is not a valid or practically advantageous means of accurately comparing intra-individual changes in

BDNF from one timepoint to another. A recent review highlighted several methodological factors contributing to statistical and sampling errors in studies evaluating saliva biomarkers in healthy volunteers [366]. The authors described a detailed protocol to minimise the risk of these errors including: screening questionnaires, detailed inclusion/exclusion criteria before sampling, grouping subjects by flow rate, and strict controls during saliva collection [366]. The current study intentionally did not adopt these recommendations because such strict methods prevent research from taking place under ecologically valid conditions. For example, it is recommended that participants rinse their mouth with water at least 10 minutes before saliva collection [365], however, adherence from neurological patients presenting for care in busy clinical environments to this recommendation when collecting saliva samples is unlikely. For this reason, participants in the current study were instructed to rinse their mouth immediately before saliva collection and to provide the sample after all the water was swallowed. This compromise may explain why 14% of samples collected were below the LOD threshold for BDNF, as it is possible that these samples did not contain saliva, rather residual water that had not been swallowed by the participant. True absence of BDNF within saliva due to low concentrations and/or Val66Met polymorphism are also possible explanations [129], although, these seem less likely since two of the samples below the LOD were collected from separate participants who demonstrated BDNF concentrations at all other timepoints evaluated. This example presents a considerable shortcoming of measuring BDNF, and other protein biomarkers, via passive drool under clinical conditions; even if necessary controls are put in place there is still a chance that what we are trying to measure will not be present within the collection tube. While collecting saliva by passive drool offers a non-invasive means of measuring BDNF this advantage appears to be offset by the risk of sampling error during collection [366].

If saliva does not offer practical advantages for measuring protein biomarkers over blood for clinical research, and still requires strict methodological controls during sampling, it raises the question why use saliva in the first place? While blood draws are invasive, they guarantee that the protein of interest will be collected (given it is present in blood to begin with). The biggest hurdle facing research using blood draws is the fear of needles. Approximately 20% of adults in the USA avoided vaccination due to fear of needles [379], and a recent meta-analysis reported that 18-27% of hospital employees and healthcare workers avoid vaccination for the same reason [367]. Furthermore, the meta-analysis indicated that most young children and 20-50% of adolescents fear needles [367]. If adults working in healthcare sectors avoid vaccination due to their fear of needles there is very low likelihood that these individuals, let alone children, would consent to participate in voluntary research involving blood draws. A recent thesis [380] quantified BDNF from capillary-drawn blood samples acquired by finger prick using a lancet. Perhaps this method could serve as a practical middle ground for the collection of blood in clinical settings because it would not require a trained phlebotomist or a needle, and the normalization of finger pricks to measure blood sugar, lactate, etc. may mean patients might be more willing to volunteer for research [381, 382].

Alternatively, microRNAs – non-coding RNAs that play a key role in gene expression and regulation of neurotrophins such as BDNF [67] – can be collected and measured easily in saliva using swabs and appears to be a new and more promising technique than measuring protein concentrations via passive drool [68]. Encouraging findings from studies evaluating the utility of microRNAs to serve as diagnostic biomarkers for mild traumatic brain injury suggest that patterns in peripheral microRNAs may overcome limitations associated with protein biomarkers [68, 69]. Future work investigating the validity and reliability of quantifying BDNF protein concentrations in capillary blood or gaining insight into BDNF expression through related salivary-microRNAs could lead to greater understanding intra-individual changes in peripheral BDNF within neurologically compromised patients. Advancement of individualised clinical management for these debilitating conditions may be possible through greater knowledge of how intra-individual changes in BDNF relate to clinical outcomes.

Limitations

There are several limitations to consider for our study. First, the low sample size prevents any concrete conclusions, but pilot studies are necessary to justify whether further allocation of time and resources is reasonable based on preliminary findings, which does not appear to be the case in this scenario. Second, strict adherence to the protocol described by Bhattarai et al [366] may have yielded clearer trends in salivary-BDNF concentrations in relation to time of sampling and storage. Even if this were the case, results produced using strict laboratory protocols are unlikely to be reproduced in ecologically valid environments. Third, all samples were collected on the same day, future research evaluating blood-based BDNF concentrations or salivary-microRNAs related to BDNF expression should also evaluate reproducibility of findings across multiple days. Lastly, linear regressions fit to the standards were used to estimate concentrations of BDNF within the saliva samples collected. Higher R-squared accuracy of these standard curves could have been obtained using four parameter logistic (4PL) regression, however sample concentrations outside the range of the standards used cannot be approximated using this method. Adjustments in sample dilution from 1:4 to 1:16 brought most samples within the range of the standards. A minority of samples were still above the top standard, and further dilution would have pushed other samples below the LOD. Since accurate standard curves were obtained using linear regression, we adopted this approach of extending the regression equation to approximate sample concentrations above the top standard to avoid losing this data. This approach was not applied to samples below the LOD threshold, as it is possible that the true value was in fact zero; whereas samples above the top standard were simply beyond the upper limit of the assay procedure to some degree. It is possible that extrapolating above the top standard for some samples could have introduced error when calculating salivary-BDNF concentrations.

Conclusion

This pilot study did not provide evidence to justify using saliva as a valid and practical means of evaluating intra-individual variation in BDNF protein concentrations. No consistent trends in salivary-BDNF concentrations were observed in relation to time of sampling or sample stability as evident in the high degree of variation within and between participants. These results should not discourage future research into the potential clinical applications of BDNF as a biomarker to advance understanding of neurological conditions; rather our findings highlight that the best method to measure BDNF in ecologically valid environments remains elusive. Future studies should replicate the experiments within this study using venous and capillary blood samples or salivary-microRNAs related to BDNF expression to determine if clearer intra-individual trends in BDNF variation can be observed.

Personal development as a researcher resulting from Chapter 7

- How to design an experimental study to represent clinical conditions.
- Acquisition of wet lab skills.
- Learning how to execute an optimised ELISA procedure.
- Advantages and disadvantages of optimised vs kit-based ELISAs.
- Advantages and disadvantages of sampling biomarkers using blood and saliva.
- Methodological trade-offs between conditions that promote precise measurement of a biomarker versus conditions that are representative of where the biomarker could offer value.

Link between Chapters 7 and 8

Chapter 7 highlighted potential issues with sampling error when measuring BDNF concentration in saliva collected via passive drool under clinically representative conditions. While the sample size was limited Chapter 7 suggested that salivary-BDNF cannot be used to make intra-individual comparisons from one clinical timepoint to another for SOBI patients. Saliva samples were collected for Chapter 8 from a cohort of SOBI patients presenting for treatment at a specialised SOBI clinic since no studies have investigated whether salivary-BDNF could serve as a non-invasive method to understand differences in SOBI patient recovery outcomes. Chapter 8 specifically attempted to provide initial evidence for the following three hypotheses: 1) BDNF concentrations would be lowest upon initial clinical presentation, and that increases in BDNF would be observed at later timepoints; 2) BDNF and symptom burden would share a negative relationship within and between timepoints, with higher BDNF concentrations associated with lower symptom reports; and 3) higher BDNF concentrations would be observed at initial assessment and the first follow-up in participants who became asymptomatic in ≤14-days compared to those who took >14-days to recover. Group-level timepoint analyses were conducted in Chapter 8 based on the findings of Chapter 7 under the assumption that sampling-related variations in salivary-BDNF may balance out from timepoint to timepoint at a group-level and reveal preliminary trends between symptom burden and BDNF concentrations. If salivary-BDNF acquired under ecologically valid conditions were to be related to symptom reports, it could become a non-invasive and practical means to make inferences about how the underlying neuroplastic environment explains differences in recovery outcomes. Furthermore, this would provide an objective neurophysiological biomarker to track SOBI recovery, which could be used in clinical investigations evaluating the potential benefit of nutritional interventions for SOBI patients.

Chapter 8: Does salivary-BDNF relate to symptom burden over the course of clinical recovery following sport-originated brain injury?

This chapter comprises the following paper submitted to *Neurorehabilitation and Neural Repair:* **McGeown, J. P.**, Hume, P. A., Quarrie, K.L., Theadom, A., Kara, S., & Dulson, D.K. Does salivary-BDNF relate to symptom burden over the course of clinical recovery following sport-originated brain injury?

Author contribution

McGeown, J. P. 80%, Hume, P. A. 5%, Quarrie, K.L. 5%, Theadom, A. 5%, Kara, S. 5%, Dulson, D.K. 5%.

Overview

Background. There is limited understanding of how the brain's neuroplastic environment, assessed via brain-derived neurotrophic factor (BDNF), contributes to differences in symptom resolution and clinical recovery trajectories following mild traumatic brain injuries (mTBI). **Objective.** To understand how salivary-BDNF relates to symptom reports for sport originated brain injury (SOBI) patients at clinical recovery timepoints. **Methods.** Participants received clinical management for mTBI in line with international recommendations. At each clinical timepoint participants completed the Sport Concussion Assessment Tool symptom scale and provided a saliva sample. An optimised enzyme-linked immunosorbent assay was used to quantify salivary-BDNF protein levels. Exploratory data analysis evaluated trends in how BDNF changed over clinical recovery and how BDNF related to subjective symptom burden. **Results.** 167 saliva samples were collected from 67 participants. There were no clear relationships between salivary-BDNF concentrations (measured via passive drool under ecologically valid clinical conditions) and SOBI-related symptom reports measured at four clinical recovery timepoints. **Conclusion.** Sampling error appeared to be an inherent risk of quantifying BDNF protein in saliva under clinically realistic conditions. There is a need to identify methods that balance accurate quantification of BDNF with clinical practicality to investigate the clinical utility of BDNF as a biomarker of recovery in mTBI patients.

Introduction

An estimated 27 million traumatic brain injuries (TBIs) occur globally each year [23], and approximately 95% of these TBIs are non-fatal [383] . The majority of TBI survivors sustained a mild traumatic brain injury (mTBI) that did not lead to skull fracture or positive neuroimaging, accompanied by a Glasgow Coma Score between 13-15, and associated with symptom complaints [19, 27]. Recovery outcomes are highly variable following mTBI, with patients experiencing spontaneous resolution of symptoms and reintegration into normal daily activities within days of the injury, through to experiencing debilitating functional and behavioural deficits for weeks, months, or years post-mTBI [33]. Age, female sex, preinjury history of mental health or migraines, ADHD and learning disabilities, and acute/subacute symptom severity have all been associated with worse mTBI recovery outcomes [39]. However, there are no definitive explanations about how the patient's underlying neurophysiological environment contributes to why some patients experience rapid symptom resolution and clinical recovery while the rest experience prolonged complications resulting in reduced quality of life and that place a significant burden on the healthcare system [35-37].

Neuroplasticity is the ability of individual neurons and complex neuronal networks to adapt and reorganise synapses throughout life and after CNS injury [129, 384]. Neuroplasticity could be an explanation for the heterogeneity of mTBI recovery trajectories. Neuroplasticity has been described as the biological substrate for neurorehabilitation [385], and is mediated by a family of homologous neuropeptides termed neurotrophins [143, 386]. In addition to neuroplasticity, neurotrophins play key regulatory roles in neurogenesis, neuronal survival, and memory consolidation by promoting long-term potentiation [129, 143]. Brain-derived neurotrophic factor (BDNF) is the most widely studied neurotrophin involved in these neurophysiological functions and has been extensively researched in human and animal studies involving healthy and neuropathological populations [129, 386]. Compared to healthy controls, differences in serum/plasma BDNF concentrations have been reported for patients with neurodegenerative conditions such as Alzheimer's disease and psychiatric disorders including depression and schizophrenia [129]. Intervention strategies such as environmental enrichment and exercise programs have been associated with improved performance on functional outcome measures related to increases in blood-based BDNF concentrations [129]. Despite research evaluating BDNF in humans with some neurological conditions, relatively few studies have examined the diagnostic and prognostic potential of BDNF for patients with TBI [65, 373, 387-392]. Collectively, the evidence for the utility of BDNF appears to be mixed, although nearly all these studies had small sample sizes and notable methodological limitations.

The most encouraging discriminative and prognostic results for BDNF has arisen from a multi-centre study [65] with 311 TBI cases and 150 controls. BDNF was measured via serum from excess clinical blood samples taken from patients presenting with mild, moderate, or severe TBI on the same day as their injury and who

met criteria for a head CT scan [65, 393]. Day-of-injury serum BDNF concentrations discriminated between cases with TBI and controls with a very high degree of accuracy (0.94-0.96 AUC). Recovery outcomes at six months were assessed in a sub-cohort of 76 participants. Cases of all severities, who had day-of-injury BDNF concentrations below the 1st percentile of the controls, were at a higher odds of incomplete recovery at six months compared to cases above this threshold. mTBI patients with BDNF levels below this limit had a higher odds of incomplete recovery than patients with moderate and severe TBI. The authors recommended that future research assess how longitudinal changes in BDNF related to clinical improvements and recovery after TBI. It was recommended that future efforts evaluate BDNF levels after sport-originated brain injury (SOBI) because of the well documented relationship between exercise and BDNF [112]. Replication of the conditions under which BDNF was collected in this study are likely to be challenging in many clinical settings for several reasons. First, all participants presented to hospital on the same day as their injury, however many mTBI patients (particularly with SOBI) do not seek medical attention until several days post-injury due to delayed symptom onset [43] or non-resolution of initial symptoms [27]. Second, while not clearly stated, most or all the participants were likely inpatients since they all met criteria for CT scan which revealed that many had an intracranial abnormality. However, a high proportion of patients with mTBI do not meet criteria for a CT scan reducing representativeness of their sample [394]. The resources available within hospitals/inpatient facilities often exceed those of outpatient clinics. Lastly, both reasons allowed BDNF to be quantified from excess serum samples that were collected for other clinical purposes. Logistical constraints and the prevalent aversion to needles [367, 379] within the general population makes it difficult to conduct research in real-world outpatient settings to investigate the potential utility of biomarkers such as BDNF. Blood-based biomarkers are widely used in many clinical applications but only after an abundance of research has demonstrated a high degree of validity, reliability, and clinical utility.

BDNF can also be measured in saliva [368, 369] and a moderate relationship between salivary-BDNF and serum-BDNF concentrations has been previously reported for participants with mTBI [373], although this relationship appears to be weak in healthy individuals [369, 373, 375]. Interestingly, blood-based BDNF concentrations relate with functional outcomes in individuals with neurological conditions, even though the correlation between serum/plasma BDNF and levels of BDNF within brain tissue is unclear [363, 364]. Consequently, it seems plausible that salivary-BDNF may represent a practical alternative to allow greater sampling to expedite our knowledge of how BDNF changes in relation to subjective symptom reports over the course of clinical recovery after mTBI/SOBI. Greater understanding of these relationships could provide a window into the neuroplastic environment of individuals with mTBI and may advance our ability to manage and rehabilitate these patients more effectively.

The aim of the current study was to understand how salivary-BDNF related to symptom reports for sport-originated mTBI patients at multiple timepoints over their clinical recovery.

We hypothesised that: 1) BDNF concentrations would be lowest upon initial presentation to the clinic and would increase over clinical recovery; 2) Higher BDNF concentrations would be associated with lower symptom reports; 3) Patients who became asymptomatic in ≤14-days would have higher BDNF concentrations at initial assessment and the first follow-up compared to patients who took >14-days to recover.

Methods

Research design and participants

This study was conducted according to the ethical standards of the Declaration of Helsinki and both institutional (AUTEC 18/374) and health and disability committee (HDEC 18/NTA/108) ethical approvals were obtained. Participants provided written consent to having their data used for research and publication. Participant assent and parental consent was acquired for participants <16 years old. All participants' data were de-identified prior to extraction/data analysis to ensure confidentiality.

Participant data were collected from single specialist clinic between April 2019 and July 2019. The cohort of patients diagnosed with SOBI were followed from their initial clinical presentation through one or more follow-up appointments until they achieved clinical recovery criteria. All participants received usual clinical care according to a previously described protocol [315].

Participant self-reported symptom burden was collected at the beginning of each appointment using the Sports Concussion Assessment Tool (SCAT-5 [73]) symptom scale. Participants used a Likert scale from 0 (no symptom) to 6 (severe symptom) to indicate which of 22 symptoms commonly associated with SOBI they were experiencing and the respective severity. The total number of symptoms with a severity >0 indicated the Positive Symptom Total (PST; maximum of 22), and the sum of the severities provided a Symptom Severity Score out of 132. Five additional composite scores (migraine, cognitive-emotional, sleep-emotional, neurological, and undefined feelings) [395] were calculated for only 51/67 (76%) participants due to the clinic database limitations.

Upon initial clinical presentation the supervising physician conducted a thorough history and physical examination. Participants received education, written guidance, and individualised management according to international recommendations. Follow-up appointments were scheduled every 7-14 days to evaluate the status of the participant's recovery to determine if they were ready to begin a graduated return to play protocol or if their treatment plan required modification. Participants were monitored in this manner until they met clinical recovery criteria defined as: (1) asymptomatic (defined as Symptom Severity Score ≤ 5 for males and ≤ 6 for females [83]); (2) demonstrated exercise tolerance, and (3) any abnormalities identified during the initial physical examination had resolved [315].

Unstimulated saliva samples were collected via passive drool from patients (letting saliva pool in their mouth while limiting orofacial movement) at the end of each clinical appointment. Once enough saliva had pooled in the mouth, a small plastic straw and gravity were used to collect the sample into a microcentifuge eppendorfs. It was difficult for some participants to provide 1 ml of saliva without bubbles, so to avoid disturbing clinical flow the minimum sample required for analysis was 0.5 ml. After collection, samples were immediately placed and kept on ice until transported to an off-site laboratory for processing and storage.

Factors that could affect the BNDF concentration including the time of collection, when the participant last ate, drank fluid other than water, brushed their teeth, exercised, and when they woke/how many hours of sleep they got the night before sampling were recorded so they could be considered during statistical modeling.

Since data collection took place in a functioning clinical environment it was not possible to standardise the timing of appointments across participants based on number of days post-injury. For this reason, data were aggregated into timepoints with Timepoint A representing the initial clinical assessment and Timepoint B, C, and D representing the first, second, and third follow-up appointments, respectively.

The research protocol was specifically designed to be easy to implement in a clinical setting (as would be the case if BDNF was found to be a useful biomarker) to avoid disrupting clinical flow and over-burdening participants. The need for flexibility, however, did mean that a saliva sample was not always possible for every participant at every clinical timepoint. Participant data were included in this study if a saliva sample was collected and symptom burden measured at Timepoint A combined with at least one further timepoint, and if the participant remained within the clinical protocol until discharge. These compromises preserved clinical flow and provided enough data to evaluate our three hypotheses.

Salivary-BDNF processing

At the end of each clinical day, samples kept on ice were then transported to an off-site laboratory for processing in the same order in which they were collected. Samples were centrifuged at 4000 rpm for 20 minutes at 4^{o}C and then split into 250 µl aliquots where a protease inhibitor cocktail (Sigma-Aldrich) was added to a final dilution of 1:100. Samples were briefly vortexed and then stored at -80^{o}C until batch analysed in duplicate according to an optimised sandwich Enzyme-Linked Immunosorbent Assay (ELISA) procedure [369]. The amount of BDNF (pg/ml) was calculated using a linear regression fitted to the standards of each plate [369]. Standard curves for the eight plates required to analyse these samples produced R^2 values ranging from 0.92 to 0.99, and the mean coefficient of variance for the 167 samples analysed in duplicate was 0.06, indicating valid and reliable execution of the ELISA procedure. However, 11 participants had at least one sample where salivary-BDNF was below the limit of detection (LOD; 60 pg/ml)

of the ELISA. In total six, seven, and three samples were below the LOD at Timepoint A, B, and C, respectively.

Statistical analysis

Medians and interquartile ranges were calculated for all demographic and clinical variables due to highly skewed distributions. The exception was days post-injury presented as mean ±SD.

Trends in timepoint-to-timepoint changes in salivary-BDNF concentrations (Hypothesis 1) were assessed using point plots. The relationship between salivary-BDNF concentrations and symptom burden scores were plotted using repeated measures correlation plots [396] with R^2 calculated for each timepoint (Hypotheses 1 and 2). Boxplots were used to inspect if participants who tended to become asymptomatic faster (≤14-days post-injury) demonstrated higher initial salivary-BDNF or greater change from timepoint A to B compared to participants with longer days to recovery (>14-days post-injury – Hypothesis 3).

Upon detailed inspection and discussion amongst the authors, the plan to use mixed modelling to account for confounding factors and fixed/random effects was not completed due to the apparent absence of any trends observed across these figures.

Results

Results are presented for 67 participants (15% females; median age 19 years [IQR: 16, 24] who provided a combined 167 saliva samples. Participants presented for initial assessment a median of 10 [IQR: 5, 12] days post-injury and required a median of 25 [IQR: 14, 36] days to become asymptomatic. The descriptive data for salivary-BDNF and symptom scores at each clinical timepoint are provided in Table 8.1.

Hypothesis 1 was not supported as there was no clear evidence that BDNF concentrations were lowest upon initial presentation to the clinic and increased over clinical recovery. The point plot depicted in Figure 8.1 shows no clear trend in BDNF changes from Timepoint A to further timepoints when measured in saliva under ecologically valid conditions.

Hypothesis 2 was not supported as there were no clear relationships between salivary-BDNF concentration and the seven symptom burden scores as measured by R^2 (PST: 0.003; symptom severity: 0.005; migraine: 0.000; cognitive-emotional: 0.006; sleep-emotional: 0.003; neurological: 0.000; undefined feelings: 0.000). Figure 8.2 visualises the lack of relationships between salivary-BDNF and the seven symptom burden scores at each clinical timepoint.

Hypothesis 3 was not supported as there was no evidence for higher BDNF concentrations at initial assessment and the first follow-up for patients who became asymptomatic in ≤14-days compared to patients who took >14-days to recover. Figure 8.3 shows a complete overlap in the distribution of salivary-

BDNF concentrations at Timepoints A and B for the 23 participants (34%) who became asymptomatic in ≤14-days compared to the 44 (66%) who took >14-days.

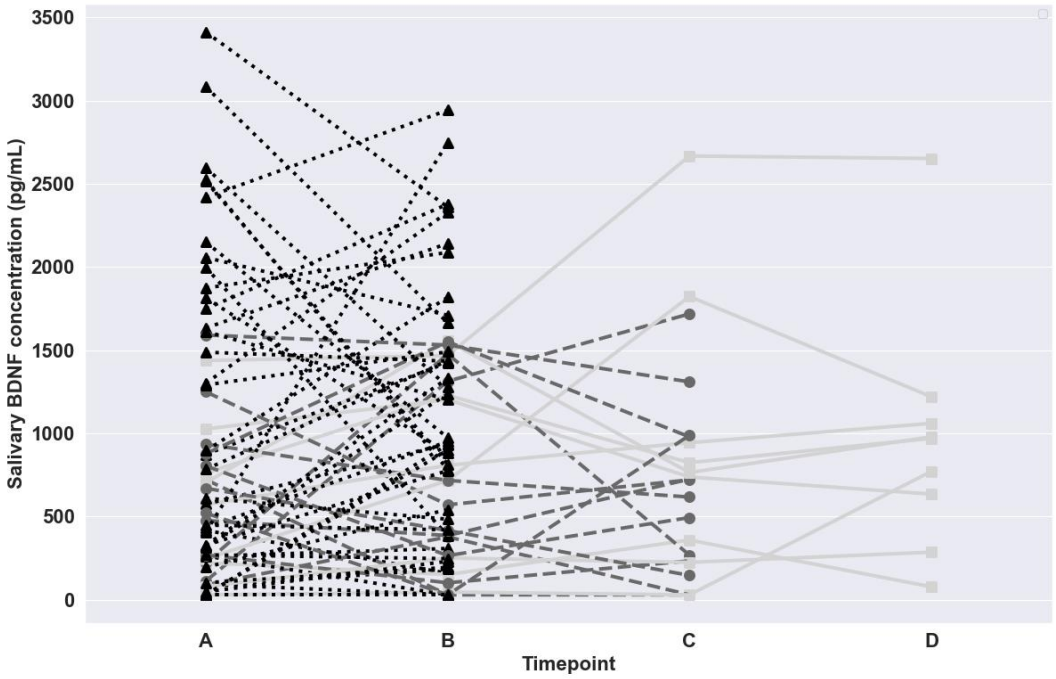


Figure 8.1 Pointplot illustrating changes in salivary-BDNF concentrations from timepoint to timepoint for each participant.

Table 8.1. Descriptive data for salivary-BDNF and symptom scores at each clinical timepoint.

	Timepoint A (n=67)	**Timepoint B (n=65)**	**Timepoint C (n=24)**	**Timepoint D (n=12)**
Days post-injury[a]	9.61±4.85	20.54±6.73	35.88±9.23	56.00±14.75
Salivary-BDNF concentration (pg/ml)[b]	671 [254, 1540]	881 [309, 1468]	732 [260, 982]	704 [431, 1000]
Positive Symptom Total[b]	9 [6, 14]	2 [0, 7]	2 [0, 7]	5 [4, 8]
Symptom Severity Score[b]	17 [8, 30]	3 [0, 10]	4 [0, 10]	7 [4, 8]
Migraine composite[b]	2 [0, 4]	0 [0, 1]	0 [0, 2]	1 [0, 2]
Cognitive-Emotional composite[b]	6 [3, 14]	1 [0, 3]	2 [0, 3]	2 [0, 3]
Sleep-Emotional composite[b]	11 [6, 20]	3 [0, 8]	2 [1, 6]	6 [4, 8]
Neurological composite[b]	3 [1, 6]	0 [0, 2]	0 [0, 1]	1 [0, 2]
Undefined feelings composite[b]	2 [1, 5]	0 [0, 1]	0 [0, 1]	0 [0, 1]

[a] mean ± SD; [b] median [interquartile range]

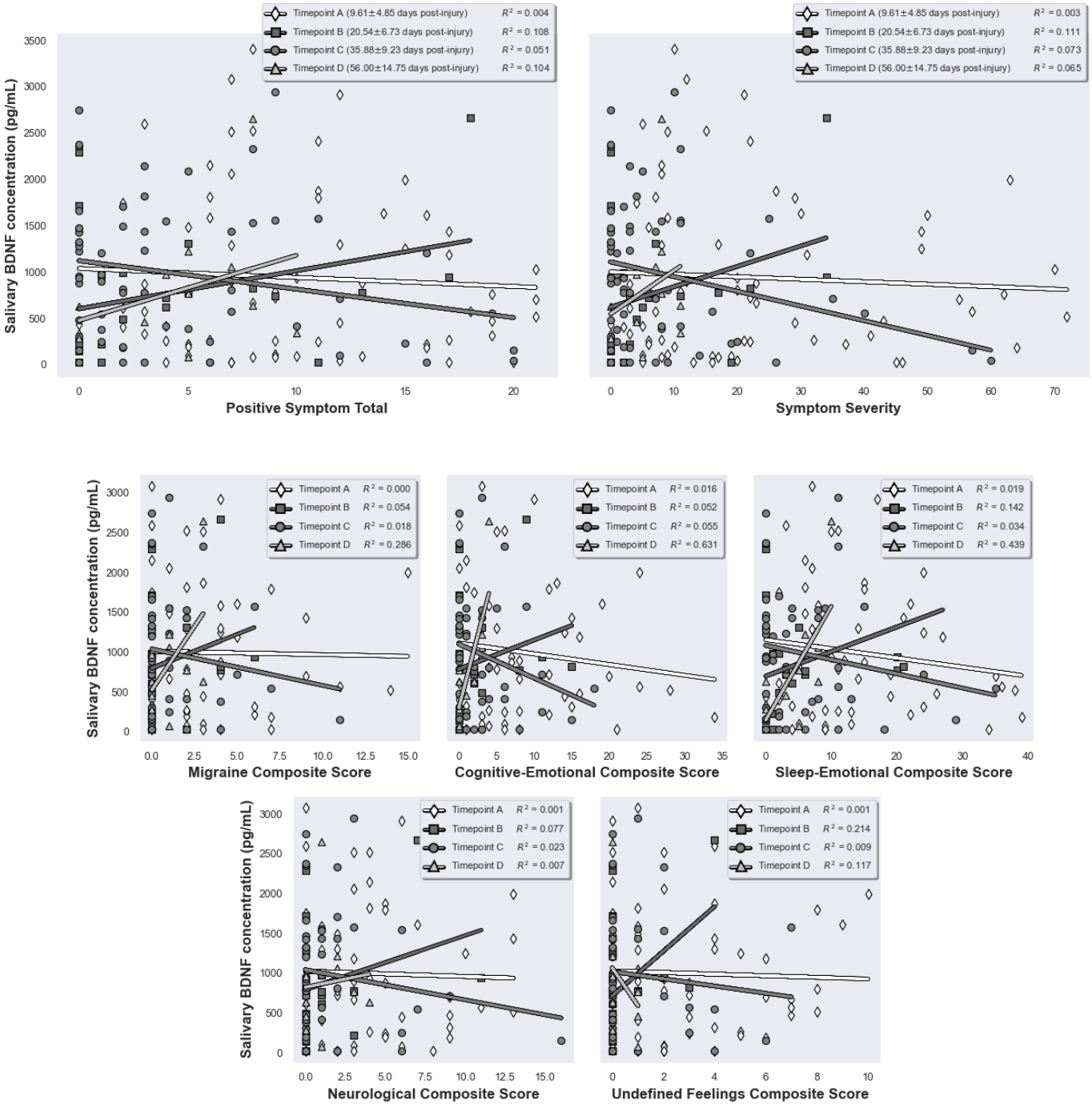


Figure 8.2. Repeated measures correlation plots visualising the relationship at each clinical timepoint between salivary-BDNF concentrations and the seven symptom burden scores calculated using the SCAT-5 symptom scale.

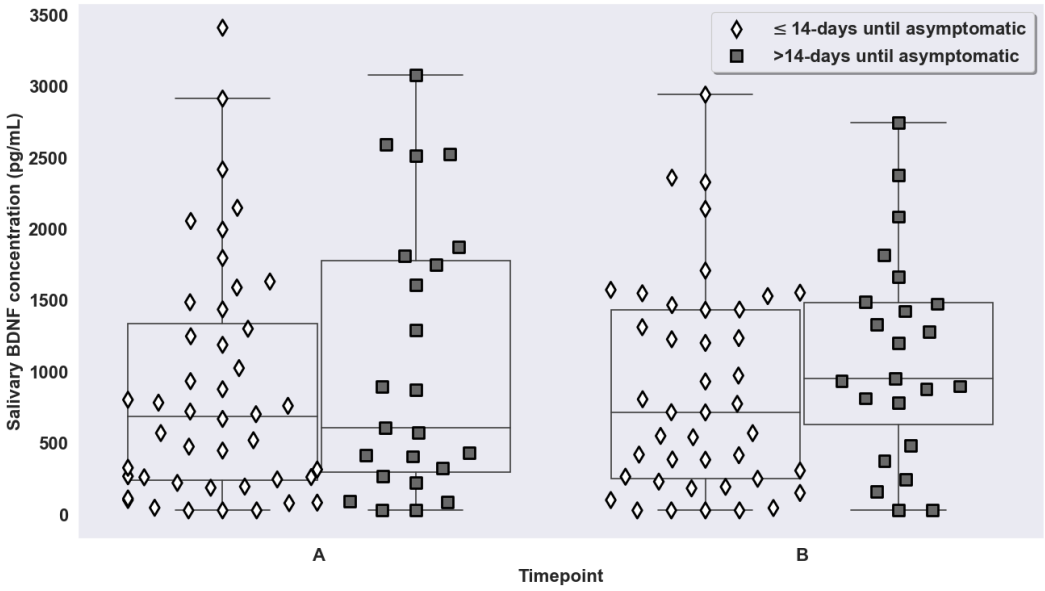


Figure 8.3 Boxplots depicting the distribution of salivary-BDNF concentrations between participants who became asymptomatic in ≤14-days vs >14-days at Timepoints A and B.

Discussion

The case against salivary-BDNF

The aim of the current study was to assess changes in salivary-BDNF concentrations at multiple timepoints over the course of clinical recovery following SOBI and to evaluate how BDNF related to subjective symptom reports at each timepoint. Our data did not support any of our initial hypotheses nor show any other discernable trend in how BDNF (measured via passive drool under ecologically valid conditions) changed over the course of clinical recovery in relation to symptom reports.

The methodological compromises made to evaluate BDNF non-invasively within a functioning clinical environment introduced too much variation to validly track group-level changes in BDNF concentrations over multiple clinical timepoints. Previous reviews have indicated that the risk of sampling error is highest during the collection and processing of saliva samples and have described detailed methodologies to minimise these risks in studies attempting to measure biomarkers in saliva [365, 366]. No study had prospectively evaluated whether passive drool sampling under clinically realistic conditions could demonstrate saliva as a practical and non-invasive alternative to blood to accelerate our understanding of BDNF's role in recovery from TBI and other neurological conditions. For example, it is generally recommended that rinsing of the mouth take place at least 10 minutes before sampling [365, 366], instead in our study participants were instructed to rinse their mouth immediately before sampling to preserve clinical flow. This practical compromise may explain why 10% of samples were below the ELISA LOD, as it is

possible that these samples contained residual water from the rinse rather than saliva. If all these samples below the LOD were observed at Timepoint A, in participants who took >14-days to become asymptomatic and reported a high symptom burden then these samples would have supported our hypotheses; but as it stands these samples were observed at Timepoints A, B, and C and had no relation to symptom burden nor recovery outcomes. Our results appear to confirm the need for strict methodological controls when measuring protein biomarkers in saliva via passive drool. However, this example highlights that even if collection conditions are standardised there is still a risk of sampling error because of the active involvement of the participant/patient in providing the sample. The non-invasive advantage of measuring BDNF in saliva appears to be counteracted by the inherent risk of sampling error even if standardised collection methodologies are in place. For these reasons, there is no justification for the use of saliva collected via passive drool in place of blood in studies attempting to quantify BDNF protein concentrations in real-world clinical populations.

Research has highlighted that reliance upon self-reported symptoms after mTBI, and particularly in the case of SOBI, can be challenging due to the non-specific nature of symptoms associated with this injury [83, 84] and the tendency of athletes to underreport or minimise their symptomology [6, 7]. Issues with symptom reporting could explain the lack of observed relationships with salivary-BDNF in addition to the variation in the BDNF due to sampling error. Given international recommendations promote ongoing use of subjective symptom reports as a cornerstone tool in the clinical management of mTBI/SOBI [39, 43], there is a need to identify neurophysiological measures that correlate with changes in symptomology.

Noting potential sampling error for BDNF, and variability in patient symptom reporting, there could also be no relationship between BDNF and clinical recovery/symptom burden following mTBI/SOBI. While this is the first study that has tried to evaluate the relationship between BDNF (measured via blood or saliva) and symptom reports after SOBI, previous works have demonstrated relationships between circulating BDNF concentrations and depressive symptoms [397] and cognitive function in individuals with bipolar disorder [398] and schizophrenia [399, 400]. Korley et al. [65] demonstrated that BDNF measured in serum on the day of TBI could identify patients at higher odds of negative recovery outcomes as measured by the Rivermead Post Concussion Symptoms Questionnaire. Based on these findings from other areas of the literature, there is theoretical justification to expect some degree of association between symptom burden (at least the cognitive-emotional symptom composite [395]) and BDNF concentrations in patients recovering from SOBI. It could be that salivary-BDNF does not relate to functional outcomes as has been reported in previous studies using blood-based measures of BDNF in other neurological conditions [129].

Future directions

Our findings have highlighted that more work is needed to identify and develop methods that account for logistical limitations in outpatient settings while maintaining high validity and reliability. Future

investigations are warranted despite the presented results given BDNF as an indicator of neuroplasticity has been suggested as important in several high-quality reviews [112, 129, 384-386, 401-404]. Theoretically, high peripheral BDNF concentrations are indicative of a favourable neuroplastic environment within the central nervous system. Such an environment would be advantageous after TBI by promoting the restoration of injured neuronal networks while also facilitating compensatory processes such as recruitment and retraining of networks less affected by injury [385]. This line of thought suggests that impaired neuroplasticity would be associated with worse functional recovery after TBI while greater plasticity would be advantageous for recovery (as reflected in our hypotheses for this study). This view may be overly simplistic as it does not account for the activity-dependent nature of neuroplasticity [402]. It is possible that a high degree of plasticity in the presence of sub-optimal sensory input and/or behavioural experience may have maladaptive consequences [402], which has been described as the dark side of plasticity [403]. These maladaptive consequences of plasticity may be a source of persistent complications in TBI patients; wherein heightened neuroplasticity is present while favourable task-specific rehabilitation strategies are absent; through which self-propagating negative compensations and behaviours are reinforced over time and become increasingly difficult to positively redirect. Low neuroplasticity could actually be beneficial if TBI patients do not seek treatment or are receiving sub-optimal management. Conversely, strategies that can deliver optimal task-specific rehabilitation modalities when a high degree of neuroplasticity is present could have profound effects on the recovery and quality of life for patients suffering from neurological conditions including TBI. However, the development of these concepts is almost entirely informed by evidence from pre-clinical studies. The relationship between circulating (peripheral) BDNF and the degree of neuroplasticity in the CNS remains poorly understood.

Clearly much research is needed to translate neuroplasticity concepts from laboratory studies to clinical settings. Large cohorts will be necessary to unravel these relationships in a clinical setting due to the heterogeneity of patients, conditions, and outcomes. The reliance on venous blood draws to sample BDNF as a proxy of neuroplasticity continues to be a hurdle. There is a considerable fear of needles within the public, causing even healthcare workers to avoid vaccinations [367, 379]. Logistical challenges of blood sampling aside, it seems unlikely that the large numbers of participants needed to understand the role of neuroplasticity in TBI recovery will voluntarily consent for research involving repeated venous blood sampling. Measurement of BDNF in saliva does not appear to be a solution to this issue. Perhaps acquisition of capillary-drawn blood using finger pricks can balance accurate quantification of BDNF with greater clinical practicality [380]. Future studies should investigate whether adequate validity and reliability is observed between BDNF measured in blood collected from capillaries via finger prick against venepuncture.

Studies are needed to understand intra-individual variation in BDNF concentrations when blood sampling takes place at different times of day to determine whether valid and reliable results can be obtained in environments where standardised time of sampling are not possible. While not explicitly stated in their

article, the results reported by Korley et al. [65] appear to support this notion because participants were TBI patients presenting to hospital who likely had blood draws shortly after admission. Moreover, the promising results from their paper were based on BDNF measured on the same day of injury. Studies that capture patients on the day of TBI and then follow these patients through clinical recovery in outpatient settings with measurement of BDNF are needed to understand the temporal profile of neuroplasticity following TBI.

Rather than directly quantifying protein concentrations, measurement of microRNAs could be another option for future studies; as microRNAs play a key role in the expression and regulation of proteins, including BDNF [67]. Recent evidence suggests that microRNAs are a useful diagnostic biomarker for mTBI and can be non-invasively measured using oral swabs [68, 69]. Acquisition of microRNAs by this method and their correlation to mTBI-related symptom reports over the course of clinical recovery has yet to be explored. The hypotheses put forward in this study remain to be answered; replication of the current study using blood measures or microRNAs may provide insight into the relationships between symptom reports and BDNF concentrations over the course of clinical recovery after mTBI.

Limitations

The main limitation of this study was the intentional methodological compromise made to preserve clinical flow and ecological validity. Previous reviews have discussed several factors that should be controlled for when obtaining saliva samples to measure biomarkers (i.e., immediate storage, standardised time of collection, time of last food/drink) [365, 366]. Strict adherence to these recommendations was not possible in the clinical environment due to logistical and scheduling constraints. For this reason, we intentionally did not try to control for these factors during sample acquisition to evaluate if any group-level relationships between symptom reports and salivary-BDNF concentrations were observed over the course of clinical recovery when collected under clinically realistic constraints. This approach prevented within-participant comparisons because changes in salivary-BDNF concentrations between timepoints may be due to different collection conditions. The current study assumed that sampling-related variations in salivary-BDNF would balance out from timepoint to timepoint at a group level and reveal preliminary trends between symptom burden and BDNF concentrations.

The decision to not perform advanced statistical modelling to control for confounding factors/effects could be viewed as a limitation. In this scenario, advanced modelling was deemed inappropriate based on a lack of relationships observed following exploratory data analysis. The variation in the number of days post injury to the Timepoint A data collection, and the days between appointments affecting Timepoints B, C and D are a limitation due to clinical scheduling constraints. Had exploratory analysis revealed preliminary relationships the number of days post-injury would have been accounted for in the advanced modeling.

Conclusion

There were no clear relationships between salivary-BDNF concentrations (measured via passive drool under ecologically valid clinical conditions) and SOBI-related symptom reports measured at four clinical recovery timepoints. The most plausible explanation for these unexpected findings is the inherent risk of sampling error when measuring BDNF in saliva. For this reason, we do not recommend investment of time and resources towards the quantification of BDNF in saliva in future clinical studies. There is a need to identify methods that balance accurate quantification of BDNF with clinical practicality to investigate the clinical utility of BDNF as a biomarker of recovery in mTBI patients.

Chapter 9: Summary and conclusions

General summary and discussion of findings

This PhD aimed to evaluate how a diverse collection of evolving neurophysiological approaches to assessing and managing mTBI might translate from bench to bedside for SOBI. To address this aim evidence was provided towards answering the questions "what factors influence time to recovery following SOBI?" and "what is the translational potential of neurophysiological approaches to advance clinical management of SOBI?". Prioritisation of ecological validity and clinical practicality were considered throughout the design, implementation, analysis, and interpretation of the studies within this thesis so that if positive findings were observed there would be a high degree of confidence that these findings could feasibly integrate into functioning clinical environments. A general summary discussing the key findings of this thesis is provided below.

Section 1: Factors that influence time to recovery post-SOBI

Shifts in attitudes and media attention about the seriousness and potential long-term risks of SOBI led Participant A to contact the research team about persistent symptoms he was experiencing that he attributed to a history of SOBI which resulted in the case study presented in Chapter 2. Participant A, age 40, self-reported a history of 10 SOBIs the most recent of which occurred more than 10 years before consenting for the case study. Through a series of thorough and multi-disciplinary assessments, it was identified that Participant A's symptoms were consistent with otolithic dysfunction and disembarkment syndrome for which an exercise program was prescribed to recalibrate the otolith in a manner that would reduce symptomology. Over eight years had elapsed from the onset of Participant A's symptoms to the initiation of this treatment program. Based on the temporal relationship between Participant A's symptom onset and his last SOBI, his symptoms were seemingly unrelated to his history of SOBI. This case study showcases that claims about the potential long-term effects of a history of SOBI may lead to biased attribution of persistent symptoms to SOBI by patients and clinicians. The consequence of this bias (whether conscious or unconscious) combined with the non-specific nature of SOBI-related symptomology are imprecise diagnostic approaches that may not consider alternate explanations for symptomology. While raised awareness surrounding the seriousness of SOBI and potential long-term consequences of unidentified/unaddressed injuries is positive; these efforts in awareness need to be matched by initiatives to provide resources and management to resulting individuals who believe they are suffering chronic deficits based on their previous engagement in sport. Chapter 2 also provides an example of the level of thoroughness and interdisciplinary collaboration required to identify and treat chronic deficits that may or may not be due to SOBI. Structured management pathways are needed to help individuals with lingering

symptoms seemingly related to SOBI, otherwise attention is being drawn to an issue without providing an effective solution.

While Chapter 2 highlighted the weaknesses and needs of current pathways in the longer term, Chapter 3 identified the strengths of an acute specialist SOBI service implementing current best practice recommendations with the aim to prevent longer term difficulties. Specifically, clinical data collected during the initial assessment of SOBI (approximately one-week post-injury) was used to develop a prognostic model to predict which patients had a higher odds of requiring >14-days to become asymptomatic. The threshold was set at 14 days for modeling in this study based on widely cited figures from previous consensus statements that 80-90% of SOBI patients will experience spontaneous clinical recovery by this time [93, 97]. Predictor variables considered for inclusion in the final model included: patient demographic information (age, sex, sport resulting in SOBI, years of education, number of previous mTBI/SOBI); SCAT-5 testing results (symptom burden composite scores, neurocognitive score, balance testing score); and clinical examination findings (PSC, presentation of autonomic dysfunction, pre-diagnosed psychological condition/illness). A final model consisting of sex, initial positive symptom total, and PSC were variables collected during initial clinical assessment of SR-mTBI that demonstrated strong predictive capacity to identify patients with >14-day and ≤14-day recovery trajectories on both training (Accuracy: 81%; AUC: 0.90) and validation (Accuracy: 76%; AUC: 0.85) datasets. This was the first study to apply PSC criteria at initial clinical presentation, rather than 21-days post-injury, which turned out to be a strong predictor of recovery trajectory. In particular, athletes with a combination of multiple PSCs or those with predominantly vestibulo-ocular or cervicogenic symptoms had greater odds of experiencing slower recovery.

The results of Chapter 3 add to a growing body of literature that the neurocognitive and balance assessments within the SCAT-5 lack clinical utility when administered beyond the first 2-3 days post-injury [73, 87, 88]. Similar performance on these measures was observed when participants were symptomatic and asymptomatic and no clinically meaningful differences were evident between those with >14-day and ≤14-day recovery trajectory. Scheduling constraints and/or delayed symptom onset [43] means that many patients do not present to a specialist clinic like the one where this study took place within the timeframe where the neurocognitive and balance assessments of the SCAT-5 provide value. SOBI patients were scheduled for 45-minute appointments for an initial assessment at the clinic within this study, there is an opportunity to reduce appointments to ~35 minutes by omitting SCAT-5 neurocognitive and balance testing [73, 87, 88]. If a clinic were to have limited capacity for 20 initial assessments per week (each requiring 45 minutes), this small change could mean increased weekly capacity and reduced wait times for another six SOBI patients. Alternatively, this time could be better allocated to the Vestibular/Ocular Motor Screening assessment that takes 5-10 minutes to complete and can help earlier identification of vestibulo-ocular symptomology [165, 169, 405, 406].

Interestingly, despite participants receiving acute and individualised care in line with current recommendations, >50% of the sample required >14-days to become asymptomatic. Although a related, work [315] showed that 75% and 94% of patients within this model were recovered by four and eight weeks, respectively. These outcomes were notably better than those reported from a cohort of predominantly SOBI patients seen at a similar clinic in the United States between 2010-2012 where 38% and 80% of patients recovered by 30 and 90 days post-injury [407]. Collectively these findings challenge the notion that the majority of SOBI patients will recover within two weeks and indicate that statements about recovery expectations require updating. There could be prevalence-incidence bias in these figures since patients within these two cohorts were those who presented to specialist clinics. However, in our cohort, the majority (60%) of participants were injured playing rugby. In New Zealand there is a mandatory stand-down period of 21-days for rugby players who sustain SOBI [408]. Medical clearance is required before these players can return-to-play, so even players with limited symptomology presented to our clinic, increasing the representativeness of the sample. While not explicitly stated in the article, this American cohort likely received rest-based management for SOBI based on consensus recommendations at the time; while the New Zealand cohort received management based on progressive cognitive and physical loading combined with individualised exercise programs to target mechanisms of symptomology. Additionally, the high proportion of individuals who experienced recovery beyond 14-days post injury in Chapter 3 is in line with findings from population-based and emergency department studies within the wider mTBI literature [33, 409, 410]. The results of the work related to Chapter 3 highlight the effectiveness of an acute management pathway for SOBI and suggest this is a better approach than current “wait and see” methods, particularly in New Zealand where the Accident Compensation Corporation will cover 80% of an injured individuals’ salary while they cannot work [411]. Given the prevalence of burden weeks, months, or years post-mTBI/SOBI outlined in Chapter 1 that may prevent a patient from working, an acute model that leads to 94% being recovered within eight weeks could have significant advantages to patients and the economy.

Finally, the findings from Chapters 2 and 3 indicated symptoms with a vestibulo-ocular origin are a risk factor for ongoing impairments in function for patients who have experienced SOBI. Training and education to screen for these symptoms, and structured treatment pathways, are needed to reduce the chance of these symptoms going unaddressed for years as seen with Participant A.

Section 2: Translational potential and clinical utility of neurophysiological approaches to advance management of SOBI

Section 1 provided in depth insight into the strengths, weaknesses, and gaps in clinical services for individuals who have sustained a SOBI in New Zealand. In particular, acute services of multi-disciplinary clinical teams, with speciality working with SOBI, that implement progressive loading and exercise-based management can greatly reduce the number of patients who experience symptoms beyond eight weeks post-SOBI. The expense and burden of SOBI could be further reduced by a two-pronged model incorporating both reactive and proactive arms. Reactive treatments being those that address specific symptoms after they have evolved (i.e., sub-symptom threshold exercise, vestibulo-ocular physiotherapy, cognitive behavioural therapy), while proactive treatments interrupt the mechanisms that lead to symptoms. For example, an EpiPen is a proactive treatment for anaphylaxis [412]. If an individual is exposed to an allergen that triggers anaphylactic pathophysiology they will experience severe symptoms as a result, but if an EpiPen is administered shortly after exposure the epinephrine blunts the pathophysiological response in a manner that reduces or prevents the development of symptoms [413, 414]. Currently there are no proactive treatments for mTBI/SOBI. Section 2 Part A (Chapter 4) was conducted to identify the most promising nutritional intervention candidate (analogous to the EpiPen) to blunt the neurophysiological consequences (analogous to anaphylactic pathophysiology) following TBI (analogous to allergen exposure).

A clinical model built around a specialist clinic can lead to the majority of SOBI patients being rehabilitated by eight weeks post-SOBI. Realistically, however, many communities will not have the resources and clinicians to establish one of these clinics and geographical, socioeconomic, and logistical barriers may prevent referrals, thus the task of managing SOBI falls on clinicians with more general skillsets. The lack of objective assessment tools to assist the assessment and management of SOBI places a great deal of pressure on these clinicians with limited training. While the clinical studies in Section 2 Part B took place in a specialised SOBI clinic, they were conducted with non-specialist clinicians in mind to evaluate the translational potential and clinical utility of neurophysiologically based assessments methods to provide objective data to assist SOBI management. Chapters 5-8 also provided a means of assessing whether the neurophysiologically based measures could be used to assess the potential of a nutritional intervention to reduce the proportion of patients who experience slower recovery. Figure 9.1 illustrates the contributions that Section 2 of this thesis has made to clinical practice and the literature as a whole.

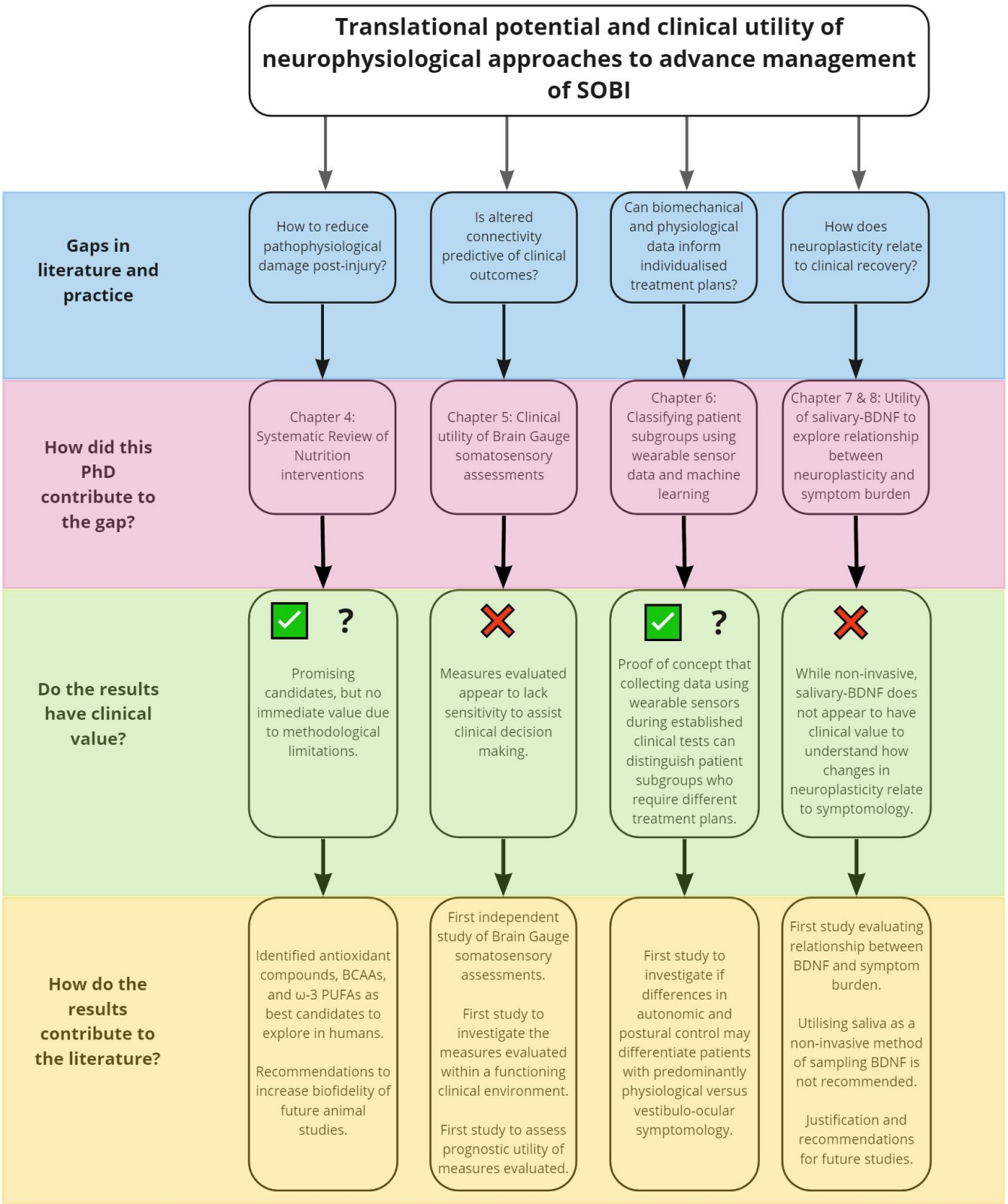


Figure 9.1 How the results of Section 2 contributed to clinical practice and the literature.

Part A

Chapter 4 revealed that no human studies have evaluated a nutritional intervention on neurophysiological outcomes following TBI, thus all knowledge on this topic is derived from pre-clinical animal studies. The systematic review identified that the nutritional interventions that had been investigated up to July 2019 included: natural compounds with anti-oxidant properties; branched chain amino acids; creatine; fasting and caloric restriction; ketogenic diet, multi-supplements; ω-3 polyunsaturated fatty acids; and a variety of natural compounds used in traditional eastern medicine. All of these nutritional interventions were associated with favourable outcomes when compared to control, but several methodological caveats combined with the absence of negative findings indicated risk of bias and threats to biofidelity. Animal models of injury and disease produce foundational knowledge into the pathophysiology of a given condition and provide an opportunity to test interventions targeting the pathological mechanisms. A main takeaway of Chapter 4 was the discordance between the design, methodology, and outcome measures in many of the animal studies which may hinder the translation of these nutritional interventions to clinical populations. With these limitations taken into account, supplementation with anti-oxidant compounds, branched chain amino acids, and ω-3 polyunsaturated fatty acids (specifically docosahexaenoic acid – DHA) appeared to be the most promising nutritional interventions worth exploring in humans. While development of a novel pharmaceutical agent requires extensive pre-clinical study before exploring the feasibility of such an intervention to benefit humans [415], modification of diet is generally considered safe in humans if contraindications are accounted for. Therefore, while our understanding of nutritional interventions for TBI in animal models is incomplete, there is proof of concept to suggest that modification of nutrition may benefit mTBI patients, and precedence to progress both pre-clinical and clinical knowledge in parallel [300].

Based on the findings of this review, DHA supplementation appeared to be the most promising nutritional intervention post-TBI as DHA combats oxidative stress and apoptosis and provides the necessary building blocks to repair phospholipid bilayers (primarily consisting of DHA) damaged by lipid peroxidation while also upregulating neuroplasticity to promote healing [218, 234, 238, 242, 243, 248, 252]. Since no studies have included neurophysiological outcome measures in the investigation of nutritional interventions in humans, identification of objective neurophysiological outcome measures that can be realistically administered in clinical environments was necessary before exploring the feasibility of DHA supplementation to complement current SOBI management. Chapter 3 presented a prognostic model that could accurately identify SOBI patients more likely to require >14-days to recover, which could be used to identify patients who may most benefit from a nutritional intervention like DHA supplementation. But this model relies on clinical experience and honest symptom reporting, which as already discussed, is not always the case. Thus, a more objective means of predicting patients likely to experience slower recovery trajectories using neurophysiological outcome measures would be advantageous.

Part B

In Chapter 5 the clinical utility of somatosensory assessments delivered by the Brain Gauge were evaluated. SOBI triggers oxidative stress and lipid peroxidation which impairs neurotransmission and propagates network dysfunction [21, 61]. The Brain Gauge was chosen based on prior evidence that the somatosensory assessments within the testing protocol provide insights into functional connectivity that can detect differences between healthy individuals and those with mTBI/SOBI [122, 123, 135]. In the context of evaluating DHA to improve outcomes post-SOBI, the Brain Gauge appeared to be a potentially objective method to predict those likely to recover more slowly and to track recovery in response to an intervention. Discriminative capacity of Brain Gauge somatosensory assessments was also evaluated as this would be extremely helpful to make diagnostic decisions in cases where symptom onset is delayed or symptom reports might be untrustworthy. The complete Brain Gauge somatosensory assessment protocol required 20 minutes to complete and thus had to be reduced to ~10 minutes to preserve clinical flow. Logistical barriers limit the accessibility of baseline performance for SOBI assessment tools, meaning clinicians need to rely on normative data to assist their decisions [81]. The reduced protocol was determined based on the availability of previously published values of how healthy individuals perform on Brain Gauge somatosensory assessments so that this data could be pooled to create preliminary normative data for statistical comparisons. Data were available from two or more studies for temporal order judgement, temporal order judgement with confounding stimulus, and duration discrimination [122, 133, 135, 318], thus these were the tasks completed by the SOBI participants in Chapter 5. Using this approach Chapter 5 indicated poor discriminative (48-63% classification accuracy) and prognostic utility (47% accuracy) of the Brain Gauge somatosensory assessments evaluated. Similarly, no differences in TOJ or DUR performance were present between SOBI patients and the normative data when testing took place when participants were symptomatic versus when they were asymptomatic. Only temporal order judgement with confounding stimulus detected differences between participants and normative data at both timepoints. The findings of Chapter 5 did not justify recommendations for the use of Brain Gauge temporal order judgement, temporal order judgement with confounding stimulus, or duration discrimination in clinics tasked with managing SOBI patients, nor to adopt these measures as a means of evaluating the potential efficacy of DHA supplementation to improve SOBI recovery outcomes.

These findings were in contrast to claims on the Brain Gauge website (https://www.corticalmetrics.com/) that this device can assist clinicians when monitoring SOBI patients and when making return-to-play decisions without the need for a baseline. The references used to support these claims are from investigations that were not conducted under clinically realistic conditions and compared non-injured baseline data to data collected post-SOBI [122, 123]. In one of these studies a high degree of discriminative accuracy between baseline and post-SOBI performance was observed using Brain Gauge tasks that were not evaluated in Chapter 5 (reaction time variability and amplitude discrimination) because there is no

published data about how healthy controls perform on these measures [123]. Again, claims are made on the website that data from over 1000 healthy controls has been collected for all BG-SA tasks [416], but this data is not published in a peer-reviewed article, and was not provided when requested for Chapter 5. Collectively the claims about the clinical utility of the Brain Gauge do not match the peer-reviewed evidence. It appears the commercialisation and marketing of this product has outpaced the clinical science. Chapter 5 provides an important example about the need for independent research when a device has been developed to fill a clinical need such as providing more objective means of assessing SOBI; and how results can differ when testing occurs in controlled laboratory settings versus under ecologically valid conditions.

Where Chapter 5 evaluated whether components of the Brain Gauge protocol could fill clinical needs for a more objective method to assist SOBI diagnosis, prognosis, and evaluation of recovery; Chapter 6 explored if the collection of biomechanical and physiological data with a wearable sensor during an established clinical test could aid clinicians when identifying subgroups of SOBI patients who require different treatment pathways. Differences in which neuronal networks are compromised after SOBI are hypothesised to contribute to the development of clinically observed symptom clusters [12, 61, 75, 124, 138, 139]. Similarly, disruption in neurotransmission due to SOBI has been attributed to observed differences in autonomic regulation and postural control during gait between healthy individuals and SOBI patients [12, 124, 127, 128, 140], but there had yet to be a study to evaluate whether differences in these functions could discriminate between SOBI patients with divergent symptom profiles. Using a convolutional neural network deep learning algorithm, Chapter 6 presented the first evidence demonstrating that features within electrocardiogram and accelerometer signal collected during a Buffalo Concussion Treadmill Test can accurately (up to 82% accuracy, 0.89 AUC, 0.6 sensitivity, 0.92 specificity, 0.75 PPV, 0.85 NPV) distinguish SOBI patients with physiological versus vestibulo-ocular PSC. Since Chapter 6 was the first study to attempt to classify SOBI subgroups using a deep learning algorithm trained with sensor data, I elected to focus on these two PSCs since Section 1 highlighted that physiological PSC is the most prevalent while vestibulo-ocular symptomology was associated with worse recovery outcomes. These encouraging preliminary findings come with the caveat that while it appears features within electrocardiogram and accelerometer signal *can* separate these two groups, deep learning analytical methods like convolutional neural networks do not provide insight into *which* features are used in classification [340]. There would be value in a "black box" model that could accurately identify SOBI patients with different PSCs, however, in my opinion there would be greater value in disentangling which features achieve classification so that then those features may lead to insights regarding severity of impairments or how impairments respond to intervention.

An ability to complete functional tasks that would normally be trivial like tolerating light/noise, engaging in exercise, reading a book, or regulating balance/gait can be impaired by mTBI/SOBI induced disruptions in

neuronal networks [61]. In Chapter 5 the Brain Gauge was used to evaluate the ability of the somatosensory cortex to detect differences in light vibrations to the fingertips. This testing paradigm is meant to provide an indirect method to make inferences about the processing capacity and functional connectivity of the brain [317, 417]. Conversely, in Chapter 6 physiological and biomechanical data was collected directly during a testing protocol that simulated day-to-day tasks (aerobic exercise and regulation of balance/gait) that patients commonly report are impaired post-SOBI [61, 75, 138]. Comparing the outcomes of Chapters 5 and 6, the findings of Chapter 6 provide a strong example for the value of acquiring objective data during functional tasks that are disrupted by SOBI over a paradigm that indirectly assesses brain function. Until there is a true "gold-standard" method of measuring brain function following mTBI/SOBI (i.e., functional MRI) that tools like the Brain Gauge can be validated against, time may be better spent identifying existing clinical tests to which an affordable sensor can be added to detect subtle deficits during these tasks. Chapter 6 indicates the value of the addition of electrocardiography and an accelerometry to treadmill stress testing. Another potential application could be combining the commonly used Vestibulo/Ocular Motor Screening assessment with eye tracking technology to detect subtle impairments in eye movements [165, 169, 405, 406, 418]. The advantage of these approaches is acquiring objective data but doing so during an assessment that clinicians already use regularly in practice, meaning there would be minimal increases in the time a clinician would have to allocate to learn and/or deploy the assessment.

One further lesson that Chapter 6 provides is the value of utilising a complete time series signal. In works that were used as rationale for Chapter 6, electrocardiogram and accelerometer data were analysed using metrics that summarised the entire signal as a single metric such as the standard deviation of the R-R interval [12, 124] and sway volume [127]. While this approach makes for simpler statistical analysis (i.e., independent t-tests) it does not make use of the whole time series and runs the risk of losing important features within the signal that may contain valuable information. Given the complexities and heterogeneity of SOBI, it is logical to speculate that our knowledge of these intricacies would increase more rapidly by utilising the entire signal, rather than reducing a large number of datapoints into a single summary measure. Advances in machine learning algorithms allow whole signal analysis and present an exciting opportunity for collaboration between clinical researchers and data scientists. Overall, further development and validation of the insights gained from Chapter 6 may lead to an objective, accurate, generalisable, and easy to implement method of assisting the identification of SOBI patients requiring different treatment pathways. Such a method could be particularly useful when geographical and socioeconomic barriers hinder referral to a specialist clinic.

BDNF is a key regulator of neuroplasticity which provides the biological substrate for neurorehabilitation [129, 384, 385]. One of the studies reviewed in Chapter 4 showed that a diet containing a high amount of anti-oxidants from blueberries led to increases in BDNF that were associated with improved behavioral

outcomes in animals post-TBI not observed in the control group [226]. Additionally, two studies included in Chapter 4 reported increased BDNF levels in animals compared to control following a DHA enriched diet [151, 242]. These studies highlight BDNF as a biomarker of interest to evaluate whether a nutritional intervention like DHA supplementation upregulates neuroplasticity following SOBI in a way that might improve recovery outcomes. BDNF is typically measured in humans via serum and plasma samples collected by invasive blood draws. The logistical constraints of acquiring blood combined with the fear of needles within the population represents a considerable barrier to understanding the role of BDNF in the recovery of SOBI patients [367, 379]. In two previous pilot studies conducted as part of my Master's degree, I measured salivary-BDNF concentrations in healthy controls and individuals with persistent mTBI-related symptoms before and after an exercise program [371, 419]. There were noteworthy differences in mean salivary-BDNF concentrations between the healthy and mTBI sample both before (mTBI approximately half of healthy) and after (mTBI approximately one quarter of healthy) the intervention [371, 419]. In these pilot studies, saliva samples were acquired under highly controlled conditions which is generally recommended when measuring concentrations of proteins like BDNF via saliva [365, 366]. Having observed these preliminary differences in salivary-BDNF concentrations between participants suffering with mTBI-related symptoms and healthy controls in my Master's, Chapters 7 and 8 of this thesis were conducted to evaluate if measuring BDNF non-invasively via saliva under clinically realistic conditions could be used to track and understand differences in SOBI recovery outcomes.

In Chapter 7 an experimental methodology was developed to explore how timing of sampling and delays in sample storage over the course of normal clinical hours (09:00-17:00) might influence the interpretation of intra-individual changes in salivary-BDNF concentrations. This was necessary because strict control over timing of sample collection and subsequent delays in storage were not feasible in the specialised outpatient clinic where data collection for Chapter 8 took place. For example, a patient might present for their initial assessment and provide a saliva sample at 09:30 in a fasted state, whereas their follow-up appointment may be scheduled at 14:00 and they may have eaten lunch shortly before providing a sample. Due to a lack of laboratory resources at the clinic, the samples would have to be stored on ice until the end of the clinical day when they could be transported to an off-site laboratory to be processed and placed in long-term storage at -80^{o}C. Assuming samples arrived at the lab by 18:00 this would mean that the sample from the initial assessment was stored on ice for ~8.5 hours, whereas the follow-up sample would have been on ice for only four hours. The experiment in Chapter 7 was necessary to evaluate if similar trends in intra-individual variation of salivary-BDNF concentrations were observed between healthy participants to inform whether within-participant timepoint to timepoint comparisons could be made in Chapter 8 when samples were acquired under clinically realistic conditions from SOBI patients. The results of Chapter 7 from healthy participants suggest that measurement of BDNF protein concentrations via saliva is not a valid or practically advantageous means of accurately comparing intra-individual changes in BDNF from one timepoint to

another. Chapter 7 also highlighted the risk of sampling error when trying to measure BDNF in saliva collected via passive drool under clinically realistic conditions. Even with these negative findings, given the low sample size in Chapter 7 and that no study had evaluated whether salivary-BDNF relates to mTBI/SOBI symptom burden a clinical investigation into the potential utility of salivary-BDNF was warranted.

Chapter 8 was the first study to investigate whether relationships exist between BDNF (measured by blood or saliva) and SOBI-related symptom reports over the course of clinical recovery. No clear relationships were observed between salivary-BDNF concentrations (measured via passive drool under ecologically valid clinical conditions) and SOBI-related symptom reports measured at multiple timepoints throughout clinical recovery. Valid and reliable execution of the biochemical assay to quantify BDNF in saliva was achieved in both Chapters 7 and 8. Building on Chapter 7, the most plausible explanation for these unexpected findings in Chapter 8 was the inherent risk of sampling error when measuring BDNF in saliva. Even though both Chapter 7 and 8 produced findings in contrast with a priori expectations, both of these works are valuable contributions to the literature. When explaining to participants in Chapter 8 why they were being asked to provide a saliva sample, and that a blood draw was the alternative method of measuring BDNF, the majority of participants expressed they would not have volunteered had there been a blood draw, which is in agreement with previous reports about the fear of needles [367, 379]. Had valid and accurate non-invasive measurement of BDNF protein concentrations via saliva been observed, it would have provided a means to expedite our clinical understanding the role of BDNF-mediated neuroplasticity plays in recovery from TBI and other neurological injuries/conditions. As it stands, evidence provided in both Chapters 7 and 8 about the risk of sampling error when measuring BDNF via saliva should discourage future researchers from allocating time and resources to attempt to answer their neuroplasticity-related hypotheses with this method. Chapters 7 and 8 highlighted that a clinical solution to acquire a large number of biological samples from which BDNF can be measured to answer research questions like those within these chapters remains elusive. Overall, based on the abundance of promising findings from studies that measured BDNF in neurological populations in blood [129], concluding that no relationship exists between BDNF and symptom burden throughout clinical recovery post-SOBI would be premature.

Future directions

As mentioned in Chapter 1, this thesis intended to culminate with an investigation into the feasibility of a nutritional intervention to positively alter SOBI patients at higher odds of longer recovery trajectories post-SOBI, but the lockdowns caused by the COVID-19 pandemic forced this final study to be omitted. While Chapter 4 highlighted risk of bias and issues with biofidelity of findings from pre-clinical studies evaluating nutritional interventions for TBI, there was sufficient justification to being exploring the feasibility of a nutritional intervention to complement current clinical best practice management of SOBI. Chapter 4 highlighted ω-3 polyunsaturated fatty acids (specifically DHA) as an intervention to combat inflammation,

reduce oxidative stress, assist in cell membrane repair, and restore impaired neurotransmission following brain injury. Multiple reviews have discussed the mechanistic rationale for why ω-3 polyunsaturated fatty acids should positively benefit the recovery of TBI patients [214, 293, 420, 421], but no study has evaluated the effectiveness of this nutritional intervention post-mTBI/SOBI in humans. Supplementation with ω-3 polyunsaturated fatty acids is widely considered safe and has been evaluated in clinical trials for Alzheimer's patients and as an intervention to aid athletic performance and recovery [422, 423]. Overall, a clinical trial to investigate the efficacy of ω-3 polyunsaturated fatty acid supplementation to improve recovery following SOBI appears justified, however, before doing so the feasibility of adding ω-3 supplementation to current best practice must be evaluated. Appendix 2 provides a complete research protocol for the feasibility study that was supposed to conclude this thesis. The main aim of the feasibility study was to identify the strengths and weaknesses of the proposed methodology (method of administration, timing, dosage, and duration) to inform the design of future clinical trials. The secondary aim was to provide preliminary evidence about the efficacy of the intervention on clinical and neurophysiological outcome measures compared to placebo. Neurophysiological outcome measures were a key feature of this feasibility study as the study would likely have been underpowered to detect clear differences using clinical outcome measures, whereas the neurophysiological measures were meant to allow comparisons with a higher degree of sensitivity. Salivary-BDNF and Brain Gauge somatosensory assessments were meant to be the neurophysiological outcome measures for these comparisons, as the animal research showed ω-3 supplementation increased BDNF levels while reducing the damaging effects of TBI that lead to impaired neurotransmission [218, 234, 238, 242, 243, 248, 252]. The results of Chapters 5, 7, and 8 clearly indicate that these measures would not have provided useful data to evaluate the effect of ω-3 supplementation to improve SOBI recovery outcomes. The feasibility study presented in Appendix 2 deserves to be conducted in the future, but refinement of neurophysiological outcome measures is required to address both research aims.

The simplest solution would be to acquire venous blood samples at each timepoint in the feasibility study to evaluate BDNF and other circulating biomarkers, but as discussed at length, this would make recruitment more challenging [367, 379]. As outlined in Chapters 7 and 8, research into the validity and reliability quantifying biomarkers like BDNF using capillary blood collected via finger prick is warranted. Alternatively, salivary-microRNAs appear more resilient to the limitations of salivary-protein concentrations and offer exciting potential as a biomarker for future investigations like the one suggested in Appendix 2 [68, 69, 424]. Chapter 6 provides an example of how to collect valuable objective neurophysiological data during a clinical evaluation of functional tasks that are commonly impaired post-SOBI. Further development of how to use sensors during treadmill stress testing to classify different subgroups of patients is warranted and may also lead to a method to track changes in impaired autonomic function and sensorimotor integration overtime. Pairing eye tracking technology with the Vestibular/Ocular Motor Screening or an accelerometer

with the Balance Error Scoring System may also provide means to collect high resolution objective data with minimal disruption of clinical flow.

The diversity of the studies within this thesis have identified several further recommendations for future animal studies and clinical investigations. Due to the differences in lifespan a more thorough understanding of the temporal profile of TBI pathophysiology between animal models (i.e., mice and rats) and humans is needed [281]. For example if the temporal profile of TBI pathophysiology is related to lifespan then initiating an intervention one hour post-injury in a rat would be analogous to beginning the intervention approximately one day post-injury in a human which could be clinically feasible [281]. However, if TBI pathophysiology is independent of lifespan positive outcomes from an animal study where an intervention began one hour following TBI would have limited biofidelity. Advances in understanding of these temporal relationships could significantly improve interpretation of how findings from animal models of TBI relate to patient outcomes.

There is a general need for future studies evaluating new outcome measures or interventions for mTBI/SOBI to be conducted under more clinically representative conditions. Section 2 provides several examples of how different findings can be when research occurs in highly controlled settings versus under ecologically valid conditions. Of course, controlled research is necessary to develop a new measure/intervention, but if the intent of the research is to demonstrate the potential of the method to improve clinical practice this needs to be reflected in the design of the study (i.e., time constraints, limited exclusion criteria, no baseline testing, etc.).

Many of the studies that were used as justification for the investigations in Section 2 Part B only provided within-group and between-group differences using t-tests and analysis of variance [12, 122, 124, 127, 128, 133, 135]. The literature would benefit from more studies that incorporate discriminative and prognostic modeling techniques in addition to/in favor of null hypothesis testing. These modeling techniques provide metrics (sensitivity, specificity, positive predictive value, negative predictive value) with which many clinicians are familiar [353, 354], and provide insight into how that outcome measure might help the clinician diagnose or predict the recovery outcomes of a new SOBI patient.

Overall, Section 2 of this thesis highlighted that the translational potential of neurophysiological approaches (at least those evaluated) to advance clinical management of SOBI in the near future is low. Incorporating sensors into existing clinical tests appears to be the most promising avenue and further work into the combined application of wearable sensors and machine learning algorithms may lead to more objective means to assist clinical decision making. But in the meantime, an acute model of care delivered by trained clinicians is this most promising candidate to reduce the proportion of SOBI patients who go on to experience prolonged symptom burden and reduced quality of life. Nearly all SOBI patients who presented

to an acute specialist SOBI service were recovered by eight weeks. However, this clinic was in New Zealand's largest city where it was possible to have a multi-disciplinary team working side by side. Research is needed to determine how to scale an acute clinical model like the one in which this PhD was embedded so it can be deployed in smaller more remote communities where it may not be possible to have multiple clinicians fully dedicated to treating SOBI patients working in the same space.

Limitations

Assumptions, methodologies, and analytical approaches that could be viewed as limitations of each individual study within this PhD have been discussed at length within each respective chapter. To avoid repetition of these limitations, this section acknowledges six main limitations that are common across multiple chapters of this thesis.

First, with the exceptions of Chapters 2 and 4, all findings were generated from samples of patients who experienced mTBI due to a sport-originated cause. Given SOBI accounts for only ~20% of all mTBIs, the generalisability of these findings to the broader mTBI population is unclear. Although all injuries were due to sport, the samples were inclusive for age, sex, and pre-diagnosed psychological conditions/illnesses (attention deficit hyperactivity disorder, anxiety, depression, or any other mental health conditions) which may improve generalisability to the wider population.

Second, data for Chapters 3, 5, 6, and 8 were collected in a specialist clinical environment that may not be reflective of general practice. In this model SOBI patients typically presented for initial clinical assessment four to 14-days post-injury depending on scheduling constraints so findings may not apply to clinical environments that see patients on the day of injury or in clinics that see patients with more chronic issues several weeks/months following SOBI. The proportion of participants who experienced longer recovery trajectories may be inflated in these samples since the patients were those who presented for specialist care. Alternatively, the positive recovery outcomes associated with this model of care may be over optimistic than what would be obtained through a non-specialist pathway. Chapters 5, 6, and 8 were specifically conducted to try and identify methods that would assist clinicians with more general skillsets who work with SOBI patients.

Third, the analyses and interpretations in Chapters 3, 5, 6, and 8 were all largely reliant on symptom reporting. Throughout this document the limitations and issues with symptom reports, specifically in SOBI patients, have been discussed including: the non-specific nature of common mTBI/SOBI related symptoms and their co-morbidity with other conditions [29, 30, 32, 82-86]; delayed onset of symptoms [43]; and the tendency in athletes to minimise symptoms [4-8]. In the case of mTBI/SOBI "asymptomatic" does not necessarily mean completely symptom free as some symptoms commonly associated with mTBI are prevalent in healthy individuals [83-86] (Table 5.1), therefore within asymptomatic was defined as

symptom severity ≤ 5 for males and ≤ 6 for females within the SOBI service participants received [83, 315]. Any one of the issues could influence the interpretation of the results of these studies. Yet, despite these limitations symptom reports are still the main tool clinicians and researchers have to manage and understand outcomes in SOBI patients [39, 43]. Until the identification of a more objective outcome measure SOBI research will continue to rely on measurement of symptoms.

Fourth, 14-days post-injury was set as the threshold to differentiate between patients who experience clinical recovery within the "expected" timeframe versus those who go on to experience prolonged symptom burden in Chapters 3, 5, and 8. This threshold was based on figures from previous consensus statements that suggested 80-90% of SOBI patients should be clinically recovered by this milestone [93], but recovery may be expected to take up to one month for children and adolescents [43]. These guidelines indicate that if SOBI-related symptoms persist beyond these timepoints then patients should then be referred for specialist evaluation. It was my belief that allowing children/adolescents to experience debilitating symptoms for a month before initiating referral for further treatment was inappropriate, thus a more conservative prognostic threshold of 14-days was applied to all participants. Days until asymptomatic was the key criteria used when determining which threshold label a participant received as symptom normalisation was a main factor when determining clinical recovery within the service where participants were managed. Scheduling constraints meant that the number of days until asymptomatic recorded within each dataset typically relied on the participant to self-report what day their symptoms resolved which was confirmed by the SCAT-5 symptom scale on the day of their final appointment. Inaccuracies in the reporting of days until asymptomatic could have influenced analyses. Another limitation of a binary threshold is it sets a discrete boundary that may lead to arbitrary differences in labelling for participants who ultimately experienced recovery in a similar amount of time i.e., 13 days versus 15 days. However, 75% of the participants in Chapter 3 who were above the 14-day threshold required 19 or more days to recovery, which suggests this threshold does a good job separating different recovery trajectories. The difference between 14 and 19 days to recover may seem trivial, but this could mean an extra week of school/work missed which I believe to be a clinically meaningful amount of time. Overall, it may be worth evaluating multiple thresholds in future investigations to see how this influences findings.

Fifth, had stricter inclusion/exclusion criteria and methodological control been prioritised over ecological validity it have reduced the amount of noise and variability in the data and led to positive findings for Chapters 5, 7, and 8. But as outlined, outpatient clinical environments often do not have the resources to perform baseline testing, optimally collect biological samples, nor the ability to withhold treatment from a patient who has a "confounding" condition like attention deficit hyperactivity disorder. The findings of these studies provide an example of the challenge presented to researchers to identify assessment tools that provide valid and reliable results under real-world constraints.

Finally, neurophysiological outcomes were not directly evaluated in Chapters 5-8. Instead, the framework provided in Figure 1.1 [61] was used to identify peripheral assessment targets that would allow inferences to be made about the upstream network and cellular environment following SOBI. This approach was adopted under the assumption that peripheral measures (i.e., sensitivity to tactile stimulus, acceleration during gait, and salivary-BDNF) are in fact representative of the state of the central nervous system (i.e., functional connectivity, sensorimotor integration/vestibular function, and neuroplasticity). Direct measurement of these neurophysiological functions would be optimal, but currently these methods are either highly invasive (i.e., *ex vivo* evaluation of slices of brain tissue from rodents) or underdeveloped and extremely expensive (i.e., functional MRI or diffusion tensor imaging). As it stands, peripheral measures are the most feasible means of gaining insight into the neurophysiological environment within the brain following-SOBI.

Conclusions

This PhD aimed to evaluate how a diverse collection of evolving neurophysiological approaches to assessing and managing mTBI might translate from bench to bedside for SOBI by answering the questions "what factors influence time to recovery following SOBI?" and "what is the translational potential of neurophysiological approaches to advance clinical management of SOBI?", the main conclusions that can be derived from this PhD are as follows.

Section 1 showed that insufficiencies in clinical pathways to be the greatest modifiable factor that influences recovery outcomes post-SOBI in New Zealand. A lack of a thorough assessment can lead to a patient with a history of SOBI to experience debilitating symptoms for years from a potentially unrelated cause. Conversely, an acute model of care delivered by clinicians who are experienced with SOBI can significantly reduce the number of patients who go on to experience symptoms beyond eight weeks. How to scale and implement such a clinical model in smaller communities without specialist clinicians is unclear and requires further attention. Identification of subgroups of SOBI patients based on their PSC appears to be another key factor related to recovery. Patients with vestibulo-ocular symptomology appear particularly vulnerable to negative outcomes, therefore there is a need to educate and train clinicians with how to recognise and treat these symptom profiles more effectively. Overall, current best practice relies heavily on clinical experience/training and honest symptom reporting on behalf of the patient and would benefit from more objective methods to assist clinical decision making. Section 1 also added to growing the body of literature indicating that figures claiming the majority of SOBI patients will be recovered by 14-days post-injury are out of date and require updating as it appears closer to 50% of patients will experience symptoms beyond this timeframe.

The hope for Section 2 was to provide practical recommendations to clinicians in regards to proactive interventions to reduce the proportion of patients who take >14-days to recover and objective assessment

methods that could assist: diagnostic and prognostic decision making; accurate classification of SOBI subgroups that have different underlying impairments responsible for symptomology; and understanding why some patients recover faster than others. Instead, Section 2 highlighted a number of methodological issues that are likely to prevent the translational of neurophysiological approaches to the benefit of current clinical practice in the foreseeable future. Nutritional interventions, in particular ω-3 polyunsaturated fatty acid supplementation, appear to have merit as a potential method to reduce the secondary damage of mTBI/SOBI in a manner that might reduce the amount of time required to recover but the biofidelity of the animal models providing this evidence is low. Neither salivary-BDNF or Brain Gauge somatosensory assessments appear to offer utility to improve clinical assessment of SOBI, nor to evaluate the potential benefit of ω-3 supplementation. The potential value of using wearable sensors and machine learning algorithms to collect and analyse objective data during an already established clinical test is the most promising finding of Section 2 but requires a great amount of development. Overall, the evidence does not suggest that nutritional interventions or neurophysiological assessments will be coming to the aid of clinicians and their SOBI patients in the future. It has been said that approximately 17 years elapses before novel evidence is integrated within clinical practice [425-427]. The findings of section 2 provided examples as to why translation of promising initial findings into practice can take so long. Future studies need to prioritise study designs that emphasise biofidelity and ecological validity so that findings are more likely to translate. In the interim, responsibility falls on healthcare organisations, educational institutes, and sporting bodies to improve the education, standards, and pathways moving forward to reduce the burden caused by persistent complications following SOBI.

Reflections

Clinical lessons and observations

An advantage of being embedded within a functioning clinical environment for a large part of this PhD is the knowledge I have gained through interactions with hundreds of mTBI/SOBI patients, and countless conversations with clinicians, researchers, administrators, athletes, and parents that extends beyond the findings presented in Chapters 2 through 8. This section is meant to synthesise two key observations that evolved as a result of these interactions, which are not directly related to the specific research questions of this thesis but are relevant given the overall theme of my work is to advance the management of SOBI. These lessons are based on my own observations and opinions which I believe deserve to be documented so that they might be considered/investigated in the future.

There appears to be considerable issues with consistent and accurate coding of mTBI in New Zealand. Only physicians can diagnose concussion/mTBI, but at the clinical service where data collection took place referrals were received from general practitioners, emergency department physicians, school nurses, and physiotherapists. Of the patients who I assessed that were referred to us by another healthcare professional injury codes included neck sprain, head contusion, concussion, head injury, and mTBI. In the context of our SOBI service, many referrals came from physiotherapists who performed an on-field assessment during training or competition and recognised the signs and symptoms of mTBI but had to code neck sprain or head contusion to submit their Accident Compensation Corporation referral forms. These examples of the variability and inaccuracy of mTBI coding highlight that the prevalence and burden of these injuries is likely being underestimated.

Similarly, I observed inconsistencies in the advice healthcare practitioners provided by patients referred to our service. Generally, patients who were referred by a general practitioner or emergency department physician were instructed to rest until their symptoms went away. Conversely, some of the patients who had been referred by physiotherapists had been instructed to rest for the first 48 hours, but then to gradually reintegrate cognitive, physical, and sensory load. Scheduling constraints meant that some patients did not present for initial assessment at our service until approximately two weeks post-injury, so patients who received out of date recommendations engaged in a prolonged period of cognitive and physical rest. Many of these patients were confused when we explained that progressive loading was key to their rehabilitation as this conflicted with the messaging from their first point of contact. The Accident Compensation Corporation SportSmart guidelines likely compound this confusion as they also still recommend athletes who sustain SOBI rest until they are symptom free.

Collectively, I believe these two examples indicate a need to empower physiotherapists in the rehabilitation of mTBI/SOBI. Firstly, it is understandable why mTBI diagnosis must be made by a physician, as they are best equipped to rule out more severe neurological sequalae. In a sporting context, physiotherapists are much more accessible than physicians at a grassroots level and are present at many games and tournaments. If physiotherapists could use a "probable mTBI" code to enter a claim into the system that could be later confirmed by a physician it would allow more accurate estimation of the prevalence of these injuries in New Zealand. Secondly, given their knowledge and training of how to progressively load injured tissues and modify exercise programs, physiotherapists are best equipped to develop and deliver treatment programs for SOBI patients. Based on my observations, the current system disenfranchises physiotherapists even though they seem to be more up to date with SOBI literature and better prepared to effectively deliver treatment. There is a need to update and standardise the advice mTBI/SOBI patients receive across healthcare providers, and efforts to empower physiotherapists to play a greater role in the management of SOBI are necessary.

Personal reflection

This thesis began with a personal statement about my past experiences with SOBI and how it has led to a career in research. It only seems appropriate to bookend this document with a personal reflection about the PhD journey. Over the last three years I have begun to think that research topics are like a forest. Each study is a leaf on a brittle twig, but a researcher's cumulative work becomes a strong branch, the branches of colleagues and collaborators unite to form the trunk of a tree, and a collection of trees creates a forest of knowledge. It may be cliché but at the outset of this PhD I naively believed that my ideas and studies were going to immediately make an impact so the original title of my thesis was "Optimising the clinical management of sport-originated brain injury". There was a point during this PhD where I began to doubt myself and my work because of the "negative" findings that went against my hopes/expectations, but I remembered this quote from the late Hall of Fame basketball coach Morgan Wooten:

> It's often been said that you learn more from losing than you do from winning. I think, if you're wise, you learn from both. You learn a lot from a loss. You learn what is it that we're not doing to get to where we want to go. It really gets your attention and it really motivates the work ethic of your team when you're not doing well.

At the end of this journey I realise that both losses and wins strengthen my branch in the forest of knowledge and equip me with the skills and knowledge necessary to conduct high quality and ecologically valid clinical research in the future. Finally, when I was struggling with my own SOBI-related complications I stumbled upon a video from motivational speaker Eric Thomas, in which there was a quote that turned my life and well-being around that said "You have to want to succeed as bad as you want to breathe". Through all the ups and downs of my life so far, I want the concluding words of this PhD journey to be that I feel as though I can breathe at last.

Appendix 1:
Additional methodological details

This thesis adhered to the AUT Pathway 2 format wherein each study was written as a stand alone journal article to be published in an academic journal. Due to journal word count limitations, it was not always possible to include all methodological details in each chapter, therefore the purpose of this appendix is to provide greater methodological clarity where not previously communicated.

Clinical model

As mentioned in Chapter 1, data collection for Chapters 3, 5, 6, and 8 took place during an ACC pilot program to evaluate the effectiveness of an evidence-based acute care clinical model for athletes who suffered a SOBI. A flow diagram depicting how the clinical model operated is provided in Figure 10.1.

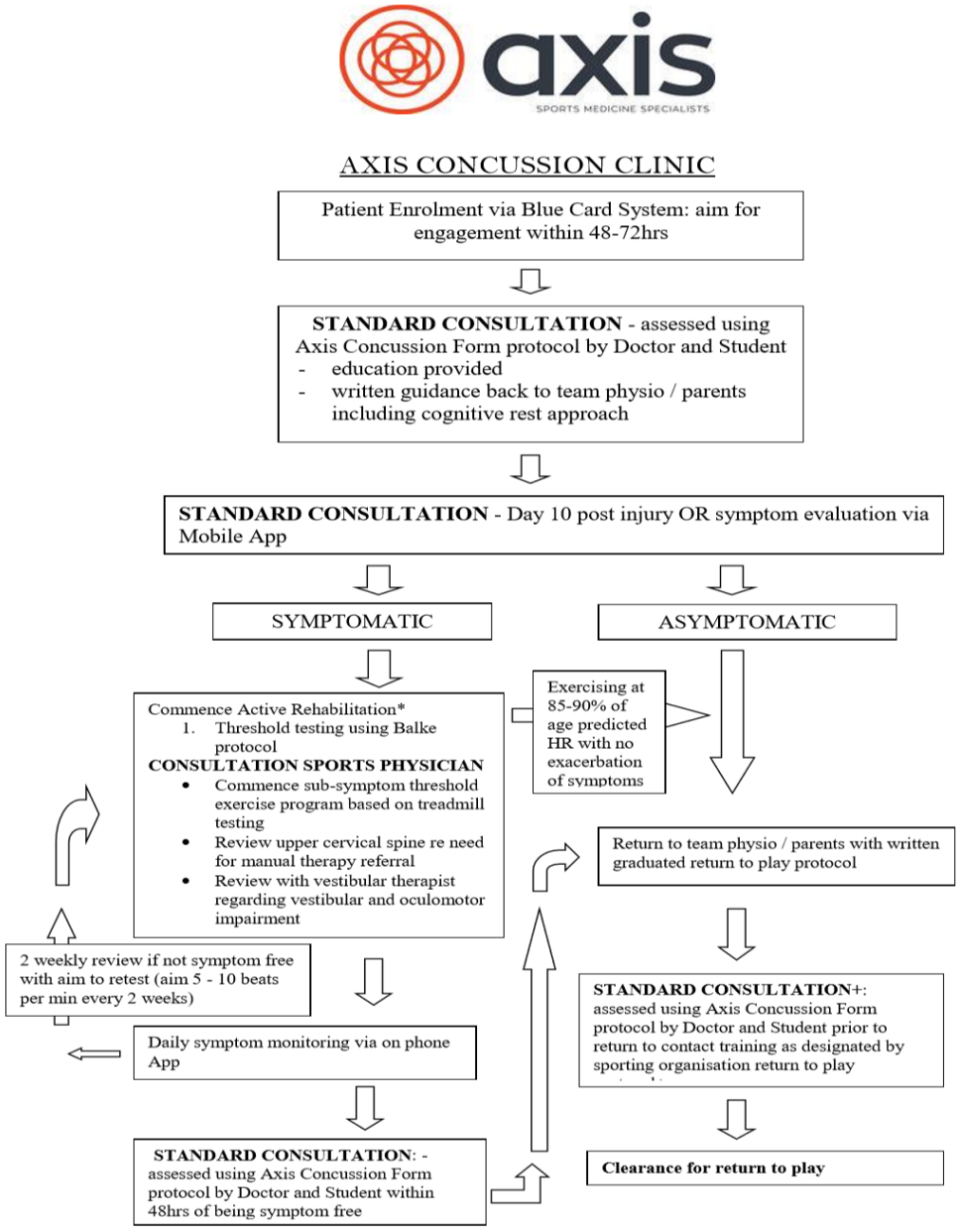


Figure 1. Clinical pathway for SOBI patients who participated in research as part of this thesis.

All patients/participants had a minimum of two clinical appointments: an initial clinical assessment wherein a comprehensive examination was performed by the supervising physician according to evidence-based recommendations [43, 75] and at least one follow-up to determine whether the athlete was clinically recovered and ready to begin a graduated return-to-play protocol [43]. Following the initial clinical assessment patients received education about their injury in lay language including: what an mTBI/SOBI is and why it is serious, what to expect in terms of recovery, and advice on how to progressively reintroduce physical, mental, and sensory stimuli. If patients presented for their initial clinical assessment in the first 24-48 hours post-injury they were also educated to engage in cognitive and physical rest for the first 48-72 hours before progressively reintroducing stimuli.

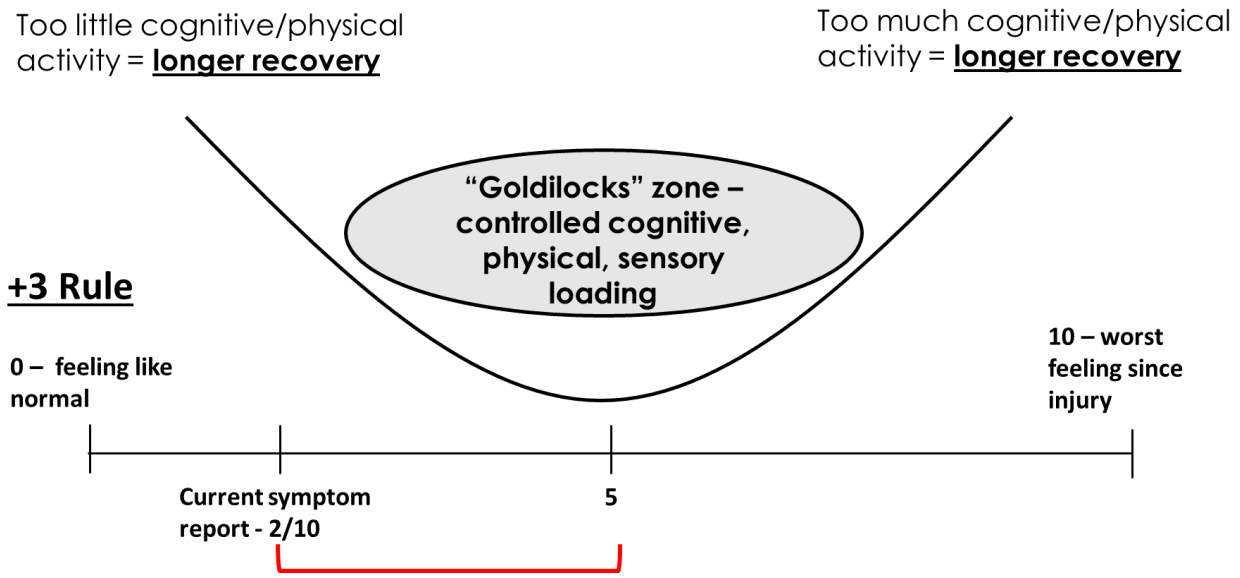


Figure 2. Framework provided to participants to progressively reintroduce loading post-SOBI.

Figure 2 provides a visualisation of how patients were educated to progressively reintroduce loading following SOBI. Before engaging in a physical (i.e., aerobic exercise), cognitive (i.e., doing homework), or sensory (i.e., having lunch in a crowded place) stimuli patients were advised to assess their current symptom burden on a scale of 0-10 (0 representing they feel like their normal self before their injury and 10 feeling their worst since the injury). Once they determined their current state of burden, they were instructed that a slight increase in symptoms by 1-2 points on the 0-10 scale was acceptable but any increase beyond 3 points meant that they should take a break or withdraw from that activity. This provided the patient with a framework to control their symptom burden while reintegrating into day-to-day activities.

When patients presented for their follow-up assessment they were re-evaluated. Those patients whose symptoms had alleviated (defined as symptom severity ≤ 5 for males and ≤ 6 for females [83]), who did not present with any abnormalities upon clinical examination, and who reported tolerance of physical and

cognitive exertion began the graduated return-to-play protocol. Patients who remained symptomatic completed exertional testing following the Buffalo Concussion Treadmill Testing protocol [89, 90]. Based on their clinical examination, symptom burden, and performance on exertion testing patients received an individual treatment plan based on the findings and recommendations of the following studies [13, 14, 43, 75, 90, 91, 94-96, 100-102, 104, 106, 161, 194]. These patients with prolonged recovery were then scheduled for a follow-up assessment every two weeks until their symptom burden was alleviated, and they were able to tolerate normal day-to-day activities.

Outcome measures

Table 1 provides a summary of the outcomes evaluated in the chapters of this thesis along with the how the outcome was measured, how long it took to acquire data for each outcome, and the reliability/validity of each of the tools/techniques that were utilised.

Table 1. Summary of outcomes evaluated throughout the thesis.

Outcome of interest	Measurement tool/technique	Acquisition time	Reliability + Validity
Symptom burden Chapters 3, 5, 6, & 8	Sport Concussion Assessment Tool 5th version (SCAT-5) symptom questionnaire	10 minutes	Reliability + Validity: Echemendia et al. 2017 [65]
Predominant symptom cluster Chapters 3 & 6	Clinical examination + symptom burden	20-30 minute multi-disciplinary clinical assessment	Determined based on criteria presented by Ellis et al. 2015 [67]. Reliability and validity not established but criteria based on evidence-based practice.
Cortical connectivity measured via somatosensory function Chapter 5	Brain Gauge device	15-20 minute testing battery	Reliability: Tannan et al. 2007 [416] Validity: Tommerdahl et al. 2016 [111]
Autonomic control Chapter 6	BioHarness - electrocardiography	During 15-20 minute exertion protocol as part of clinical practice	Reliability: Johnstone et al. 2012 [336] Validity: Johnstone et al. 2012 [337]
Postural control Chapter 6	BioHarness - accelerometry	During 15-20 minute exertion protocol as part of clinical practice	Reliability: Johnstone et al. 2012 [336] Validity: Johnstone et al. 2012 [337]
Neuroplasticity Chapters 7 & 8	Optimised ELISA technique to quantify salivary brain-derived neurotrophic factor	2 minutes to collect saliva	Reliability + Validity: Mandel et al. 2011 [359]

Sample size considerations

This section presents details regarding sample size estimations that were performed during the planning phase of each study that utilised quantitative data analyses within this thesis. A priori sample sizes were determined for all hypothesis generating studies that prospectively collected data using G*Power calculations [428, 429] or recommendations from the statistical literature (Chapters 5-8).

Chapter 3

Retrospective clinical data was available from 568 patients who presented with and received treatment for SOBI from the clinical service within which this PhD was embedded. Since the objective of Chapter 3 was to develop a prognostic multivariable model to predict the binary outcome >14-day versus ≤14-day recovery trajectory logistic regression was selected as the model of choice. Published guidelines indicate that observational studies utilising multivariable logistic regression should have a minimum sample size of n = 500 to estimate the parameters of the target population [430], which was achieved with our retrospective sample.

Chapter 5

In Chapter 5 several analyses were performed to evaluate the clinical utility of the three Brain Gauge somatosensory assessments that were evaluated. The primary analysis for this study/chapter was assessing the prognostic capability of the somatosensory assessments conducted during the initial clinical assessment to match or outperform the accuracy of the model developed with clinical data in Chapter 3 to predict >14-day versus ≤14-day recovery trajectory. Since data were to be prospectively collected for Chapter 5, we anticipated that some participants who completed testing at initial assessment may be lost to follow-up and therefore their recovery trajectory status may be unknown. We were interested in evaluating if differences in somatosensory function measured by the Brain Gauge could predict participants who recovered in the “expected” amount of time (<14-days), those who experienced “delayed” recovery (>14-days), and those who were lost to follow-up, as the ability to predict these three groups would offer clinical value. Since the outcome was no longer binary we elected to utilise a classification and regression tree machine learning algorithm to evaluate the prognostic utility of Brain Gauge somatosensory assessments. Previous work utilising simulations indicated that with a minimum sample size of 80 annotated observations classification machine learning algorithms can yield predictions with ≥80% accuracy [431]; therefore, the minimum target sample size for Chapter 5 was n = 80 participants with initial assessment data.

The secondary analysis was to assess the individual utility of each Brain Gauge somatosensory task to discriminate between participants who sustained SOBI and a reference sample of pooled data from previous investigations that used healthy controls. Univariable logistic regressions were used to evaluate if task performance could accurately discriminate between healthy controls and SOBI participants. Based on sample size tables presented by Hsieh, a minimum total sample size of 164 (with equal proportion of healthy and SOBI cases) would also be satisfactory to detect an odds ratio of 1.5 or greater with $\alpha = 0.05$ and 80% power [432].

The final analyses in Chapter 5 evaluated within and between-group performance differences for the Brain Gauge tasks. Using G*Power 3.1 a priori estimation indicated a sample of n = 54 would be necessary to detect a difference between task performance at initial assessment and clinical discharge with a moderate effect of 0.4, $\alpha = 0.05$, and 80% power. To detect group differences between participants with SOBI and a simulated healthy control sample with a moderate effect of 0.4, $\alpha = 0.05$, and 80% power a total sample size of n = 208 was estimated by G*Power [428, 429].

Chapter 6

To evaluate whether features within physiological and biomechanical data could discriminate between SOBI participants who had a predominantly physiological versus vestibulo-ocular symptom profile a convolutional neural network machine learning algorithm was utilised. There did not appear to be any clear recommendations for sample size considerations for these types of machine learning algorithms using time series data. Additionally, since Chapter 7 was the first study to explore this objective there were no prior studies from which to base our sample size. Previous work has used wearable accelerometers and machine learning to classify different human activities such as sitting, standing, walking, running, etc. It appeared that accurate human activity classification (>80%) could be achieved with time series data from as few as five participants, which has been recently summarised in a comprehensive review by Narayanan et al. [433]. Based on these findings, our minimum target was to obtain data from five SOBI participants with physiological symptomology and five with vestibulo-ocular symptomology for Chapter 6.

Chapter 7

In Chapter 7 two experiments were conducted first to assess how the time of day at which saliva samples were collected contributes to intra-individual variation in salivary-BDNF concentrations and second to evaluate the stability of salivary-BDNF when immediate processing and storage is not possible. Budgeting constraints meant that the sample sizes for each experiment were limited based on the amount of consumable materials that could be purchased within the budget. Since the two experiments in Chapter 7 were in effect reliability studies, sample sizes for each experiment were based on the guide to determine sample sizes for intraclass correlation coefficients (ICC) presented by Bujang & Baharum [434]. This guide

indicated that for obtaining five observations per subject in Experiment 1 a minimum of 3-5 subjects would be necessary to detect moderate to good reliability (ICC 0.6-0.8) with 80% power. Since Experiment 1 included both males and females we endeavored to collect five saliva samples from five males and five females for a total of 50 samples. Due to consumable restraints we only planned to investigate the stability of salivary-BDNF at three time points instead of five. The guide indicated that for three observations per subject 4-8 participants would be necessary to establish moderate to good reliability (ICC 0.6-0.8) with 80% power.

Chapter 8

Since Chapter 8 was the first study to explore the relationship between BDNF and symptom burden following mTBI/SOBI we hypothesised that a negative linear relationship would exist between these variables. Using G*Power 3.1, a minimum sample size of n = 46 observations would be necessary to establish a moderate bivariate relationship (0.4) between BDNF and symptom burden with $\alpha = 0.05$ and 80% power [428, 429].

Additional notes regarding sample size

Data collection for Chapter 5, 6, and 8 all took place in parallel while embedded within the clinical service described earlier. Data collection was to coincide with rugby season as this was when the clinic had the highest volume of new SOBI patients presenting for management (~20-25 new patients per week). Using the sample size considerations for each of the chapters outlined above, the aim was to obtain data from a cohort of 120 participants. Based on the time constraints of a PhD, and an anticipated recruitment rate of 50%, a period of five months was allotted for this data collection. Based on previous data that showed 94% of patients would be recovered by 8 weeks post-injury we planned to bring new participants into the study for the first three months of data collection, leaving the final two months to follow the last participants recruited until they were recovered. Additionally, since Chapter 3 demonstrated that 50% of participants experience symptoms beyond 14 days and that within the clinical model only patients/participants who remained symptomatic beyond 10-14 days performed exertion testing we anticipated a maximum sample size of n = 60 for Chapter 6.

All the chapters utilising quantitative data analyses achieved the minimum a priori sample size except Chapter 5. The prognostic and discriminative analyses were slightly underpowered with data analysed from n = 79 participants rather than >80 participants. Data were collected from 85 participants, but malfunctions with the Brain Gauge meant that data from six participants was not available for these analyses. Both the within- and between-group comparisons in Chapter 5 were also underpowered which may have affected the results that were reported.

Appendix 2:
Protocol for ω-3 supplementation feasibility study

Title: "Feasibility of omega-3 supplementation in sport-related mTBI"

Lay title: "Can omega-3s assist concussion recovery?"

Coordinating investigator: Josh McGeown

Primary contact and co-investigator: Professor Patria Hume

Co-investigators: Dr Ken Quarrie, Dr Alice Theadom, Dr Stephen Kara, Dr Mark Fulcher, Hannah Crosswell

Research protocol and methodology

Rationale

Our recent study suggests that approximately 50% of patients with sport-related mild traumatic brain injury (SR-mTBI) will take >14-days to achieve symptom resolution even when they have received current best practice from a dedicated SR-mTBI clinic [316]. Extended recovery timelines place additional burden on the medical system and on the patient in the form of disrupted daily activities and prolonged symptomology. To date, no pharmaceutical interventions have the capacity to mitigate the secondary phase of injury following SR-mTBI characterised by disrupted neurophysiological mechanisms responsible for normal brain function as pharmaceuticals can only target one neurophysiological pathway. Nutritional strategies demonstrate therapeutic promise to attenuate a number of neurophysiological impairments post-injury. Specifically, pre-clinical evidence demonstrates omega-3 fatty acid supplementation following brain injury can combat inflammation, reduce oxidative stress, assist in cell membrane repair, and restore impaired neurotransmission. Omega-3 supplementation is widely considered safe and has been evaluated in clinical trials in athletic populations along with cardiovascular disease and Alzheimer's patients. Presently, no clinical trials have been conducted following SR-mTBI to evaluate omega-3 supplementation effect on mechanisms responsible for symptom burden. Based on this evidence, a clinical investigation into the potential effect of omega-3 supplementation to attenuate impaired neurophysiology and improve recovery outcomes following SR-mTBI is warranted. However, the feasibility of adding omega-3 supplementation to current best practice must be evaluated before initiating a large scale clinical trial.

Questions

Can seven days of omega-3 supplementation following sport-related mild traumatic brain injury (SR-mTBI) complement current best practice to potentially reduce the proportion of patients that experience complicated symptom recovery?

What are the strengths and weaknesses of the method of administration, timing, dosage, and duration of omega-3 supplementation post-SR-mTBI?

Are differences in clinical and/or neurophysiological outcomes measures observed between the omega-3 and placebo groups?

Purpose

The purpose of this study is to evaluate the feasibility of integrating omega-3 supplementation into current clinical best practice for the management of SR-mTBI. Subsequently, the main aim is to identify the strengths and weaknesses of the proposed methodology to inform the design of future clinical trials evaluating the potential effect of omega-3 supplementation to reduce the proportion of SR-mTBI patients who experience complicated symptom recovery. This investigation will provide preliminary evidence regarding the effect of omega-3 supplementation on clinical and neurophysiological outcome measures compared to placebo.

Participants and recruitment

Participants in this study will be deidentified patients presenting a SR-mTBI to an ACC funded SR-mTBI clinic. A poster advertising the project will be placed in the waiting room of the clinic to inform potential participants of the project. The first 30-40 willing participants who meet inclusion criteria will be selected to participate if they were diagnosed with SR-mTBI by a physician with speciality in the management of SR-mTBI within one week of their injury. Once the physician concludes the initial clinical evaluation, patients who appear to meet inclusion criteria will be verbally informed of the study by Josh McGeown and asked if they would like to receive more information. Interested patients will receive the information sheet and will have to opportunity to ask Josh McGeown questions about what participation entails. If patients wish to participate in the study, written informed consent will be obtained. Following consent and entry into the

study participants will be randomised into the omega-3 supplement or placebo group. No other patient sampling method will be performed to increase the number of participants. Non-participation in the study will not affect patient assessment or treatment in anyway. See Appendix A. for a flowchart detailing how the research fits within clinical practice and what will happen for patients who do or do not wish to participate.

Inclusion/Exclusion

Participants will be included if they are suspected to have sustained a SR-mTBI during sport or physical activity, resulting in a referral to the ACC funded Axis Sports Concussion Clinic and diagnosed with SR-mTBI by Dr Stephen Kara or another clinic physician. Both males and females aged 16 years and older will be eligible for inclusion. Specifically, participants in this study will be SR-mTBI patients who present for SR-mTBI evaluation at a dedicated SR-mTBI clinic within one week of their injury and who, based on their initial clinical evaluation, appear likely to take >14-days to experience symptom resolution which represents a complicated recovery trajectory. This will be determined primarily by clinical judgement of the supervising physician, and assisted by a predictive model developed from historical clinic data [316]. This criteria is necessary because historical data indicate approximately 50% of patients will experience complicated recovery despite receiving best practice management. Hence the aim is to evaluate the feasibility and utility of omega-3 supplementation to reduce the proportion of individuals who experience >14 day (complicated) symptom recovery. If omega-3 supplementation shows promise in this regard, it may serve as a useful strategy to decrease the burden of complicated SR-mTBI for patients themselves, and on the healthcare system.

Participants will not be eligible to participate if they present for their initial appointment more than one week after their injury. Following SR-mTBI impairments in mechanisms that regulate normal brain function are present for approximately for approximately 14-days post-injury; in some individuals these impairments are hypothesized to cause unfavourable compensations which underly persistent symptoms. Therefore, theoretically, omega-3 supplementation within this window may assist in resolving these impairments and prevent subsequent issues. Participants will be excluded if they report hypersensitivity/allergy to fish and/or shellfish as the omega-3s in this study will be derived from fish sources. Additionally, if participants report a known lactose intolerance they will be excluded because the drink preparation contains lactose. Participants will be excluded if they have a pre-diagnosed mental illness, or if they have a pre-diagnosed

neurological condition (i.e. epilepsy, cerebral palsy), or if they are currently taking any blood-thinning medication. Finally, pregnant women will be excluded as there is presently no evidence to support the safety of the omega-3 dosage proposed for this study during pregnancy.

Methods

Initial clinical assessment

During the initial SR-mTBI clinic consultation clinicians will conduct standard clinical service delivery to evaluate SR-mTBI injury characteristics, patient sociodemographic characteristics, patient injury history, and any other clinically relevant information. Next, participants will be asked to complete the Sport Concussion Assessment Tool (SCAT5) to quantify elements of patient history, SR-mTBI symptom examination, and brief screening of cognitive function. As part of standard clinical service, all patients/participants will receive: SR-mTBI education to better understand their injury; and written guidance will be provided to the patient regarding cognitive and physical rest strategies for the initial phase of SR-mTBI recovery.

After completing standard clinical service delivery, patients who meet inclusion criteria and consent to participate will be randomised into either the omega-3 supplement or placebo group. Following randomisation, participants will complete additional measures for research purposes. First, their bodyweight will be measured and they will be asked to complete a brief questionnaire (4 questions) to gain insight into some of their habitual eating habits. Second, once the questionnaire is completed participants will provide a small saliva sample (~2mL) via passive drool. This saliva sample will be immediately placed on ice and transported to a freezer to preserve the sample until analysis at a later date. Lastly, participants will complete Brain Gauge testing, requiring approximately 10 minutes, to evaluate somatosensory function (See Figure 1.). To avoid overburdening clinicians in a busy clinical environment, collection of all research related measures will be conducted by primary researcher, Josh McGeown, in a private area within the clinic. Specifics relating to outcome measures are discussed in further detail in the "Assessments and outcome measures" section below. Standard clinical service delivery typically requires 40-45 minutes during the initial assessment, participation will include an additional 20-25 minutes to obtain consent, collect the research measures, and provide the participant with their omega-3 supplement or placebo.

When participants have completed the additional research measures, they will be provided with their allocation of either omega-3 or placebo juice (details below in "Intervention" section). Instructions for drinking their respective juice (outlined in the participant information sheet) will be explained verbally before they depart the clinic. Participants will have the opportunity to ask questions, and will receive Josh McGeown's contact information in case questions arise at a later time.

Intervention

This study will employ a double-blinded, randomised, parallel group design to investigate the feasibility and potential utility of omega-3 supplementation to complement current best practice compared to a placebo. At the end of the initial appointment, patients who consent to participate will be randomised into either the omega-3 supplement or placebo group. Depending on which group they end up in they will be provided with seven days worth of omega-3 or placebo. Both the omega-3 and placebo will be delivered via a 200mL juice made by *SMARTFISH*, a Norwegian based company (see Figure 2 and supplemental documents for more information). Both the omega-3 and placebo juice are made using the same recipe with the only difference between the juices being the 1000 mg of eicosapentaenoic acid (EPA) and 1000 mg of docosahexaenoic acid (DHA) within the omega-3 juice. Both the omega-3 and placebo will be served in identical white packages. The juice has a pleasant flavour, a smoothie like consistency, and is made from fruit and berry juice. Participants will be provided with a total of 14 juice packages before they depart the initial assessment along with directions to drink one juice in the morning and another in the evening after work/school each day for a total of seven days. For the omega-3 group, this will deliver a total omega-3 supplement dosage of 4g/day (2000 mg EPA; 2000 mg DHA). By consuming the daily dosage of juice, the participant will consume an additional 360 calories of energy intake per day (~180 calories per 200 mL) and this is highlighted in the information sheet. Participants, and primary researcher Josh McGeown, will be blinded regarding which group they are assigned to.

Currently, there is no tolerable upper intake level established for omega-3s, although the European Food Safety Authority states that combined dosages of DHA and EPA up to 5g/day do not raise safety concerns for adults [435]. A systematic review of omega-3 supplementation for individuals with mild and moderate Alzheimer's disease/dementia reported limited benefit of omega-3 supplementation on cognitive outcomes. The review stated that the EPA and DHA dosages may not have been high enough to elicit positive effects, citing combined EPA/DHA dosages ranging from 1500-2300 mg/day [423]. Furthermore,

pre-clinical brain injury studies in animals evaluating different dosages of omega-3 supplementation on neurophysiological outcomes reported greater benefits at higher dosages [218, 248]. Due to the relatively small sample size we intend to recruit in this study, it will not be possible to investigate the effects of different omega-3 dosages. For this reason, and based on the related evidence presented above, we believe the best approach is to investigate if a high dose can display a preliminary effect on clinical and neurophysiological outcomes following SR-mTBI. If a trend towards a positive effect is observed at this dosage, then future large scale clinical trials could evaluate the effectiveness of lower dosages as well. It is also possible that the body weight of the participant may influence the potential effectiveness of omega-3 supplementation on SR-mTBI outcomes, however, there is limited evidence to inform omega-3 dosages based on body weight at this time. To account for this, participants will be weighed before departing the initial assessment to factor the potential influence of bodyweight into data analysis and interpretation. If participant weight does appear to influence the effectiveness of omega-3 supplementation, this information will also be invaluable in designing future clinical trials.

While omega-3 supplementation is widely considered safe, and the potential associated risks are accounted for in the exclusion criteria, there is a chance some participants may experience gastrointestinal complaints as a result of omega-3 supplementation. It will be explained in the information sheets, as well as verbally, that if the participant experiences gastrointestinal issues from the juice they are provided they can choose to no longer drink the juice. If the participant makes this decision, it will not affect their ongoing clinical assessment and treatment in anyway.

Follow-up clinical assessments

All participants/patients will be scheduled for at least one follow-up consultation approximately 7 days following their initial appointment, wherein standard clinical service delivery will be completed again. Additionally, participants will provide another saliva sample at each follow-up. Patients/participants who are not asymptomatic by the first follow-up will complete treadmill testing if requested by the supervising physician. Treadmill testing is part of standard clinical service, and is normally conducted using a standard heart rate monitor. Participants in this study will wear a will wear a sophisticated Zephyr BioHarness during treadmill testing as described in the section below. Symptomatic patients will continue with their management plan until they are symptom free. Subsequent follow-up sessions will be scheduled weekly/bi-weekly accordingly as per standard practice. Once patients/participants are asymptomatic they will receive

a written graduated return to play protocol, the second Brain Gauge testing will take place at this timepoint. Once the participant clears all stages of the return to play protocol, a final consultation will be completed wherein the supervising physician will ensure the participant is cleared to return to contact training, and full competition.

Assessments and Outcome Measures

Participants will be assessed following standard clinical service (see Figure 3 for a flow chart of the clinical management of SR-mTBI) and using the additional research measures outlined in greater detail below. Table 1 includes how the outcome measures will be explained and presented to participants in the information sheet, along with a figure visualising the timepoints when each assessment/outcome measure will be collected.

Standard clinical service

Clinical measures

Relevant patient information will be collected via clinical service delivery; meaning the SR-mTBI clinic specialists will conduct routine consultation to gain knowledge of SR-mTBI injury characteristics, patient sociodemographic characteristics, patient injury history, and any other clinically relevant information. The patient information gathered during initial consultation will be compared to patient information collected during follow-up visit(s) to the SR-mTBI clinic. Observations made by clinicians between initial consultation and follow-up visit(s) will be used to determine whether the patient has clinically recovered from SR-mTBI, or if they need to receive an individualised treatment program to address SR-mTBI symptoms. If individualised treatment and additional follow-up visits are necessary, the clinicians will record treatment parameters and follow-up visit findings until the patient is deemed clinically recovered.

SCAT-5

During the initial consultation participants will complete the SCAT5, which requires approximately 10 minutes to complete, and will be administered at each visit. The SCAT5 is a validated and standardised tool

used widely within clinical assessment of SR-mTBI. The SCAT5 is used in all SR-mTBI clinic SR-mTBI assessments. Within the SCAT5 are questions and assessment tools to clinically quantify elements of patient history, SR-mTBI symptom examination, and treatment.

Additional measures for research purposes

Objective clinical measures for research purposes will include the collection of saliva samples, assessment of somatosensory function via the Brain Gauge, and the addition of a sophisticated heart rate monitor (BioHarness) to current clinical practice of a treadmill test. Participant body weight and information about nutrition habits will be gathered to inform analysis and interpretation of results.

Saliva

Participants will be asked to provide a 2mL saliva sample into a microcentrifuge tube. This saliva sample will be analysed to measure salivary brain-derived neurotrophic factor (BDNF) concentrations in the acute recovery phase of SR-mTBI. A saliva sample will be collected during each follow-up visit to assess changes in salivary-BDNF concentrations over time during SR-mTBI recovery. Total concentrations of BDNF will be measured in picograms per millilitre (pg/mL) utilising an enzyme-linked immunosorbent assay (ELISA) technique following a previously validated protocol.

BioHarness – Treadmill stress test

Participants who are still presenting with SR-mTBI symptoms at the first follow-up visit will undergo a treadmill stress test as per standard of care (if requested by the supervising physician). Participants in this study will wear a sophisticated Zephyr BioHarness device during their stress test to gather detailed physiological data, including: heart rate, heart rate variability, breathing rate, temperature, and postural sway. The BioHarness is safe and is widely used in sport, military, and work settings. It consists of a strap and a sensor that is placed against the skin across the chest in the same manner as a standard heart rate monitor.

Brain Gauge

Somatosensory function will be measured using a Brain Gauge device. The Brain Gauge requires 10 minutes to administer and consists of a sophisticated computer mouse capable of emitting small vibrations to

evaluate tactile sensitivity and discrimination. This somatosensory information will provide insight into central processing following SR-mTBI and throughout patient recovery.

Data analysis

As a feasibility study, this investigation will likely be underpowered to detect differences in effect between the omega-3 and placebo group on clinical and neurophysiological outcomes following SR-mTBI. As a result, we will primarily conduct a descriptive analysis between the two groups to identify if any preliminary differences appear to be present. Additionally, we will interrogate the data using a mixed modeling approach. We will also qualitatively describe the strengths and weaknesses of the methodological approach taken in this feasibility study to inform how to best design future clinical trials.

Figure 1. Flowchart of how the research fits within clinical practice and what will happen for patients who do or do not participate.

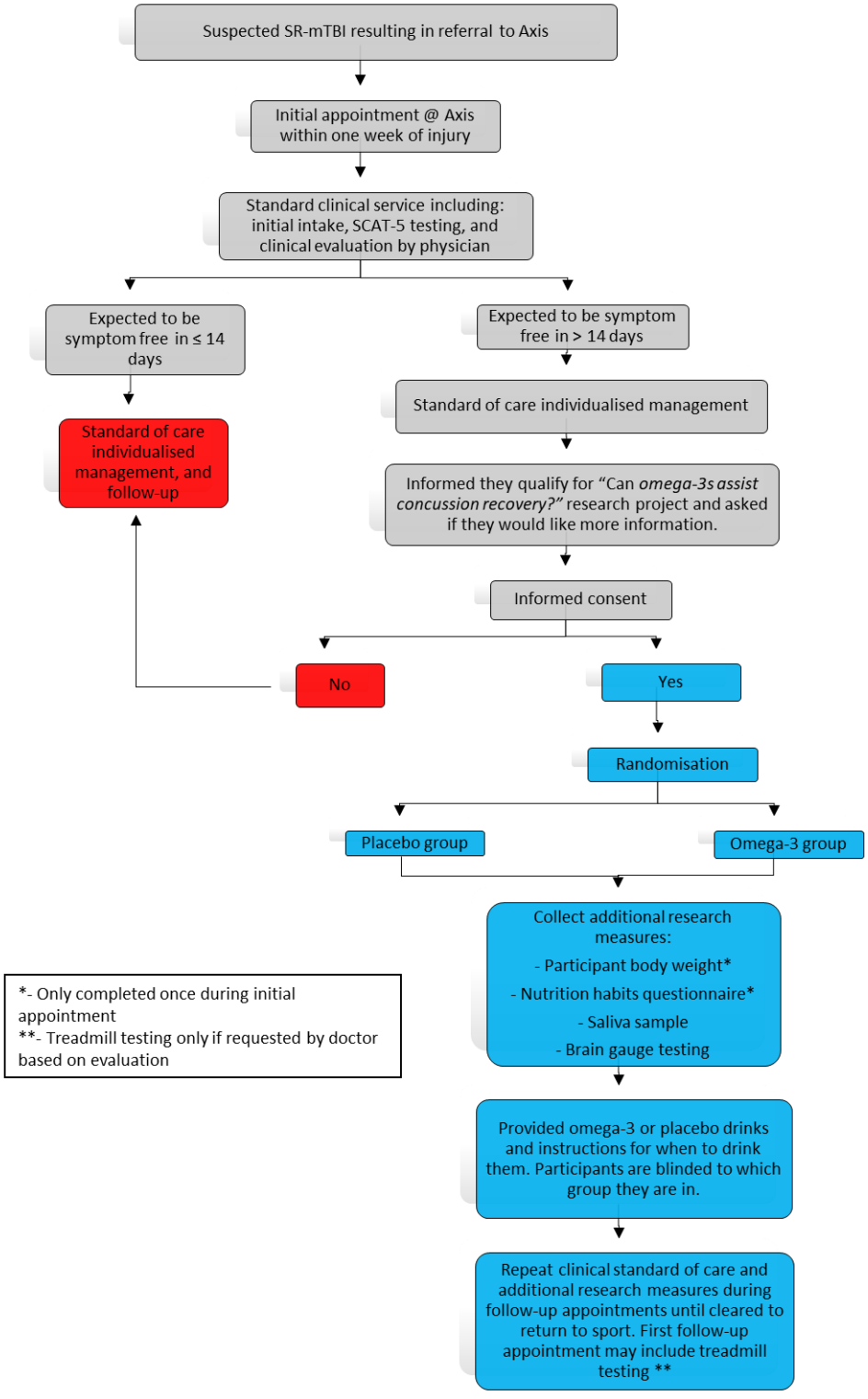


Figure 2. Snapshot of *SMARTFISH* omega-3 product information

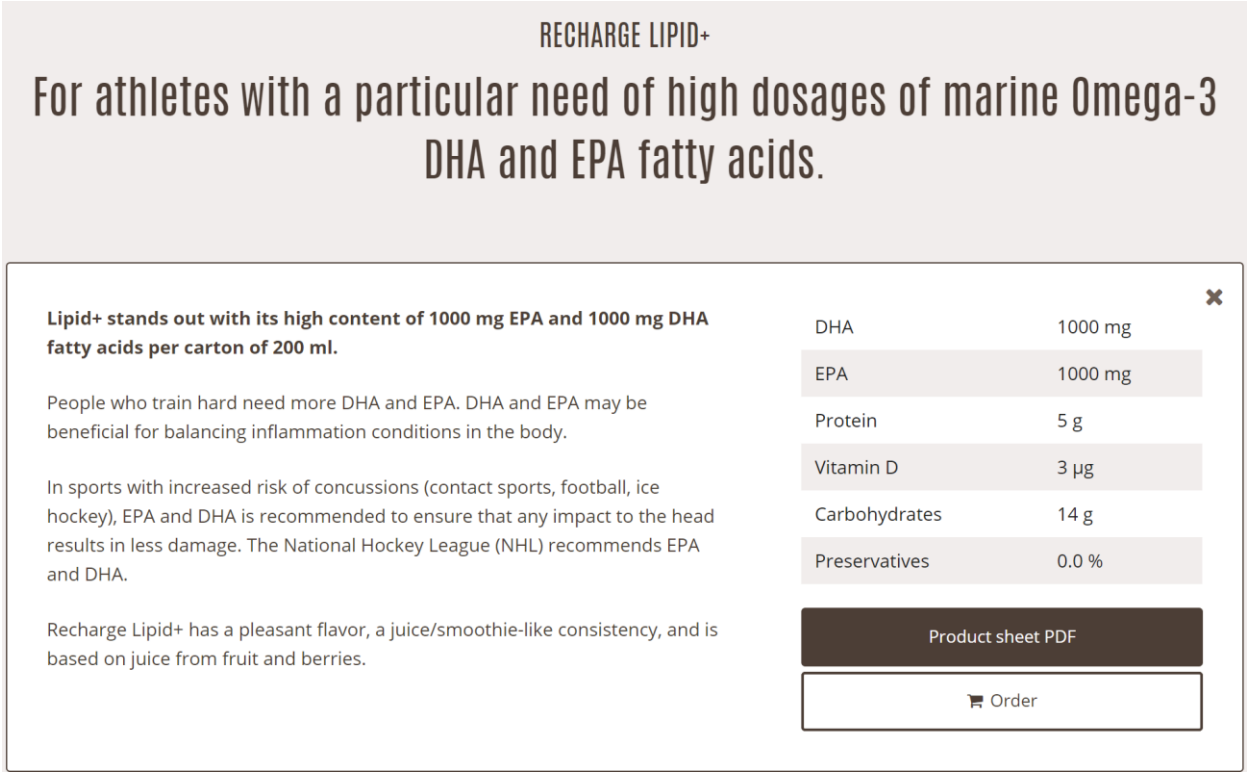


Figure 3. Outline of clinical model for SR-mTBI treatment.

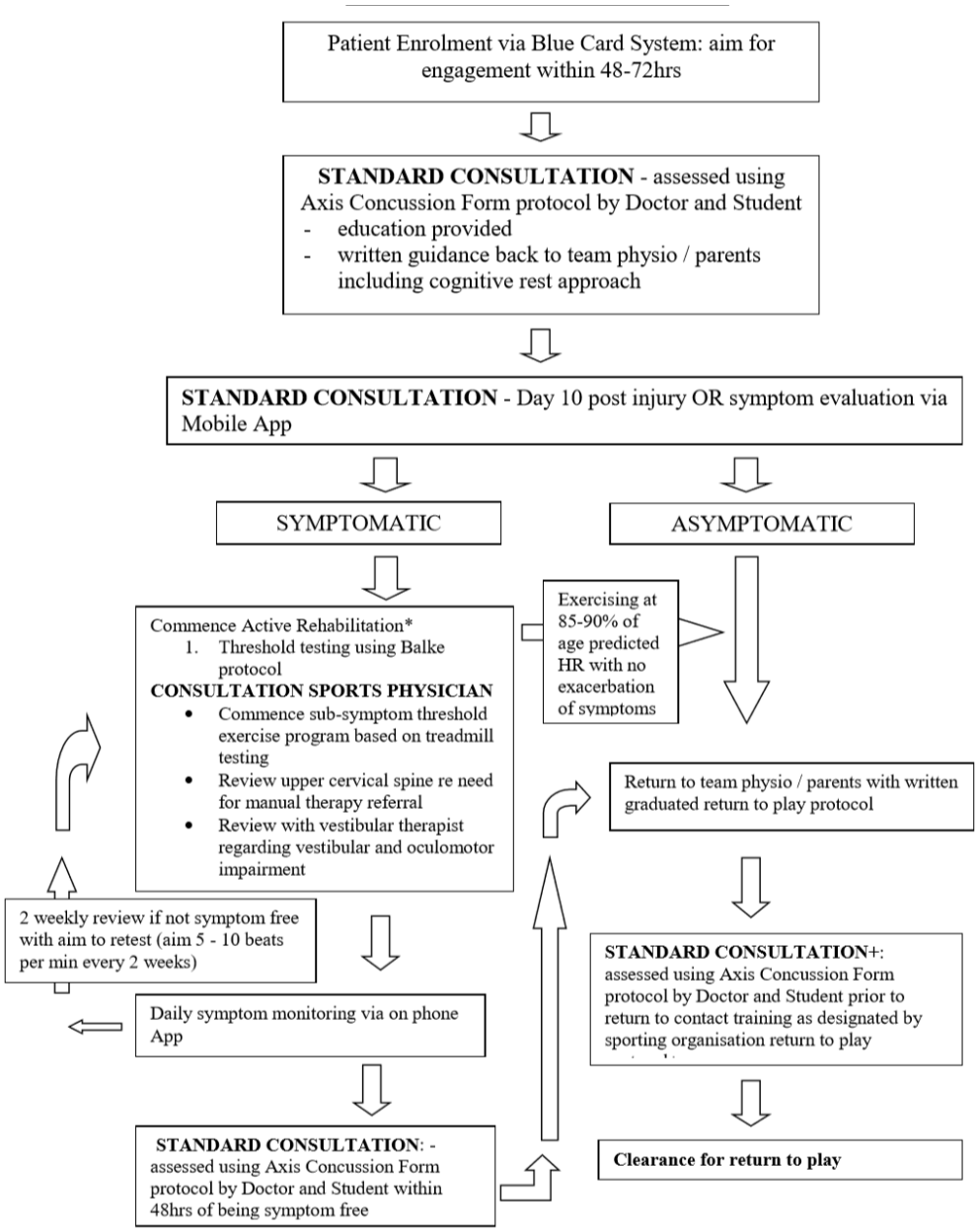
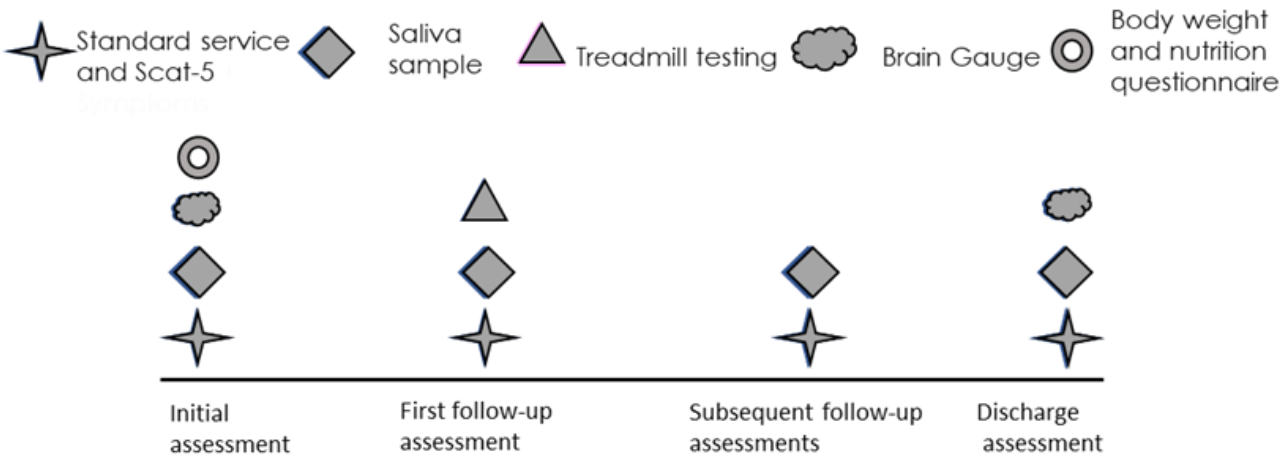


Table 1. Explanations of assessments and outcome measures for information sheet.

Assessment and how long will it take	Research team member responsible	What is it and why is it important?	Photo
1) Initial intake (10-15 mins as part of standard practice)	Hannah Crosswell or Josh McGeown	**What:** Widely used Sport Concussion Assessment Tool to assess someone after a concussion due to sport. **Why:** Measures symptoms, memory, concentration, and balance after concussion.	
2) Clinical evaluation (10-25 mins as part of standard practice)	Dr Stephen Kara or Concussion Clinic physician	**What:** Examination and questions with physician. **Why:** To collect your history, how your injury happened, perform any specific tests to figure out what is causing your symptoms.	
3) Saliva sample (1-5 mins for research purposes)	Josh McGeown	**What**: providing a small (2mL) saliva sample. **Why:** To measure amounts of a protein linked to brain health.	
4a) Additional assessments (20-25 mins for research purposes)	Josh McGeown	**What:** Short nutrition questionnaire and measuring your weight (only at the first appointment). Brain Gauge computerised testing. **Why:** Brain Gauge testing is to accurately assess how your brain is able to process and respond to stimulus to measure your reaction time and brain health. Questionnaire is to understand your normal eating habits. Measuring your weight is to see if weight influences the effect of the supplement.	
4b) Treadmill test (5-20 mins as part of standard practice – addition of special heart rate monitor for research purposes)	Hannah Crosswell or Josh McGeown	**What**: A treadmill test where you walk at a constant pace, and the incline increases every minute until your symptoms get worse or for a maximum of 18 minutes. You will wear a special heart rate monitor that will give the researchers more detailed information about your performance during the treadmill test than a regular heart rate monitor would. *** Not all participants will do this test, only if doctor thinks it is required. **Why:** This is a safe and widely used test, and it provides the doctor with valuable information to design a treatment plan for you.	

Figure 4. Outcome measures collected at each timepoint protocol



Appendix 3:
Ethical approvals

AUTEC Secretariat

Auckland University of Technology
D-88, WU406 Level 4 WU Building City Campus
T: +64 9 921 9999 ext. 8316
E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

12 March 2018

Patria Hume
Faculty of Health and Environmental Sciences

Dear Patria

Re Ethics Application: **18/45 Measuring physiological and neurological function during post-concussion syndrome: A case study**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 12 March 2021.

Non-Standard Conditions of Approval

1. The Information Sheet still refers to 'blood pressure' from finger device;
2. In the Information Sheet moderate statement about 'help with your health'.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by AUTEC before commencing your study.

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining it. You are reminded that it is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard.

For any enquiries, please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: josh.mcgeown@aut.ac.nz

AUTEC Secretariat

Auckland University of Technology
D-88, WU406 Level 4 WU Building City Campus
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E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

13 March 2018

Patria Hume
Faculty of Health and Environmental Sciences

Dear Patria

Re Ethics Application: **18/46 Assessing the incidence, management and outcomes following sport-related concussion in New Zealand. A cohort study investigating presentations to a sport-concussion service**

Thank you for providing evidence as requested, which satisfies the point raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 13 March 2021.

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/researchethics.
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Please quote the application number and title on all future correspondence related to this project.

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For any enquiries, please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: josh.mcgeown@aut.ac.nz; natalie.hardaker@acc.co.nz; Alice Theadom

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington

0800 4 ETHICS
hdecs@moh.govt.nz

6011

20 September 2018

Josh McGeown
SA 207, Level 2, AUT-Millennium Campus
17 Antares Place
Mairangi Bay
Auckland 0632

Dear McGeown

Re:	**Ethics ref:**	**18/NTA/108**
	Study title:	Investigating gender differences and the clinical utility of objective neurophysiological measures for sports-related mTBI

I am pleased to advise that this application has been *approved* by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern A Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *each given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 19 September 2019.

Participant access to ACC

The Northern A Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Dr Brian Fergus
Chairperson
Northern A Health and Disability Ethics Committee

Encl: appendix A: documents submitted appendix B: statement of compliance and list of members

Appendix A Documents submitted

Document	*Version*	*Date*
CV for CI	1.0	27 June 2018
Covering Letter	1.0	27 June 2018
PIS/CF: PIS/CF with Track Changes	2.0	05 August 2018
PIS/CF for persons interested in welfare of non-consenting participant: Child Information and Assent Form with Track Changes	2.0	05 August 2018
Protocol	1.0	01 July 2018
Investigator's Brochure: Recruitment Poster	1.0	01 July 2018
Survey/questionnaire: Sport Concussion Assessment Tool Version 5	1.0	01 July 2018
Evidence of scientific review: Reviewer feedback for CI's entire PhD proposal. Highlighted comments for chapter 5 relate to the current application.	1.0	01 July 2018
CVs for other Investigators: CV for Prof Patria Hume	1.0	03 July 2018
CVs for other Investigators: CV for Prof Patrick Neary	1.0	03 July 2018
CVs for other Investigators: CV for Natalie Hardaker	1.0	03 July 2018
CVs for other Investigators: CV for Dr Stacy Sims	1.0	03 July 2018
CVs for other Investigators: CV for Prof James Selfe	1.0	04 July 2018
CVs for other Investigators: CV for Dr Stephen Kara	1.0	04 July 2018
Application		
PIS/CF: Clean Participant Information Sheet and Consent Form	2.1	05 August 2018
PIS/CF for persons interested in welfare of non-consenting participant: Clean Child Information Sheet and Consent Form	2.1	05 August 2018
PIS/CF for persons interested in welfare of non-consenting participant: Parent Information Sheet and Consent Form with Track Changes	3.0	05 August 2018
PIS/CF for persons interested in welfare of non-consenting participant: Clean Parent Information Sheet and Consent Form	3.1	05 August 2018
Covering Letter: Cover letter summarising provisional acceptance amendments.	1.0	05 August 2018
Response to Request for Further Information		

Appendix B Statement of compliance and list of members

Statement of compliance

The Northern A Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990

 is registered (number 00008714) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Dr Brian Fergus	Lay (consumer/community perspectives)	11/11/2015	11/11/2018
Dr Karen Bartholomew	Non-lay (intervention studies)	13/05/2016	13/05/2019
Dr Christine Crooks	Non-lay (intervention studies)	11/11/2015	11/11/2018
Dr Catherine Jackson	Non-lay (health/disability service provision)	11/11/2016	11/11/2019
Ms Toni Millar	Lay (consumer/community perspectives)	11/11/2016	11/11/2019
Dr Kate Parker	Non-lay (observational studies)	11/11/2015	11/11/2018
Ms Rochelle Style	Lay (ethical/moral reasoning)	14/06/2017	14/06/2020

Unless members resign, vacate or are removed from their office, every member of HDEC shall continue in office until their successor comes into office (HDEC Terms of Reference)

http://www.ethics.health.govt.nz

Auckland University of Technology Ethics Committee (AUTEC)

Auckland University of Technology
D-88, Private Bag 92006, Auckland 1142, NZ
T: +64 9 921 9999 ext. 8316
E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

7 November 2018

Patria Hume
Faculty of Health and Environmental Sciences

Dear Patria

Re Ethics Application: **18/374 Investigating gender differences and the clinical utility of objective neurophysiological measures for sports-related mTBI**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 7 November 2021.

Standard Conditions of Approval

1. A progress report is due annually on the anniversary of the approval date, using form EA2, which is available online through http://www.aut.ac.nz/research/researchethics.
2. A final report is due at the expiration of the approval period, or, upon completion of project, using form EA3, which is available online through http://www.aut.ac.nz/research/researchethics.
3. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form: http://www.aut.ac.nz/research/researchethics.
4. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
5. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.

Please quote the application number and title on all future correspondence related to this project.

AUTEC grants ethical approval only. If you require management approval for access for your research from another institution or organisation then you are responsible for obtaining itYou are reminded that it is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard.

For any enquiries, please contact ethics@aut.ac.nz

Yours sincerely,

Kate O'Connor
Executive Manager
Auckland University of Technology Ethics Committee

Cc: josh.mcgeown@aut.ac.nz; Alice Theadom

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@health.govt.nz

05 February 2020

Mr Josh McGeown
SA 207, Level 2, AUT-Millennium Campus
17 Antares Place
Mairangi Bay
Auckland 0632

Dear Mr McGeown,

Re:	**Ethics ref:**	**19/NTB/169**
	Study title:	Does omega-3 fatty acid supplementation influence sport-related mTBI recovery? A feasibility pilot study

I am pleased to advise that this application has been *approved* by the Northern B Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern B Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *any* locality in New Zealand, it must be registered in a clinical trials registry. This should be a WHO-approved registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au) or https://clinicaltrials.gov/.

3. Before the study commences at *each given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 04 February 2021

Participant access to ACC

This clinical trial is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Section 32 of the Accident Compensation Act 2001 provides that participants injured as a result of treatment received as part of this trial will **not** be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Chairperson
Northern B Health and Disability Ethics Committee

Encl: appendix A: documents submitted appendix B: statement of compliance and list of members

Appendix A Documents submitted

Document	*Version*	*Date*
Covering Letter: Cover letter detailing changes made in response to committee feedback.	2	18 September 2019
Investigator's Brochure	1	18 September 2019
Protocol: Updated track changes version of study protocol	2.1	18 September 2019
PIS/CF: Updated track changes version of PIS/CF	2.1	18 September 2019
Evidence of scientific review: Updated peer review	2	18 September 2019
Survey/questionnaire: Brief nutrition questionnaire	1	18 September 2019
Survey/questionnaire: Sport Concussion Assessment Tool Version 5	1	18 September 2019
Omega-3 supplement product information	1	18 September 2019
CVs for other Investigators: CV for Patria Hume	1	18 September 2019
CVs for other Investigators: CV for Stephen Kara	1	18 September 2019
CV for CI: CV for Josh McGeown	1	18 September 2019
Application		19 September 2019
Response to Request for Further Information		

Appendix B Statement of compliance and list of members

Statement of compliance

The Northern B Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008715) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Mr John Hancock	Lay (the law)	14/12/2015	14/12/2018
Dr Nora Lynch	Non-lay (health/disability service provision)	24/07/2015	24/07/2022
Miss Tangihaere Macfarlane	Lay (consumer/community perspectives)	20/05/2017	20/05/2020
Mrs Kate O'Connor	Lay (ethical/moral reasoning)	14/12/2015	14/12/2018
Mrs Stephanie Pollard	Non-lay (intervention studies)	01/07/2015	01/07/2018
Mrs Leesa Russell	Non-lay (intervention studies), Nonlay (observational studies)	14/12/2015	14/12/2018
Ms Susan Sherrard	Lay (consumer/community perspectives)	19/03/2019	19/03/2022
Mrs Jane Wylie	Non-lay (intervention studies)	20/05/2017	20/05/2020

Unless members resign, vacate or are removed from their office, every member of HDEC shall continue in office until their successor comes into office (HDEC Terms of Reference)

http://www.ethics.health.govt.nz

References

1. McCrory P, Feddermann-Demont N, Dvořák J, Cassidy JD, McIntosh A, Vos PE, et al. What is the definition of sports-related concussion: a systematic review. Br J Sports Med. 2017 Jun;51(11):877-87.

2. Sharp DJ, Jenkins PO. Concussion is confusing us all. Pract Neurol. 2015;15(3):172.

3. Lumba-Brown A, Yeates KO, Sarmiento K, Breiding MJ, Haegerich TM, Gioia GA, et al. Centers for Disease Control and Prevention Guideline on the Diagnosis and Management of Mild Traumatic Brain Injury Among Children. JAMA Pediatr. 2018 Nov 1;172(11):e182853.

4. McCrea M, Hammeke T, Olsen G, Leo P, Guskiewicz K. Unreported concussion in high school football players: implications for prevention. Clin J Sport Med. 2004 Jan;14(1):13-7.

5. Delaney JS, Lacroix VJ, Leclerc S, Johnston KM. Concussions among university football and soccer players. Clin J Sport Med. 2002 Nov;12(6):331-8.

6. Meier TB, Brummel BJ, Singh R, Nerio CJ, Polanski DW, Bellgowan PS. The underreporting of self-reported symptoms following sports-related concussion. J Sci Med Sport. 2015 Sep;18(5):507-11.

7. Asken BM, McCrea MA, Clugston JR, Snyder AR, Houck ZM, Bauer RM. "Playing Through It": Delayed Reporting and Removal From Athletic Activity After Concussion Predicts Prolonged Recovery. Journal of Athletic Training (Allen Press). 2016;51(4):329-35.

8. Clark R, Stanfill AG. A Systematic Review of Barriers and Facilitators for Concussion Reporting Behavior Among Student Athletes. Journal of Trauma Nursing | JTN. 2019;26(6).

9. Carroll LJ, Cassidy JD, Holm L, Kraus J, Coronado VG. Methodological issues and research recommendations for mild traumatic brain injury: the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2004 Feb(43 Suppl):113-25.

10. Theadom A, Starkey NJ, Dowell T, Hume PA, Kahan M, McPherson K, et al. Sports-related brain injury in the general population: an epidemiological study. J Sci Med Sport. 2014 Nov;17(6):591-6.

11. Hume PA, King D, McGeown J, Theadom A. Sports-related concussion, mild traumatic brain injury, or sport-originated brain injury (SOBI): A more useful term. New Zealand Journal of Sports Medicine. 2019;45(2):64-7.

12. Gall B, Parkhouse W, Goodman D. Heart rate variability of recently concussed athletes at rest and exercise. Med Sci Sports Exerc. 2004 Aug;36(8):1269-74.

13. Leddy JJ, Cox JL, Baker JG, Wack DS, Pendergast DR, Zivadinov R, et al. Exercise treatment for postconcussion syndrome: a pilot study of changes in functional magnetic resonance imaging activation, physiology, and symptoms. J Head Trauma Rehabil. 2013 Jul-Aug;28(4):241-9.

14. Leddy JJ, Haider MN, Ellis MJ, Mannix R, Darling SR, Freitas MS, et al. Early Subthreshold Aerobic Exercise for Sport-Related Concussion: A Randomized Clinical Trial. JAMA Pediatrics. 2019;173(4):319-25.

15. Holleman GA, Hooge ITC, Kemner C, Hessels RS. The 'Real-World Approach' and Its Problems: A Critique of the Term Ecological Validity. 2020 2020-April-30;11(721).

16. Wojnarowicz MW, Fisher AM, Minaeva O, Goldstein LE. Considerations for Experimental Animal Models of Concussion, Traumatic Brain Injury, and Chronic Traumatic Encephalopathy-These Matters Matter. Front Neurol. 2017;8:240-.

17. Kent M. Traumatic Brain Injury. The Oxford Dictionary of Sports Science & Medicine. Third ed. Online: Oxford University Press; 2016.

18. Saatman KE, Duhaime A-C, Bullock R, Maas AIR, Valadka A, Manley GT, et al. Classification of traumatic brain injury for targeted therapies. J Neurotrauma. 2008;25(7):719-38.

19. Wang K, Cui D, Gao L. Traumatic brain injury: A review of characteristics, molecular basis and management. Frontiers in Bioscience - Landmark. 2016;21(5):890-9.

20. Douglas DB, Ro T, Toffoli T, Krawchuk B, Muldermans J, Gullo J, et al. Neuroimaging of Traumatic Brain Injury. Med Sci (Basel). 2018;7(1):2.

21. Romeu-Mejia R, Giza CC, Goldman JT. Concussion Pathophysiology and Injury Biomechanics. Curr Rev Musculoskelet Med. 2019 2019/02/28.

22. Jain S, Iverson L. Glasgow Coma Scale. Treasure Island (FL): StatPearls Publishing; 2020.

23. James SL, Theadom A, Ellenbogen RG, Bannick MS, Montjoy-Venning W, Lucchesi LR, et al. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology. 2019;18(1):56-87.

24. Sussman ES, Pendharkar AV, Ho AL, Ghajar J. Mild traumatic brain injury and concussion: terminology and classification. Handb Clin Neurol. 2018;158:21-4.

25. Gardner AJ, Zafonte R. Neuroepidemiology of traumatic brain injury. Handb Clin Neurol. 2016;138:207-23.

26. Barker-Collo S, Theadom A, Jones K, Feigin VL, Kahan M. Accuracy of an International Classification of Diseases Code Surveillance System in the Identification of Traumatic Brain Injury. Neuroepidemiology. 2016;47(1):46-52.

27. Feigin VL, Theadom A, Barker-Collo S, Starkey NJ, McPherson K, Kahan M, et al. Incidence of traumatic brain injury in New Zealand: a population-based study. Lancet Neurol. 2013 Jan;12(1):53-64.

28. Polinder S, Cnossen MC, Real RGL, Covic A, Gorbunova A, Voormolen DC, et al. A Multidimensional Approach to Post-concussion Symptoms in Mild Traumatic Brain Injury. Front Neurol. 2018 2018-December-19;9(1113).

29. Voormolen DC, Cnossen MC, Polinder S, Gravesteijn BY, Von Steinbuechel N, Real RGL, et al. Prevalence of post-concussion-like symptoms in the general population in Italy, The Netherlands and the United Kingdom. Brain Inj. 2019;33(8):1078-86.

30. Voormolen DC, Cnossen MC, Polinder S, von Steinbuechel N, Vos PE, Haagsma JA. Divergent Classification Methods of Post-Concussion Syndrome after Mild Traumatic Brain Injury: Prevalence Rates, Risk Factors, and Functional Outcome. J Neurotrauma. 2018 Jun 1;35(11):1233-41.

31. Cassidy JD, Cancelliere C, Carroll LJ, Cote P, Hincapie CA, Holm LW, et al. Systematic review of self-reported prognosis in adults after mild traumatic brain injury: results of the International Collaboration on Mild Traumatic Brain Injury Prognosis. Arch Phys Med Rehabil. 2014 Mar;95(3 Suppl):S132-51.

32. van der Vlegel M, Polinder S, Toet H, Panneman MJM, Haagsma JA. Prevalence of Post-Concussion-Like Symptoms in the General Injury Population and the Association with Health-Related Quality of Life, Health Care Use, and Return to Work. J Clin Med. 2021;10(4):806.

33. Theadom A, Parag V, Dowell T, McPherson K, Starkey N, Barker-Collo S, et al. Persistent problems 1 year after mild traumatic brain injury: a longitudinal population study in New Zealand. Br J Gen Pract. 2016 Jan;66(642):e16-23.

34. Theadom A, Starkey N, Barker-Collo S, Jones K, Ameratunga S, Feigin V, et al. Population-based cohort study of the impacts of mild traumatic brain injury in adults four years post-injury. PLOS ONE. 2018;13(1):e0191655.

35. Te Ao B, Brown P, Tobias M, Ameratunga S, Barker-Collo S, Theadom A, et al. Cost of traumatic brain injury in New Zealand. Neurology. 2014;83(18):1645.

36. Voormolen DC, Polinder S, von Steinbuechel N, Vos PE, Cnossen MC, Haagsma JA. The association between post-concussion symptoms and health-related quality of life in patients with mild traumatic brain injury. Injury. 2019 May;50(5):1068-74.

37. Howell DR, Wilson JC, Kirkwood MW, Grubenhoff JA. Quality of Life and Symptom Burden 1 Month After Concussion in Children and Adolescents. Clin Pediatr (Phila). 2019 Jan;58(1):42-9.

38. McKee AC, Stern RA, Nowinski CJ, Stein TD, Alvarez VE, Daneshvar DH, et al. The spectrum of disease in chronic traumatic encephalopathy. Brain. 2013 Jan;136(Pt 1):43-64.

39. Iverson GL, Gardner AJ, Terry DP, Ponsford JL, Sills AK, Broshek DK, et al. Predictors of clinical recovery from concussion: a systematic review. Br J Sports Med. 2017;51(12):941-8.

40. Emery CA, Warriyar Kv V, Black AM, Palacios-Derflingher L, Sick S, Debert C, et al. Factors Associated With Clinical Recovery After Concussion in Youth Ice Hockey Players. Orthopaedic Journal of Sports Medicine. 2021 2021/05/01;9(5):23259671211013370.

41. Zemek R, Barrowman N, Freedman SB, Gravel J, Gagnon I, McGahern C, et al. Clinical Risk Score for Persistent Postconcussion Symptoms Among Children With Acute Concussion in the ED. JAMA. 2016 Mar 8;315(10):1014-25.

42. Giza CC, Hovda DA. The new neurometabolic cascade of concussion. Neurosurgery. 2014 Oct;75 Suppl 4:S24-33.

43. McCrory P, Meeuwisse W, Dvorak J, Aubry M, Bailes J, Broglio S, et al. Consensus statement on concussion in sport-the 5(th) international conference on concussion in sport held in Berlin, October 2016. Br J Sports Med. 2017 Jun;51(11):838-47.

44. Lynall RC, Mauntel TC, Padua DA, Mihalik JP. Acute lower extremity injury rates increase after concussion in college athletes. Med Sci Sports Exerc. 2015;47(12):2487-92.

45. Eagle SR, Kontos AP, Pepping G-J, Johnson CD, Sinnott A, LaGoy A, et al. Increased Risk of Musculoskeletal Injury Following Sport-Related Concussion: A Perception–Action Coupling Approach. Sports Med. 2020 2020/01/01;50(1):15-23.

46. Field M, Collins MW, Lovell MR, Maroon J. Does age play a role in recovery from sports-related concussion? A comparison of high school and collegiate athletes. J Pediatr. 2003 May;142(5):546-53.

47. Zuckerman SL, Lee YM, Odom MJ, Solomon GS, Forbes JA, Sills AK. Recovery from sports-related concussion: Days to return to neurocognitive baseline in adolescents versus young adults. Surg Neurol Int. 2012;3:130.

48. Covassin T, Elbin RJ, Harris W, Parker T, Kontos A. The role of age and sex in symptoms, neurocognitive performance, and postural stability in athletes after concussion. Am J Sports Med. 2012 Jun;40(6):1303-12.

49. Baillargeon A, Lassonde M, Leclerc S, Ellemberg D. Neuropsychological and neurophysiological assessment of sport concussion in children, adolescents and adults. Brain Inj. 2012;26(3):211-20.

50. Shrey DW, Griesbach GS, Giza CC. The pathophysiology of concussions in youth. Phys Med Rehabil Clin N Am. 2011 Nov;22(4):577-602, vii.

51. Kleffelgaard I, Roe C, Soberg HL, Bergland A. Associations among self-reported balance problems, post-concussion symptoms and performance-based tests: a longitudinal follow-up study. Disabil Rehabil. 2012;34(9):788-94.

52. Davis GA, Anderson V, Babl FE, Gioia GA, Giza CC, Meehan W, et al. What is the difference in concussion management in children as compared with adults? A systematic review. Br J Sports Med. 2017;51(12):949.

53. Murphy B, Garcia-Roberts G. NFL players with brain trauma receive notice of settlements stripped to nothing. USA Today. 2018.

54. NHL, retired players reach $19M concussions settlement. USA Today. 2018.

55. Ingle S. Rugby players' claim for brain injury has 'less than 50/50' chance, say experts. The Guardian. 2020.

56. LoBue C, Schaffert J, Cullum CM. Chronic traumatic encephalopathy: understanding the facts and debate. Current opinion in psychiatry. 2019 Dec 27.

57. Brett BL, Wilmoth K, Cummings P, Solomon GS, McCrea MA, Zuckerman SL. The Neuropathological and Clinical Diagnostic Criteria of Chronic Traumatic Encephalopathy: A Critical Examination in Relation to Other Neurodegenerative Diseases. J Alzheimers Dis. 2019;68(2):591-608.

58. Iverson GL, Gardner AJ, McCrory P, Zafonte R, Castellani RJ. A critical review of chronic traumatic encephalopathy. Neurosci Biobehav Rev. 2015;56:276-93.

59. Gordon KE, Kuhle S. 'Reported concussion' time trends within two national health surveys over two decades. Brain Inj. 2018 2018/06/07;32(7):843-9.

60. Rosenthal JA, Foraker RE, Collins CL, Comstock RD. National High School Athlete Concussion Rates From 2005-2006 to 2011-2012. The American Journal of Sports Medicine. 2014 2014/07/01;42(7):1710-5.

61. Kenzie ES, Parks EL, Bigler ED, Wright DW, Lim MM, Chesnutt JC, et al. The dynamics of concussion: Mapping pathophysiology, persistence, and recovery with causal-loop diagramming. Front Neurol. 2018;9:203-.

62. Powers CM. The Influence of Abnormal Hip Mechanics on Knee Injury: A Biomechanical Perspective. J Orthop Sports Phys Ther. 2010 2010/02/01;40(2):42-51.

63. Giza CC, Hovda DA. The neurometabolic cascade of concussion. J Athl Train. 2001 Sep;36(3):228-35.

64. Nitta ME, Savitz J, Nelson LD, Teague TK, Hoelzle JB, McCrea MA, et al. Acute elevation of serum inflammatory markers predicts symptom recovery after concussion. Neurology. 2019 Jul 30;93(5):e497-e507.

65. Korley FK, Diaz-Arrastia R, Wu AH, Yue JK, Manley GT, Sair HI, et al. Circulating brain-derived neurotrophic factor has diagnostic and prognostic value in traumatic brain injury. J Neurotrauma. 2016 Jan;33(2):215-25.

66. McCrea M, Broglio SP, McAllister TW, Gill J, Giza CC, Huber DL, et al. Association of Blood Biomarkers With Acute Sport-Related Concussion in Collegiate Athletes: Findings From the NCAA and Department of Defense CARE Consortium. JAMA Network Open. 2020;3(1):e1919771-e.

67. Numakawa T, Richards M, Adachi N, Kishi S, Kunugi H, Hashido K. MicroRNA function and neurotrophin BDNF. Neurochem Int. 2011 2011/10/01/;59(5):551-8.

68. Hicks SD, Onks C, Kim RY, Zhen KJ, Loeffert J, Loeffert AC, et al. Diagnosing mild traumatic brain injury using saliva RNA compared to cognitive and balance testing. Clinical and Translational Medicine. 2020 2020/10/01;10(6):e197.

69. Di Pietro V, Porto E, Ragusa M, Barbagallo C, Davies D, Forcione M, et al. Salivary MicroRNAs: Diagnostic Markers of Mild Traumatic Brain Injury in Contact-Sport. Front Mol Neurosci. 2018 2018-August-20;11(290).

70. Dean PJ, Sato JR, Vieira G, McNamara A, Sterr A. Long-term structural changes after mTBI and their relation to post-concussion symptoms. Brain Inj. 2015 Jun 11:1-8.

71. Kamins J, Bigler E, Covassin T, Henry L, Kemp S, Leddy JJ, et al. What is the physiological time to recovery after concussion? A systematic review. Br J Sports Med. 2017 Jun;51(12):935-40.

72. Tayebi M, Holdsworth SJ, Champagne AA, Cook DJ, Nielsen P, Lee TR, et al. The role of diffusion tensor imaging in characterizing injury patterns on athletes with concussion and subconcussive injury: a systematic review. Brain Inj. 2021 May 12;35(6):621-44.

73. Echemendia RJ, Meeuwisse W, McCrory P, Davis GA, Putukian M, Leddy J, et al. The Sport Concussion Assessment Tool 5th Edition (SCAT5): Background and rationale. Br J Sports Med. 2017 Jun;51(11):848-50.

74. Leddy JJ, Wilier B. Use of Graded Exercise Testing in Consussion and Return-to-Activity Management. Current Sports Medicine Reports (Lippincott Williams & Wilkins). 2013;12(6):370-6.

75. Ellis MJ, Leddy JJ, Willer B. Physiological, vestibulo-ocular and cervicogenic post-concussion disorders: an evidence-based classification system with directions for treatment. Brain Inj. 2015;29(2):238-48.

76. Broglio SP, Katz BP, Zhao S, McCrea M, McAllister T. Test-Retest Reliability and Interpretation of Common Concussion Assessment Tools: Findings from the NCAA-DoD CARE Consortium. Sports Med. 2018 May;48(5):1255-68.

77. Herring SA, Cantu RC, Guskiewicz KM, Putukian M, Kibler WB, Bergfeld JA, et al. Concussion (mild traumatic brain injury) and the team physician: a consensus statement--2011 update. Med Sci Sports Exerc. 2011 Dec;43(12):2412-22.

78. National Collegiate Athletic Association. Interassociation consensus: Diagnosis and management of sport-related concussion best practices; 2016.

79. Broglio SP, Cantu RC, Gioia GA, Guskiewicz KM, Kutcher J, Palm M, et al. National Athletic Trainers' Association position statement: management of sport concussion. J Athl Training. 2014 Mar-Apr;49(2):245-65.

80. Broglio SP, Harezlak J, Katz B, Zhao S, McAllister T, McCrea M. Acute Sport Concussion Assessment Optimization: A Prospective Assessment from the CARE Consortium. Sports Med. 2019 Dec;49(12):1977-87.

81. Iverson GL, Schatz P. Advanced topics in neuropsychological assessment following sport-related concussion. Brain Inj. 2015 2015/01/28;29(2):263-75.

82. Kutcher JS, Giza CC. Sports concussion diagnosis and management. Continuum (Minneap Minn). 2014;20(6 Sports Neurology):1552-69.

83. Alla S, John Sullivan S, McCrory P. Defining asymptomatic status following sports concussion: Fact or fallacy? Br J Sports Med. 2012;46(8):562-9.

84. Asken BM, Snyder AR, Clugston JR, Gaynor LS, Sullan MJ, Bauer RM. Concussion-Like Symptom Reporting in Non-Concussed Collegiate Athletes. Arch Clin Neuropsychol. 2017;32(8):963-71.

85. Rădoi A, Poca MA, Gándara D, Castro L, Cevallos M, Pacios ME, et al. The Sport Concussion Assessment Tool (SCAT2) for evaluating civilian mild traumatic brain injury. A pilot normative study. PloS one. 2019;14(2):e0212541-e.

86. Iverson GL, Silverberg ND, Mannix R, Maxwell BA, Atkins JE, Zafonte R, et al. Factors Associated With Concussion-like Symptom Reporting in High School Athletes. JAMA pediatrics. 2015;169(12):1132-40.

87. Murray N, Salvatore A, Powell D, Reed-Jones R. Reliability and validity evidence of multiple balance assessments in athletes with a concussion. J Athl Training. 2014;49(4):540-9.

88. Teel EF, Marshall SW, Shankar V, McCrea M, Guskiewicz KM. Predicting Recovery Patterns After Sport-Related Concussion. J Athl Train. 2017 Mar;52(3):288-98.

89. Leddy JJ, Baker JG, Kozlowski K, Bisson L, Willer B. Reliability of a graded exercise test for assessing recovery from concussion. Clin J Sport Med. 2011 Mar;21(2):89-94.

90. Leddy JJ, Hinds AL, Miecznikowski J, Darling S, Matuszak J, Baker JG, et al. Safety and Prognostic Utility of Provocative Exercise Testing in Acutely Concussed Adolescents: A Randomized Trial. Clinical Journal Of Sport Medicine: Official Journal Of The Canadian Academy Of Sport Medicine. 2018;28(1):13-20.

91. Leddy JJ, Kozlowski K, Donnelly JP, Pendergast DR, Epstein LH, Willer B. A preliminary study of subsymptom threshold exercise training for refractory post-concussion syndrome. Clin J Sport Med. 2010 Jan;20(1):21-7.

92. Moser RS, Glatts C, Schatz P. Efficacy of immediate and delayed cognitive and physical rest for treatment of sports-related concussion. J Pediatr. 2012 Nov;161(5):922-6.

93. McCrory P, Meeuwisse WH, Aubry M, Cantu B, Dvorak J, Echemendia RJ, et al. Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport held in Zurich, November 2012. Br J Sports Med. 2013 Apr;47(5):250-8.

94. Schneider KJ, Leddy JJ, Guskiewicz KM, Seifert T, McCrea M, Silverberg ND, et al. Rest and treatment/rehabilitation following sport-related concussion: a systematic review. Br J Sports Med. 2017 Jun;51(12):930-4.

95. Grool AM, Aglipay M, Momoli F, Meehan WP, 3rd, Freedman SB, Yeates KO, et al. Association between early participation in physical activity following acute concussion and persistent postconcussive symptoms in children and adolescents. JAMA. 2016 Dec 20;316(23):2504-14.

96. Leddy JJ, Baker JG, Willer B. Active rehabilitation of concussion and post-concussion syndrome. Phys Med Rehabil Clin N Am. 2016 May;27(2):437-54.

97. Silverberg ND, Iaccarino MA, Panenka WJ, Iverson GL, McCulloch KL, Dams-O'Connor K, et al. Management of Concussion and Mild Traumatic Brain Injury: A Synthesis of Practice Guidelines. Arch Phys Med Rehabil. 2020 Feb;101(2):382-93.

98. Ellis MJ, Leddy J, Willer B. Multi-disciplinary management of athletes with post-concussion syndrome: An evolving pathophysiological approach. Front Neurol. 2016;7:136.

99. Leddy JJ, Sandhu H, Sodhi V, Baker JG, Willer B. Rehabilitation of concussion and post-concussion syndrome. Sports Health. 2012 Mar;4(2):147-54.

100. Kurowski BG, Hugentobler J, Quatman-Yates C, Taylor J, Gubanich PJ, Altaye M, et al. Aerobic exercise for adolescents with prolonged symptoms after mild traumatic brain injury: An exploratory randomized clinical trial. J Head Trauma Rehabil. 2017 Mar/Apr;32(2):79-89.

101. Gagnon I, Grilli L, Friedman D, Iverson GL. A pilot study of active rehabilitation for adolescents who are slow to recover from sport-related concussion. Scand J Med Sci Sports. 2016 Mar;26(3):299-306.

102. Quatman-Yates C, Cupp A, Gunsch C, Haley T, Vaculik S, Kujawa D. Physical Rehabilitation Interventions for Post-mTBI Symptoms Lasting Greater Than 2 Weeks: Systematic Review. Phys Ther. 2016;96(11):1753-63.

103. Kontos AP, Deitrick JM, Collins MW, Mucha A. Review of vestibular and oculomotor screening and concussion rehabilitation. J Athl Train. 2017 Mar;52(3):256-61.

104. Alsalaheen BA, Whitney SL, Mucha A, Morris LO, Furman JM, Sparto PJ. Exercise prescription patterns in patients treated with vestibular rehabilitation after concussion. Physiother Res Int. 2013 Jun;18(2):100-8.

105. Storey EP, Wiebe DJ, D'Alonzo BA, Nixon-Cave K, Jackson-Coty J, Goodman AM, et al. Vestibular rehabilitation is associated with visuovestibular improvement in pediatric concussion. Journal of Neurologic Physical Therapy. 2018;42(3):134-41.

106. Schneider KJ, Meeuwisse WH, Nettel-Aguirre A, Barlow K, Boyd L, Kang J, et al. Cervicovestibular rehabilitation in sport-related concussion: a randomised controlled trial. Br J Sports Med. 2014;48(17):1294.

107. Hillman CH, Erickson KI, Kramer AF. Be smart, exercise your heart: exercise effects on brain and cognition. Nat Rev Neurosci. 2008 Jan;9(1):58-65.

108. Phillips C, Baktir MA, Srivatsan M, Salehi A. Neuroprotective effects of physical activity on the brain: a closer look at trophic factor signaling. Front Cell Neurosci. 2014;8:170.

109. Carter JB, Banister EW, Blaber AP. Effect of endurance exercise on autonomic control of heart rate. Sports Med. 2003;33(1):33-46.

110. Smith PJ, Blumenthal JA, Hoffman BM, Cooper H, Strauman TA, Welsh-Bohmer K, et al. Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. Psychosom Med. 2010 Apr;72(3):239-52.

111. Borghi-Silva A, Arena R, Castello V, Simoes RP, Martins LE, Catai AM, et al. Aerobic exercise training improves autonomic nervous control in patients with COPD. Respir Med. 2009 Oct;103(10):1503-10.

112. Szuhany KL, Bugatti M, Otto MW. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. J Psychiatr Res. 2015;60:56-64.

113. Fetzner MG, Asmundson GJG. Aerobic Exercise Reduces Symptoms of Posttraumatic Stress Disorder: A Randomized Controlled Trial. Cogn Behav Ther. 2015 2015/07/04;44(4):301-13.

114. Askari J, Saberi-Kakhki A, Taheri H, Yassini SM, Hassanbeigi A. The effect of aerobic exercise on various symptoms of depression: the mediating role of quality of life. Sport Sciences for Health. 2020 2020/06/01;16(2):273-80.

115. Dimeo F, Bauer M, Varahram I, Proest G, Halter U. Benefits from aerobic exercise in patients with major depression: a pilot study. Br J Sports Med. 2001;35(2):114.

116. New Zealand Government. Accident Compensation Act. 2001.

117. Accident Compensation Corporation. Our vision and values. [cited; Available from: https://careers.acc.co.nz/info/page2#:~:text=Our%20Vision%20and%20Values,everyday%20life%20if%20we%20can.&text=Our%20vision%20is%20to%20create,incidence%20and%20impact%20of%20injury.

118. Accident Compensation Corporation. Concussion Service Operational Guidelines. Wellington, New Zealand: Accident Compensation Corporation; 2020.

119. McPherson K, Fadyl J, Theadom A, Channon A, Levack W, Starkey N, et al. Living Life After Traumatic Brain Injury: Phase 1 of a Longitudinal Qualitative Study. J Head Trauma Rehabil. 2018 Jan/Feb;33(1):E44-e52.

120. Konigs M, Beurskens EA, Snoep L, Scherder EJA, Oosterlaan J. The effects of timing and intensity of neurorehabilitation on functional outcome after traumatic brain injury: A systematic review & meta-analysis. Arch Phys Med Rehabil. 2018 Feb 8.

121. King D, Hume PA, Tommerdahl M. Use of the Brain-Gauge somatosensory assessment for monitoring recovery from concussion: A case study. Journal of Physiotherapy Research. 2018;2(1:13).

122. Tommerdahl M, Dennis RG, Francisco EM, Holden JK, Nguyen R, Favorov OV. Neurosensory Assessments of Concussion. Mil Med. 2016 May;181(5 Suppl):45-50.

123. Favorov OV, Francisco E, Holden J, Kursun O, Zai L, Tommerdahl M. Quantification of Mild Traumatic Brain Injury via Cortical Metrics: Analytical Methods. Mil Med. 2019 Mar 1;184(Suppl 1):228-36.

124. Bishop SA, Dech R, Baker T, Butz M, Aravinthan K, Neary JP. Parasympathetic baroreflexes and heart rate variability during acute stage of sport concussion recovery. Brain Inj. 2017;31(2):247-59.

125. Bishop SA, Dech RT, Guzik P, Neary JP. Heart rate variability and implication for sport concussion. Clin Physiol Funct Imaging. 2017 Nov 16.

126. Wright AD, Smirl JD, Bryk K, Fraser S, Jakovac M, van Donkelaar P. Sport-related concussion alters indices of dynamic cerebral autoregulation. Front Neurol. 2018 03/27

11/10/received

03/13/accepted;9:196.

127. Doherty C, Zhao L, Ryan J, Komaba Y, Inomata A, Caulfield B. Quantification of postural control deficits in patients with recent concussion: An inertial-sensor based approach. Clin Biomech. 2017;42:79-84.

128. Doherty C, Zhao L, Ryan J, Komaba Y, Inomata A, Caulfield B. Concussion is associated with altered preparatory postural adjustments during gait initiation. Human Movement Science. 2017;52:160-9.

129. Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. Front Cell Neurosci. 2019 2019-August-07;13(363).

130. Borich MR, Brodie SM, Gray WA, Ionta S, Boyd LA. Understanding the role of the primary somatosensory cortex: Opportunities for rehabilitation. Neuropsychologia. 2015 07/09;79(Pt B):246-55.

131. Bajaj S, Dailey NS, Rosso IM, Rauch SL, Killgore WDS. Time-dependent differences in cortical measures and their associations with behavioral measures following mild traumatic brain injury. Hum Brain Mapp. 2018 Jan 22.

132. Powers KC, Cinelli ME, Kalmar JM. Cortical hypoexcitability persists beyond the symptomatic phase of a concussion. Brain Inj. 2014;28(4):465-71.

133. Nguyen RH, Ford S, Calhoun AH, Holden JK, Gracely RH, Tommerdahl M. Neurosensory assessments of migraine. Brain Res. 2013 Mar 1;1498:50-8.

134. Pearce AJ, Kidgell DJ, Frazer AK, King DA, Buckland ME, Tommerdahl M. Corticomotor correlates of somatosensory reaction time and variability in individuals with post concussion symptoms. Somatosens Mot Res. 2020 Mar;37(1):14-21.

135. Pearce AJ, Tommerdahl M, King DA. Neurophysiological abnormalities in individuals with persistent post-concussion symptoms. Neuroscience. 2019 Apr 17;408:272-81.

136. Puts NAJ, Harris AD, Mikkelsen M, Tommerdahl M, Edden RAE, Mostofsky SH. Altered tactile sensitivity in children with attention-deficit hyperactivity disorder. J Neurophysiol. 2017 2017/11/01;118(5):2568-78.

137. Tommerdahl M, Tannan V, Holden JK, Baranek GT. Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity? Behav Brain Funct. 2008 Apr 24;4:19.

138. Akin FW, Murnane OD, Hall CD, Riska KM. Vestibular consequences of mild traumatic brain injury and blast exposure: A review. Brain Inj. 2017 Jul;31(9):1188-94.

139. Franke LM, Walker WC, Cifu DX, Ochs AL, Lew HL. Sensorintegrative dysfunction underlying vestibular disorders after traumatic brain injury: A review. J Rehabil Res Dev. 2012;49(7):985-93.

140. Slobounov S, Sebastianelli W, Hallett M. Residual brain dysfunction observed one year post-mild traumatic brain injury: combined EEG and balance study. Clin Neurophysiol. 2012 Sep;123(9):1755-61.

141. Johansson D, Malmgren K, Alt Murphy M. Wearable sensors for clinical applications in epilepsy, Parkinson's disease, and stroke: a mixed-methods systematic review. J Neurol. 2018 Aug;265(8):1740-52.

142. Nazari G, Bobos P, MacDermid JC, Sinden KE, Richardson J, Tang A. Psychometric properties of the Zephyr bioharness device: a systematic review. BMC sports science, medicine & rehabilitation. 2018;10:6.

143. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci. 2001;24:677-736.

144. Neuroplasticity. Concise Medical Dictionary Oxford University Press 2014.

145. Hennigan A, O'Callaghan RM, Kelly AM. Neurotrophins and their receptors: roles in plasticity, neurodegeneration and neuroprotection. Biochem Soc Trans. 2007 Apr;35(Pt 2):424-7.

146. Adachi N, Numakawa T, Richards M, Nakajima S, Kunugi H. New insight in expression, transport, and secretion of brain-derived neurotrophic factor: Implications in brain-related diseases. World J Biol Chem. 2014 Nov 26;5(4):409-28.

147. Rubovitch V, Pharayra A, Har-Even M, Dvir O, Mattson MP, Pick CG. Dietary Energy Restriction Ameliorates Cognitive Impairment in a Mouse Model of Traumatic Brain Injury. J Mol Neurosci. 2019;67(4):613-21.

148. Deng-Bryant Y, Prins ML, Hovda DA, Harris NG. Ketogenic diet prevents alterations in brain metabolism in young but not adult rats after traumatic brain injury. J Neurotrauma. 2011 Sep;28(9):1813-25.

149. Itoh T, Tabuchi M, Mizuguchi N, Imano M, Tsubaki M, Nishida S, et al. Neuroprotective effect of (-)-epigallocatechin-3-gallate in rats when administered pre- or post-traumatic brain injury. J Neural Transm. 2013 May;120(5):767-83.

150. Gerbatin RR, Silva LFA, Hoffmann MS, Della-Pace ID, do Nascimento PS, Kegler A, et al. Delayed creatine supplementation counteracts reduction of GABAergic function and protects against seizures susceptibility after traumatic brain injury in rats. Prog Neuropsychopharmacol Biol Psychiatry. 2019 Jun;92:328-38.

151. Wu AG, Ying Z, Gomez-Pinilla F. Dietary Strategy to Repair Plasma Membrane After Brain Trauma: Implications for Plasticity and Cognition. Neurorehabil Neural Repair. 2014 Jan;28(1):75-84.

152. Hume P, Carlson I, Hardaker N. Concussion guidelines introduction: Safeguarding against concussion harm - launch of the ACC Sport Concussion Guidelines. New Zealand Journal of Sports Medicine. 2014;41(2):61-6.

153. Hume PA, Theadom A, Lewis GN, Quarrie KL, Brown SR, Hill R, et al. A Comparison of Cognitive Function in Former Rugby Union Players Compared with Former Non-Contact-Sport Players and the Impact of Concussion History. Sports Med. 2017 Jun;47(6):1209-20.

154. Bishop SA, Neary JP. Assessing prefrontal cortex oxygenation after sport concussion with near-infrared spectroscopy. Clin Physiol Funct Imaging. 2017 Jun 18;38(4):573-85.

155. Len TK, Neary JP, Asmundson GJ, Goodman DG, Bjornson B, Bhambhani YN. Cerebrovascular reactivity impairment after sport-induced concussion. Med Sci Sports Exerc. 2011 Dec;43(12):2241-8.

156. Len TK, Neary JP, Asmundson GJG, Candow DG, Goodman DG, Bjornson B, et al. Serial monitoring of CO2 reactivity following sport concussion using hypocapnia and hypercapnia. Brain Inj. 2013 2013/03/01;27(3):346-53.

157. Smirl JD, Hoffman K, Tzeng Y-C, Hansen A, Ainslie PN. Methodological comparison of active- and passive-driven oscillations in blood pressure; implications for the assessment of cerebral pressure-flow relationships. Journal Of Applied Physiology (Bethesda, Md: 1985). 2015;119(5):487-501.

158. Ellis MJ, Cordingley D, Vis S, Reimer K, Leiter J, Russell K. Vestibulo-ocular dysfunction in pediatric sports-related concussion. J Neurosurg Pediatr. 2015 Sep;16(3):248-55.

159. McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, et al. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J Neuropathol Exp Neurol. 2009 Jul;68(7):709-35.

160. De Beaumont L, Henry LC, Gosselin N. Long-term functional alterations in sports concussion. Neurosurg Focus. 2012;33(6):1-7.

161. Leddy J, Baker JG, Haider MN, Hinds A, Willer B. A physiological approach to prolonged recovery from sport-related concussion. J Athl Train. 2017 Mar;52(3):299-308.

162. Belanger HG, Barwick FH, Kip KE, Kretzmer T, Vanderploeg RD. Postconcussive symptom complaints and potentially malleable positive predictors. Clin Neuropsychol. 2013;27(3):343-55.

163. Ellis MJ, Ritchie LJ, Koltek M, Hosain S, Cordingley D, Chu S, et al. Psychiatric outcomes after pediatric sports-related concussion. J Neurosurg Pediatr. 2015 Dec;16(6):709-18.

164. Makdissi M, Schneider KJ, Feddermann-Demont N, Guskiewicz KM, Hinds S, Leddy JJ, et al. Approach to investigation and treatment of persistent symptoms following sport-related concussion: a systematic review. Br J Sports Med. 2017 Jun;51(12):958-68.

165. Anzalone AJ, Blueitt D, Case T, McGuffin T, Pollard K, Garrison JC, et al. A positive Vestibular/Ocular Motor Screening (voms) is associated with increased recovery time after sports-related concussion in youth and adolescent athletes. Am J Sports Med. 2017 Feb;45(2):474-9.

166. Meehan 3rd WP, Mannix R, Monuteaux MC, Stein CJ, Bachur RG, Meehan WP, 3rd. Early symptom burden predicts recovery after sport-related concussion. Neurology. 2014;83(24):2204-10.

167. Theadom A, Starkey N, Barker-Collo S, Jones K, Ameratunga S, Feigin V. Population-based cohort study of the impacts of mild traumatic brain injury in adults four years post-injury. PLoS One. 2018;13(1):e0191655.

168. Merritt VC, Meyer JE, Arnett PA. A novel approach to classifying postconcussion symptoms: The application of a new framework to the Post-Concussion Symptom Scale. J Clin Exp Neuropsychol. 2015;37(7):764-75.

169. Mucha A, Collins MW, Elbin RJ, Furman JM, Troutman-Enseki C, DeWolf RM, et al. A brief Vestibular/Ocular Motor Screening (VOMS) assessment to evaluate concussions: Preliminary findings. The American journal of sports medicine. 2014;42(10):2479-86.

170. Rice DA, Kluger MT, McNair PJ, Lewis GN, Somogyi AA, Borotkanics R, et al. Persistent postoperative pain after total knee arthroplasty: a prospective cohort study of potential risk factors. Br J Anaesth. 2018;121(4):804-12.

171. Graham MH. CONFRONTING MULTICOLLINEARITY IN ECOLOGICAL MULTIPLE REGRESSION. Ecology. 2003 2003/11/01;84(11):2809-15.

172. Solomito MJ, Reuman H, Wang DH. Sex differences in concussion: a review of brain anatomy, function, and biomechanical response to impact. Brain Inj. 2018:1-6.

173. Gallagher V, Kramer N, Abbott K, Alexander J, Breiter H, Herrold A, et al. The effects of sex differences and hormonal contraception on outcomes after collegiate sports-related concussion. J Neurotrauma. 2018 Jun 1;35(11):1242-7.

174. Wunderle K, Hoeger KM, Wasserman E, Bazarian JJ. Menstrual phase as predictor of outcome after mild traumatic brain injury in women. J Head Trauma Rehabil. 2014 Sep-Oct;29(5):E1-8.

175. Baker JG, Leddy JJ, Darling SR, Shucard J, Makdissi M, Willer BS. Gender differences in recovery from sports-related concussion in adolescents. Clin Pediatr (Phila). 2016 Jul;55(8):771-5.

176. Merritt VC, Padgett CR, Jak AJ. A systematic review of sex differences in concussion outcome: what do we know? Clin Neuropsychol. 2019 Jan 8:1-28.

177. Reid D, Stuart C, Fulcher M, Hume AP, Theadom A, Whatman C, et al. Knowledge and attitudes (KA) surveys on concussion in sport: Doctors September 2017 Survey. Report #4 to Accident Compensation Corporation.: SPRINZ, Auckland University of Technology 2018.

178. Ellis MJ, Cordingley DM, Vis S, Reimer KM, Leiter J, Russell K. Clinical predictors of vestibulo-ocular dysfunction in pediatric sports-related concussion. J Neurosurg Pediatr. 2017 Jan;19(1):38-45.

179. Costello DM, Kaye AH, O'Brien TJ, Shultz SR. Sport related concussion – Potential for biomarkers to improve acute management. J Clin Neurosci. 2018 2018/10/01/;56:1-6.

180. Papa L. Potential Blood-based Biomarkers for Concussion. Sports Med Arthrosc Rev. 2016 Sep;24(3):108-15.

181. Kim HJ, Tsao JW, Stanfill AG. The current state of biomarkers of mild traumatic brain injury. JCI Insight. 2018 Jan 11;3(1).

182. Bishop SA, Dech RT, Guzik P, Neary JP. Heart rate variability and implication for sport concussion. Clin Physiol Funct Imaging. 2018;38(5):733-42.

183. Senthinathan A, Mainwaring LM, Hutchison M. Heart rate variability of athletes across concussion recovery milestones: A preliminary study. Clinical Journal Of Sport Medicine: Official Journal Of The Canadian Academy Of Sport Medicine. 2017;27(3):288-95.

184. Clausen M, Pendergast DR, Willer B, Leddy J. Cerebral Blood Flow During Treadmill Exercise Is a Marker of Physiological Postconcussion Syndrome in Female Athletes. The Journal Of Head Trauma Rehabilitation. 2016;31(3):215-24.

185. La Fountaine MF, Toda M, Testa AJ, Hill-Lombardi V. Autonomic nervous system responses to concussion: Arterial pulse contour analysis. Front Neurol. 2016;7(FEB).

186. Oldham JR, Munkasy BA, Evans KM, Wikstrom EA, Buckley TA. Altered dynamic postural control during gait termination following concussion. Gait Posture. 2016;49:437-42.

187. Degani AM, Santos MM, Leonard CT, Rau TF, Patel SA, Mohapatra S, et al. The effects of mild traumatic brain injury on postural control. Brain Inj. 2017;31(1):49-56.

188. Chatfield C. Model Uncertainty, Data Mining and Statistical Inference. Journal of the Royal Statistical Society Series A (Statistics in Society). 1995;158(3):419-66.

189. Rusnak M. Giving voice to a silent epidemic. Nature Reviews Neurology. 2013 2013/04/01;9(4):186-7.

190. Fernandes J, Arida RM, Gomez-Pinilla F. Physical exercise as an epigenetic modulator of brain plasticity and cognition. Neurosci Biobehav Rev. 2017 Sep;80:443-56.

191. Gomez-Pinilla F, Vaynman S, Ying Z. Brain-derived neurotrophic factor functions as a metabotrophin to mediate the effects of exercise on cognition. Eur J Neurosci. 2008 Dec;28(11):2278-87.

192. Griesbach GS, Hovda DA, Gomez-Pinilla F. Exercise-induced improvement in cognitive performance after traumatic brain injury in rats is dependent on BDNF activation. Brain Res. 2009 Sep 8;1288:105-15.

193. Griesbach GS, Hovda DA, Molteni R, Wu A, Gomez-Pinilla F. Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor upregulation and recovery of function. Neuroscience. 2004;125(1):129-39.

194. Baker JG, Freitas MS, Leddy JJ, Kozlowski KF, Willer BS. Return to full functioning after graded exercise assessment and progressive exercise treatment of postconcussion syndrome. Rehabil Res Pract. 2012;2012:705309.

195. Leddy JJ, Kozlowski K, Fung M, Pendergast DR, Willer B. Regulatory and autoregulatory physiological dysfunction as a primary characteristic of post concussion syndrome: implications for treatment. Neurorehabilitation. 2007;22(3):199-205.

196. Archer T, Svensson K, Alricsson M. Physical exercise ameliorates deficits induced by traumatic brain injury. Acta Neurol Scand. 2012;125(5):293-302.

197. Alsalaheen BA, Mucha A, Morris LO, Whitney SL, Furman JM, Camiolo-Reddy CE, et al. Vestibular rehabilitation for dizziness and balance disorders after concussion. J Neurol Phys Ther. 2010 Jun;34(2):87-93.

198. Bhatnagar S, Iaccarino MA, Zafonte R. Pharmacotherapy in rehabilitation of post-acute traumatic brain injury. Brain Res. 2016 2016/06/01/;1640:164-79.

199. Meehan WP, 3rd. Medical therapies for concussion. Clin Sports Med. 2011;30(1):115-ix.

200. Miller Phillips M, Reddy CC. Managing Patients with Prolonged Recovery Following Concussion. Phys Med Rehabil Clin N Am. 2016 May;27(2):455-74.

201. Plantier D, Luauté J. Drugs for behavior disorders after traumatic brain injury: Systematic review and expert consensus leading to French recommendations for good practice. Ann Phys Rehabil Med. 2016 2016/02/01/;59(1):42-57.

202. Liu Q, Li R, Qu W, Li B, Yang W, Cui R. Pharmacological and non-pharmacological interventions of depression after traumatic brain injury: A systematic review. Eur J Pharmacol. 2019 2019/12/15/;865:172775.

203. Mohamadpour M, Whitney K, Bergold PJ. The Importance of Therapeutic Time Window in the Treatment of Traumatic Brain Injury. Front Neurosci. 2019 2019-January-23;13(7).

204. Gomez-Pinilla F. The combined effects of exercise and foods in preventing neurological and cognitive disorders. Prev Med. 2011 2011/06/01/;52:S75-S80.

205. Oliver JM, Anzalone AJ, Jones MT, Kirk KM, Gable DA, Gao Y, et al. Nutritional Supplements for the Treatment and Prevention of Sports-Related Concussion - Omega 3 Fatty Acids: Evidence Still Lacking? Current Sports Medicine Reports. 2018;17(3):103-4.

206. Chakraborty S, Skolnick B, Narayan RK. Neuroprotection Trials in Traumatic Brain Injury. Curr Neurol Neurosci Rep. 2016;16(4):1-8.

207. Trojian TH, Wang DH, Leddy JJ. Nutritional supplements for the treatment and prevention of sports-related concussion-evidence still lacking. Curr Sports Med Rep. 2017 Jul/Aug;16(4):247-55.

208. Lukaski HC, Hardy G. Micronutrients in brain function, and traumatic brain injury. Curr Opin Clin Nutr Metab Care. 2013;16(6):700-2.

209. Sharma B, Lawrence DW, Hutchison MG. Branched Chain Amino Acids (BCAAs) and Traumatic Brain Injury: A Systematic Review. The Journal Of Head Trauma Rehabilitation. 2018;33(1):33-45.

210. White H, Venkatesh B. Clinical review: ketones and brain injury. Crit Care. 2011;15(2):219-.

211. Bothe MK, Stover JF. The Branched Chain Amino Acids in the Context of Other Amino Acids in Traumatic Brain Injury. Branched Chain Amino Acids in Clinical Nutrition; 2015. p. 111-25.

212. McDougall A, Bayley M, Munce SE. The ketogenic diet as a treatment for traumatic brain injury: a scoping review. Brain Inj. 2018;32(4):416-22.

213. Davies DJ. Supplementary magnesium in traumatic brain injury: where do we go from here? J R Army Med Corps. 2018 Jun 18.

214. Lewis MD. Concussions, Traumatic Brain Injury, and the Innovative Use of Omega-3s. J Am Coll Nutr. 2016;35(5):469-75.

215. Ashbaugh A, McGrew C. The role of nutritional supplements in sports concussion treatment. Curr Sports Med Rep. 2016 Jan-Feb;15(1):16-9.

216. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014 Mar 26;14:43.

217. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011 Oct 18;343:d5928.

218. Bailes JE, Mills JD. Docosahexaenoic acid reduces traumatic axonal injury in a rodent head injury model. J Neurotrauma. 2010;27(9):1617-24.

219. Cole JT, Mitala CM, Kundu S, Verma A, Elkind JA, Nissim I, et al. Dietary branched chain amino acids ameliorate injury-induced cognitive impairment. Proc Natl Acad Sci U S A. 2010;107(1):366-71.

220. Davis LM, Pauly JR, Readnower RD, Rho JM, Sullivan PG. Fasting is neuroprotective following traumatic brain injury. J Neurosci Res. 2008;86(8):1812-22.

221. Elliott JE, De Luche SE, Churchill MJ, Moore C, Cohen AS, Meshul CK, et al. Dietary therapy restores glutamatergic input to orexin/hypocretin neurons after traumatic brain injury in mice. Sleep. 2018;41(3).

222. Greco T, Glenn TC, Hovda DA, Prins ML. Ketogenic diet decreases oxidative stress and improves mitochondrial respiratory complex activity. Journal Of Cerebral Blood Flow And Metabolism: Official Journal Of The International Society Of Cerebral Blood Flow And Metabolism. 2016;36(9):1603-13.

223. Hu ZG, Wang HD, Jin W, Yin HX. Ketogenic diet reduces cytochrome c release and cellular apoptosis following traumatic brain injury in juvenile rats. Ann Clin Lab Sci. 2009;39(1):76-83.

224. Hu Z-G, Wang H-D, Qiao L, Yan W, Tan Q-F, Yin H-X. The protective effect of the ketogenic diet on traumatic brain injury-induced cell death in juvenile rats. Brain Inj. 2009;23(5):459-65.

225. Ji X, Peng D, Zhang Y, Zhang J, Wang Y, Gao Y, et al. Astaxanthin improves cognitive performance in mice following mild traumatic brain injury. Brain Res. 2017;1659:88-95.

226. Krishna G, Ying Z, Gomez-Pinilla F. Blueberry Supplementation Mitigates Altered Brain Plasticity and Behavior after Traumatic Brain Injury in Rats. Molecular Nutrition and Food Research. 2019.

227. Kumar A, Rinwa P, Dhar H. Microglial inhibitory effect of ginseng ameliorates cognitive deficits and neuroinflammation following traumatic head injury in rats. Inflammopharmacology. 2014;22(3):155-67.

228. Lim MM, Elkind J, Xiong G, Galante R, Zhu J, Zhang L, et al. Dietary therapy mitigates persistent wake deficits caused by mild traumatic brain injury. Sci Transl Med. 2013;5(215):215ra173-215ra173.

229. Liu Y, Wang R, Zhao Z, Dong W, Zhang X, Chen X, et al. Short-term caloric restriction exerts neuroprotective effects following mild traumatic brain injury by promoting autophagy and inhibiting astrocyte activation. Behav Brain Res. 2017;331:135-42.

230. Ozbal S, Cankurt U, Tugyan K, Pekcetin C, Sisman AR, Gunduz K, et al. The effects of α-lipoic acid on immature rats with traumatic brain injury. Biotech Histochem. 2015;90(3):206-15.

231. Özevren H, Irtegün S, Ekingen A, Cudi Tuncer M, Gökalp Özkorkmaz E, Deveci E, et al. Immunoexpression of vascular endothelial growth factor, β-cell lymphoma 2 and cluster of differentiation 68 in cerebellar tissue of rats treated with Ganoderma lucidum. International Journal of Morphology. 2018;36(4):1453-62.

232. Prins ML, Fujima LS, Hovda DA. Age-dependent reduction of cortical contusion volume by ketones after traumatic brain injury. J Neurosci Res. 2005;82(3):413-20.

233. Prins ML, Hovda DA. The effects of age and ketogenic diet on local cerebral metabolic rates of glucose after controlled cortical impact injury in rats. J Neurotrauma. 2009;26(7):1083-93.

234. Schober ME, Requena DF, Abdullah OM, Casper T, Beachy J, Malleske D, et al. Dietary docosahexaenoic acid improves cognitive function, tissue sparing, and magnetic resonance imaging indices of edema and white matter injury in the immature rat after traumatic brain injury. J Neurotrauma. 2016 Feb;33(4):390-402.

235. Schwartzkroin PA, Wenzel HJ, Lyeth BG, Poon CC, Delance A, Van KC, et al. Does ketogenic diet alter seizure sensitivity and cell loss following fluid percussion injury? Epilepsy Res. 2010;92(1):74-84.

236. Sharma S, Ying Z, Gomez-Pinilla F. A pyrazole curcumin derivative restores membrane homeostasis disrupted after brain trauma. Exp Neurol. 2010;226(1):191-9.

237. Shin SS, Dixon CE. Oral fish oil restores striatal dopamine release after traumatic brain injury. Neurosci Lett. 2011;496(3):168-71.

238. Su J, Quan K, Fan K, Zhang J, Liu J, Hua W, et al. High ω-3/ω-6 ratio diet could alleviate blood-brain barrier dysfunction in rat model with traumatic brain injury. Int J Clin Exp Med. 2016;9(11):21270-9.

239. Thau-Zuchman O, Gomes RN, Dyall SC, Davies M, Priestley JV, Groenendijk M, et al. Brain Phospholipid Precursors Administered Post-Injury Reduce Tissue Damage and Improve Neurological Outcome in Experimental Traumatic Brain Injury. J Neurotrauma. 2019;36(1):25-42.

240. Wang HC, Wang BD, Chen MS, Chen H, Sun CF, Shen G, et al. Neuroprotective effect of berberine against learning and memory deficits in diffuse axonal injury. Exp Ther Med. 2018;15(1):1129-35.

241. Wang Y, Zhang C, Peng W, Xia Z, Gan P, Huang W, et al. Hydroxysafflor yellow A exerts antioxidant effects in a rat model of traumatic brain injury. Mol Med Report. 2016;14(4):3690-6.

242. Wu A, Ying Z, Gomez-Pinilla F. The salutary effects of DHA dietary supplementation on cognition, neuroplasticity, and membrane homeostasis after brain trauma. J Neurotrauma. 2011;28(10):2113-22.

243. Wu A, Ying Z, Gomez-Pinilla F. Dietary strategy to repair plasma membrane after brain trauma: implications for plasticity and cognition. Neurorehabil Neural Repair. 2014 Jan;28(1):75-84.

244. Xie G, Peng W, Li P, Xia Z, Zhong Y, He F, et al. A Network Pharmacology Analysis to Explore the Effect of Astragali Radix-Radix Angelica Sinensis on Traumatic Brain Injury. BioMed Research International. 2018:1-13.

245. Xing Z, Xia Z, Peng W, Li J, Zhang C, Fu C, et al. Xuefu Zhuyu decoction, a traditional Chinese medicine, provides neuroprotection in a rat model of traumatic brain injury via an anti-inflammatory pathway. Sci Rep. 2016;6.

246. Zhang F, Wu H, Jin Y, Zhang X. Proton Magnetic Resonance Spectroscopy (H1-MRS) Study of the Ketogenic Diet on Repetitive Mild Traumatic Brain Injury in Adolescent Rats and Its Effect on Neurodegeneration. World Neurosurg. 2018;120:e1193-e202.

247. Zhao GW, Wang Y, Li YC, Jiang ZL, Sun L, Xi X, et al. The neuroprotective effect of modified "shengyu" decoction is mediated through an anti-inflammatory mechanism in the rat after traumatic brain injury. J Ethnopharmacol. 2014;151(1):694-703.

248. Zhu W, Chi N, Zou P, Chen H, Tang G, Zhao W. Effect of docosahexaenoic acid on traumatic brain injury in rats. Exp Ther Med. 2017;14(5):4411-6.

249. Saraiva ALL, Ferreira APO, Silva LFA, Hoffmann MS, Dutra FD, Furian AF, et al. Creatine reduces oxidative stress markers but does not protect against seizure susceptibility after severe traumatic brain injury. Brain Res Bull. 2012;87(2-3):180-6.

250. Xu X, Lv H, Xia Z, Fan R, Zhang C, Wang Y, et al. Rhein exhibits antioxidative effects similar to Rhubarb in a rat model of traumatic brain injury. BMC Complementary and Alternative Medicine. 2017;17(1).

251. Toklu HZ, Hakan T, Biber N, Solakoğlu S, Oğünç AV, Sener G. The protective effect of alpha lipoic acid against traumatic brain injury in rats. Free Radical Research. 2009;43(7):658-67.

252. Mills JD, Bailes JE, Sedney CL, Hutchins H, Sears B. Omega-3 fatty acid supplementation and reduction of traumatic axonal injury in a rodent head injury model: Laboratory investigation. Journal of Neurosurgery. 2011;114(1):77-84.

253. Cheng T, Wang W, Li Q, Han X, Xing J, Qi C, et al. Cerebroprotection of flavanol (-)-epicatechin after traumatic brain injury via Nrf2-dependent and -independent pathways. Free Radical Biology and Medicine. 2016;92:15-28.

254. Wei W, Wang H, Wu Y, Ding K, Li T, Cong Z, et al. Alpha lipoic acid inhibits neural apoptosis via a mitochondrial pathway in rats following traumatic brain injury. Neurochemistry International. 2015;87:85-91.

255. Jiang Z, Zhang J, Cai Y, Huang J, You L. Catechin attenuates traumatic brain injury-induced blood–brain barrier damage and improves longer-term neurological outcomes in rats. Experimental Physiology. 2017;102(10):1269-77.

256. Wang W, Li H, Yu J, Hong M, Zhou J, Zhu L, et al. Protective effects of Chinese herbal medicine Rhizoma drynariae in rats after traumatic brain injury and identification of active compound. Molecular Neurobiology. 2016 Sep;53(7):4809-20.

257. Snilstveit B, Oliver S, Vojtkova M. Narrative approaches to systematic review and synthesis of evidence for international development policy and practice. Journal of Development Effectiveness. 2012 2012/09/01;4(3):409-29.

258. de Vries RBM, Wever KE, Avey MT, Stephens ML, Sena ES, Leenaars M. The usefulness of systematic reviews of animal experiments for the design of preclinical and clinical studies. ILAR J. 2014;55(3):427-37.

259. Ma X, Aravind A, Pfister BJ, Chandra N, Haorah J. Animal Models of Traumatic Brain Injury and Assessment of Injury Severity. Mol Neurobiol. 2019 2019/08/01;56(8):5332-45.

260. Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. Nature reviews Neuroscience. 2013;14(2):128-42.

261. Siebold L, Obenaus A, Goyal R. Criteria to define mild, moderate, and severe traumatic brain injury in the mouse controlled cortical impact model. Exp Neurol. 2018 2018/12/01/;310:48-57.

262. Jackson SJ, Andrews N, Ball D, Bellantuono I, Gray J, Hachoumi L, et al. Does age matter? The impact of rodent age on study outcomes. Lab Anim. 2017;51(2):160-9.

263. Wang Y, Fan X, Tang T, Fan R, Zhang C, Huang Z, et al. Rhein and rhubarb similarly protect the blood-brain barrier after experimental traumatic brain injury via gp91phox subunit of NADPH oxidase/ROS/ERK/MMP-9 signaling pathway. Scientific Reports. 2016;6:37098-.

264. Sakellaris G, Kotsiou M, Tamiolaki M, Kalostos G, Tsapaki E, Spanaki M, et al. Prevention of complications related to traumatic brain injury in children and adolescents with creatine administration: an open label randomized pilot study. J Trauma. 2006 Aug;61(2):322-9.

265. Sakellaris G, Nasis G, Kotsiou M, Tamiolaki M, Charissis G, Evangeliou A. Prevention of traumatic headache, dizziness and fatigue with creatine administration. A pilot study. Acta Paediatr. 2008 Jan;97(1):31-4.

266. Aquilani R, Boselli M, Boschi F, Viglio S, Iadarola P, Dossena M, et al. Branched-chain amino acids may improve recovery from a vegetative or minimally conscious state in patients with traumatic brain injury: a pilot study. Arch Phys Med Rehabil. 2008 Sep;89(9):1642-7.

267. Aquilani R, Iadarola P, Contardi A, Boselli M, Verri M, Pastoris O, et al. Branched-chain amino acids enhance the cognitive recovery of patients with severe traumatic brain injury. Arch Phys Med Rehabil. 2005 Sep;86(9):1729-35.

268. Theadom A, Barker-Collo S, Jones KM, Parmar P, Bhattacharjee R, Feigin VL. MLC901 (NeuroAiD II) for cognition after traumatic brain injury: a pilot randomized clinical trial. Eur J Neurol. 2018 Aug;25(8):1055-e82.

269. Theadom A, Mahon S, Barker-Collo S, McPherson K, Rush E, Vandal AC, et al. Enzogenol for cognitive functioning in traumatic brain injury: a pilot placebo-controlled RCT. Eur J Neurol. 2013;20(8):1135-44.

270. Theadom A, Mahon S, Feigin V, Barker-Collo S, Rush E. Enzogenol supplementation for improving cognitive function post-traumatic brain injury: a pilot study. Neurorehabil Neural Repair; 2012. p. 780.

271. Noguchi H, Nishi D, Matsumura K, Hamazaki K, Hamazaki T, Matsuoka YJ. Limited effect of omega-3 fatty acids on the quality of life in survivors of traumatic injury: A randomized, placebo-controlled trial. Prostaglandins Leukotrienes Essential Fatty Acids. 2017;127:1-5.

272. Walter A, Finelli K, Bai X, Arnett P, Bream T, Seidenberg P, et al. Effect of Enzogenol(R) Supplementation on Cognitive, Executive, and Vestibular/Balance Functioning in Chronic Phase of Concussion. Dev Neuropsychol. 2017;42(2):93-103.

273. Yang DL, Xu JF. Effect of dipeptide of glutamine and alanine on severe traumatic brain injury. Chin J Traumatol. 2007 Jun;10(3):145-9.

274. Shultz SR, McDonald SJ, Vonder Haar C, Meconi A, Vink R, van Donkelaar P, et al. The potential for animal models to provide insight into mild traumatic brain injury: Translational challenges and strategies. Neurosci Biobehav Rev. 2017 May;76(Pt B):396-414.

275. Perel P, Roberts I, Sena E, Wheble P, Briscoe C, Sandercock P, et al. Comparison of treatment effects between animal experiments and clinical trials: systematic review. BMJ. 2007;334(7586):197.

276. Gupte R, Brooks W, Vukas R, Pierce J, Harris J. Sex Differences in Traumatic Brain Injury: What We Know and What We Should Know. J Neurotrauma. 2019 Nov 15;36(22):3063-91.

277. Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. Neurosci Biobehav Rev. 2014 2014/03/01/;40:1-5.

278. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol. 2000 Aug;32(1):56-67.

279. Hiskens MI, Angoa-Perez M, Schneiders AG, Vella RK, Fenning AS. Modeling sports-related mild traumatic brain injury in animals-A systematic review. J Neurosci Res. 2019 Oct;97(10):1194-222.

280. Dixon KJ. Pathophysiology of Traumatic Brain Injury. Phys Med Rehabil Clin N Am. 2017 May;28(2):215-25.

281. Agoston DV. How to Translate Time? The Temporal Aspect of Human and Rodent Biology. Front Neurol. 2017;8:92-.

282. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG, Group NCRRGW. Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol. 2010;160(7):1577-9.

283. Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, et al. A call for transparent reporting to optimize the predictive value of preclinical research. Nature. 2012 Oct 11;490(7419):187-91.

284. Hooijmans CR, Rovers M, de Vries RB, Leenaars M, Ritskes-Hoitinga M. An initiative to facilitate well-informed decision-making in laboratory animal research: report of the First International Symposium on Systematic Reviews in Laboratory Animal Science. Lab Anim. 2012 Oct;46(4):356-7.

285. Collins FS, Tabak LA. Policy: NIH plans to enhance reproducibility. Nature. 2014 Jan 30;505(7485):612-3.

286. Mathias JL, Alvaro PK. Prevalence of sleep disturbances, disorders, and problems following traumatic brain injury: a meta-analysis. Sleep Med. 2012 Aug;13(7):898-905.

287. Shichiri M. The role of lipid peroxidation in neurological disorders. J Clin Biochem Nutr. 2014;54(3):151-60.

288. Stoica BA, Faden AI. Cell death mechanisms and modulation in traumatic brain injury. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2010;7(1):3-12.

289. Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology. 2000 Sep;7(3):153-63.

290. Kuriakose M, Younger D, Ravula AR, Alay E, Rama Rao KV, Chandra N. Synergistic Role of Oxidative Stress and Blood-Brain Barrier Permeability as Injury Mechanisms in the Acute Pathophysiology of Blast-induced Neurotrauma. Sci Rep. 2019 2019/05/22;9(1):7717.

291. Ljubisavljevic S. Oxidative Stress and Neurobiology of Demyelination. Mol Neurobiol. 2016 Jan;53(1):744-58.

292. Luissint A-C, Artus C, Glacial F, Ganeshamoorthy K, Couraud P-O. Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. Fluids Barriers CNS. 2012;9(1):23-.

293. Michael-Titus AT, Priestley JV. Omega-3 fatty acids and traumatic neurological injury: from neuroprotection to neuroplasticity? Trends Neurosci. 2014 Jan;37(1):30-8.

294. Lo Van A, Sakayori N, Hachem M, Belkouch M, Picq M, Lagarde M, et al. Mechanisms of DHA transport to the brain and potential therapy to neurodegenerative diseases. Biochimie. 2016 2016/11/01/;130:163-7.

295. Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors. 2004 Sep;22(3):123-31.

296. Hempstead BL. Brain-derived neurotrophic factor: Three ligands, many actions. Trans Am Clin Climatol Assoc. 2015;126:9-19.

297. Quain AM, Khardori NM. Nutrition in Wound Care Management: A Comprehensive Overview. Wounds : a compendium of clinical research and practice. 2015 Dec;27(12):327-35.

298. Tipton KD. Nutritional Support for Exercise-Induced Injuries. Sports medicine (Auckland, NZ). 2015;45 Suppl 1:S93-S104.

299. Wild T, Rahbarnia A, Kellner M, Sobotka L, Eberlein T. Basics in nutrition and wound healing. Nutrition. 2010 2010/09/01/;26(9):862-6.

300. Jordan S, Tung N, Casanova-Acebes M, Chang C, Cantoni C, Zhang D, et al. Dietary Intake Regulates the Circulating Inflammatory Monocyte Pool. Cell. 2019 2019/08/22/;178(5):1102-14.e17.

301. Wu A, Ying Z, Gomez-Pinilla F. Exercise facilitates the action of dietary DHA on functional recovery after brain trauma. Neuroscience. 2013 Sep 17;248:655-63.

302. King D, Hume PA, Hardaker N, Pearce A, Cummins C, Clark T. Traumatic brain injuries in New Zealand: National Insurance (Accident Compensation Corporation) claims from 2012 to 2016. J Neurol Sci. 2019;399:61-8.

303. Bashir S, Vernet M, Yoo W-K, Mizrahi I, Theoret H, Pascual-Leone A. Changes in cortical plasticity after mild traumatic brain injury. Restor Neurol Neurosci. 2012;30(4):277-82.

304. Castellanos NP, Paúl N, Ordóñ Ez VE, Demuynck O, Bajo R, Campo P, et al. Reorganization of functional connectivity as a correlate of cognitive recovery in acquired brain injury. Brain. 2010;133(8):2365-81.

305. Iraji A, Benson RR, Welch RD, O'Neil BJ, Woodard JL, Ayaz SI, et al. Resting State Functional Connectivity in Mild Traumatic Brain Injury at the Acute Stage: Independent Component and Seed-Based Analyses. J Neurotrauma. 2015;32(14):1031-45.

306. Mayer AR, Mannell MV, Ling J, Gasparovic C, Yeo RA. Functional connectivity in mild traumatic brain injury. Hum Brain Mapp. 2011 2011/11/01;32(11):1825-35.

307. Meehan SK, Mirdamadi JL, Martini DN, Broglio SP. Changes in Cortical Plasticity in Relation to a History of Concussion during Adolescence. Frontiers In Human Neuroscience. 2017;11:5-.

308. Meier T, Bellgowan P, Mayer A, Meier TB, Bellgowan PSF, Mayer AR. Longitudinal assessment of local and global functional connectivity following sports-related concussion. Brain Imaging & Behavior. 2017;11(1):129-40.

309. Militana A, Donahue M, Sills A, Solomon G, Gregory A, Strother M, et al. Alterations in default-mode network connectivity may be influenced by cerebrovascular changes within 1 week of sports related concussion in college varsity athletes: a pilot study. Brain Imaging & Behavior. 2016;10(2):559-68.

310. Moore RD, Broglio SP, Hillman CH. Sport-related concussion and sensory function in young adults. J Athl Train. 2014 Jan-Feb;49(1):36-41.

311. Seeger TA, Kirton A, Esser MJ, Gallagher C, Dunn J, Zewdie E, et al. Cortical excitability after pediatric mild traumatic brain injury. Brain Stimulation. 2017;10(2):305-14.

312. Stevens MC, Lovejoy D, Kim J, Oakes H, Kureshi I, Witt ST. Multiple resting state network functional connectivity abnormalities in mild traumatic brain injury. Brain Imaging & Behavior. 2012;6(2):293-318.

313. Xiao H, Yang Y, Xi J-H, Chen Z-Q. Structural and functional connectivity in traumatic brain injury. Neural regeneration research. 2015;10(12):2062-71.

314. Purves D. The somatic sensory system: Touch and proprioception. In: Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia A, White LE, editors. Neuroscience. 5th ed. Sunderland, Massachusetts USA: Sinauer Associates, Inc; 2012. p. 189-207.

315. Kara S, Crosswell H, Forch K, Cavadino A, McGeown J, Fulcher M. Less Than Half of Patients Recover Within 2 Weeks of Injury After a Sports-Related Mild Traumatic Brain Injury: A 2-Year Prospective Study. Clin J Sport Med. 2020 Mar;30(2):96-101.

316. McGeown JP, Kara S, Fulcher M, Crosswell H, Borotkanics R, Hume PA, et al. Predicting Sport-related mTBI Symptom Resolution Trajectory Using Initial Clinical Assessment Findings: A Retrospective Cohort Study. Sports Med. 2019 2019/12/16.

317. Holden JK, Nguyen RH, Francisco EM, Zhang Z, Dennis RG, Tommerdahl M. A novel device for the study of somatosensory information processing. J Neurosci Methods. 2012 Mar 15;204(2):215-20.

318. Jones CB, Lulic T, Bailey AZ, Mackenzie TN, Mi YQ, Tommerdahl M, et al. Metaplasticity in human primary somatosensory cortex: effects on physiology and tactile perception. J Neurophysiol. 2016;115(5):2681-91.

319. Higgins J, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions: Version 5.1.0 [updated March 2011]. The Cochrane Collaboration; 2011.

320. Whelan R. Effective Analysis of Reaction Time Data. The Psychological Record. 2008 2008/07/01;58(3):475-82.

321. Ratcliff R. Group reaction time distributions and an analysis of distribution statistics. Psychol Bull. 1979 May;86(3):446-61.

322. Fernandez LM, Vadillo MA. Flexibility in reaction time analysis: Many roads to a false positive? Royal Society Open Science. 2020;7(2).

323. McGrath S, Sohn H, Steele R, Benedetti A. Meta-analysis of the difference of medians. Biometrical journal Biometrische Zeitschrift. 2020 Jan;62(1):69-98.

324. Lovell MR, Iverson GL, Collins MW, Podell K, Johnston KM, Pardini D, et al. Measurement of symptoms following sports-related concussion: reliability and normative data for the post-concussion scale. Appl Neuropsychol. 2006;13(3):166-74.

325. Hastie T, Tibshirani R, Friedman JH. Chapter 9 Additive Models, Trees, and Related Methods. The Elements of Statistical Learning: Data Mining, Inference, and Prediction: Springer; 2009.

326. Miller NR, Yasen AL, Maynard LF, Chou L-S, Howell DR, Christie AD. Acute and longitudinal changes in motor cortex function following mild traumatic brain injury. Brain Inj. 2014 Sep;28(10):1270-6.

327. Henry LC, Tremblay J, Tremblay S, Lee A, Brun C, Lepore N, et al. Acute and chronic changes in diffusivity measures after sports concussion. J Neurotrauma. 2011 Oct;28(10):2049-59.

328. Czerniak SM, Sikoglu EM, Liso Navarro AA, McCafferty J, Eisenstock J, Stevenson JH, et al. A resting state functional magnetic resonance imaging study of concussion in collegiate athletes. Brain imaging and behavior. 2015;9(2):323-32.

329. Guay S, De Beaumont L, Drisdelle BL, Lina J-M, Jolicoeur P. Electrophysiological impact of multiple concussions in asymptomatic athletes: A re-analysis based on alpha activity during a visual-spatial attention task. Neuropsychologia. 2018 Jan;108:42-9.

330. Johnson B, Zhang K, Gay M, Horovitz S, Hallett M, Sebastianelli W, et al. Alteration of brain default network in subacute phase of injury in concussed individuals: resting-state fMRI study. Neuroimage. 2012 Jan 2;59(1):511-8.

331. Slobounov SM, Gay M, Zhang K, Johnson B, Pennell D, Sebastianelli W, et al. Alteration of brain functional network at rest and in response to YMCA physical stress test in concussed athletes: RsFMRI study. Neuroimage. 2011;55(4):1716-27.

332. Thériault M, De Beaumont L, Gosselin N, Filipinni M, Lassonde M. Electrophysiological abnormalities in well functioning multiple concussed athletes. Brain Inj. 2009;23(11):899-906.

333. De Beaumont L, Mongeon D, Tremblay S, Messier J, Prince F, Leclerc S, et al. Persistent motor system abnormalities in formerly concussed athletes. J Athl Train. 2011 May-Jun;46(3):234-40.

334. Leddy John J. Buffalo Concussion Test - Instruction Manual.

335. Leddy JJ, Baker JG, Merchant A, Picano J, Gaile D, Matuszak J, et al. Brain or strain? Symptoms alone do not distinguish physiologic concussion from cervical/vestibular injury. Clin J Sport Med. 2015 May;25(3):237-42.

336. Galbraith H, Quesnele J, Kenrick-Rochon S, Grenier S, Baldisera T. What are the knowledge, attitudes and beliefs regarding concussion of primary care physicians and family resident physicians in rural communities? Journal of Concussion. 2020 2020/01/01;4:2059700220974548.

337. Yorke AM, Littleton S, Alsalaheen BA. Concussion Attitudes and Beliefs, Knowledge, and Clinical Practice: Survey of Physical Therapists. Phys Ther. 2016 Jul;96(7):1018-28.

338. Reid D, Stuart C, Fulcher M, Hume AP, Theadom A, Whatman C, et al. Knowledge and attitudes (KA) surveys on concussion in sports: Doctor september 2017 survey. Report #4 to Accident Compensation Corporation: SPRINZ, Auckland University of Technology; 2018.

339. Russell B, McDaid A, Toscano W, Hume P. Moving the Lab into the Mountains: A Pilot Study of Human Activity Recognition in Unstructured Environments. Sensors (Basel). 2021;21(2):654.

340. Sidey-Gibbons JAM, Sidey-Gibbons CJ. Machine learning in medicine: a practical introduction. BMC Med Res Methodol. 2019 2019/03/19;19(1):64.

341. Ignatov A. Real-time human activity recognition from accelerometer data using Convolutional Neural Networks. Applied Soft Computing. 2018 2018/01/01/;62:915-22.

342. Clouthier AL, Ross GB, Graham RB. Sensor Data Required for Automatic Recognition of Athletic Tasks Using Deep Neural Networks. 2020 2020-January-21;7(473).

343. Attia ZI, Kapa S, Lopez-Jimenez F, McKie PM, Ladewig DJ, Satam G, et al. Screening for cardiac contractile dysfunction using an artificial intelligence–enabled electrocardiogram. Nat Med. 2019 2019/01/01;25(1):70-4.

344. Esteva A, Robicquet A, Ramsundar B, Kuleshov V, DePristo M, Chou K, et al. A guide to deep learning in healthcare. Nat Med. 2019 2019/01/01;25(1):24-9.

345. Haider MN, Leddy JJ, Wilber CG, Viera KB, Bezherano I, Wilkins KJ, et al. The Predictive Capacity of the Buffalo Concussion Treadmill Test After Sport-Related Concussion in Adolescents. 2019 2019-April-24;10(395).

346. Borg G. Borg's perceived exertion and pain scales. Champaign, IL, US: Human Kinetics; 1998.

347. Johnstone JA, Ford PA, Hughes G, Watson T, Garrett AT. Bioharness() multivariable monitoring device: Part. Ii: Reliability. J Sports Sci Med. 2012;11(3):409-17.

348. Johnstone JA, Ford PA, Hughes G, Watson T, Garrett AT. Bioharness() multivariable monitoring device: Part. I: Validity. J Sports Sci Med. 2012;11(3):400-8.

349. Cust EE, Sweeting AJ, Ball K, Robertson S. Machine and deep learning for sport-specific movement recognition: a systematic review of model development and performance. J Sports Sci. 2019 2019/03/04;37(5):568-600.

350. Kis M. Reliability of a New Test of Balance Function in Healthy and Concussion Populations. Journal of functional morphology and kinesiology. 2020 Feb 14;5(1).

351. Hou Y-R, Chiu Y-L, Chiang S-L, Chen H-Y, Sung W-H. Development of a Smartphone-Based Balance Assessment System for Subjects with Stroke. Sensors (Basel). 2019;20(1):88.

352. Saito T, Rehmsmeier M. The Precision-Recall Plot Is More Informative than the ROC Plot When Evaluating Binary Classifiers on Imbalanced Datasets. PLOS ONE. 2015;10(3):e0118432.

353. Hajian-Tilaki K. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. Caspian J Intern Med. 2013 Spring;4(2):627-35.

354. Trevethan R. Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice. Frontiers in public health. 2017;5:307-.

355. Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S. New insights into brain BDNF function in normal aging and Alzheimer disease. Brain Res Rev. 2008 Nov;59(1):201-20.

356. Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry. 2008;64(6):527-32.

357. Molendijk ML, Spinhoven P, Polak M, Bus BA, Penninx BW, Elzinga BM. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). Mol Psychiatry. 2014 Jul;19(7):791-800.

358. Green MJ, Matheson SL, Shepherd A, Weickert CS, Carr VJ. Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis. Mol Psychiatry. 2011 Sep;16(9):960-72.

359. Fernandes BS, Gama CS, Ceresér KM, Yatham LN, Fries GR, Colpo G, et al. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. J Psychiatr Res. 2011 Aug;45(8):995-1004.

360. Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, et al. Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Prog Neuropsychopharmacol Biol Psychiatry. 2006 Dec 30;30(8):1529-31.

361. Gejl AK, Enevold C, Bugge A, Andersen MS, Nielsen CH, Andersen LB. Associations between serum and plasma brain-derived neurotrophic factor and influence of storage time and centrifugation strategy. Sci Rep. 2019;9(1):9655-.

362. Polacchini A, Metelli G, Francavilla R, Baj G, Florean M, Mascaretti LG, et al. A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. Sci Rep. 2015 12/10/online;5:17989.

363. Sartorius A, Hellweg R, Litzke J, Vogt M, Dormann C, Vollmayr B, et al. Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. Pharmacopsychiatry. 2009 Nov;42(6):270-6.

364. Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, et al. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. Int J Neuropsychopharmacol. 2011;14(3):347-53.

365. Granger DA, Johnson SB, Szanton SL, Out D, Schumann LL. Incorporating salivary biomarkers into nursing research: an overview and review of best practices. Biol Res Nurs. 2012;14(4):347-56.

366. Bhattarai KR, Kim H-R, Chae H-J. Compliance with Saliva Collection Protocol in Healthy Volunteers: Strategies for Managing Risk and Errors. Int J Med Sci. 2018;15(8):823-31.

367. McLenon J, Rogers MAM. The fear of needles: A systematic review and meta-analysis. J Adv Nurs. 2019 Jan;75(1):30-42.

368. Mandel AL, Ozdener H, Utermohlen V. Identification of pro- and mature brain-derived neurotrophic factor in human saliva. Arch Oral Biol. 2009 Jul;54(7):689-95.

369. Mandel AL, Ozdener H, Utermohlen V. Brain-derived neurotrophic factor in human saliva: ELISA optimization and biological correlates. J Immunoassay Immunochem. 2011;32(1):18-30.

370. Vrijen C, Schenk HM, Hartman CA, Oldehinkel AJ. Measuring BDNF in saliva using commercial ELISA: Results from a small pilot study. Psychiatry Res. 2017 2017/08/01/;254:340-6.

371. McGeown JP, Zerpa C, Lees S, Niccoli S, Sanzo P. Implementing a structured exercise program for persistent concussion symptoms: a pilot study on the effects on salivary brain-derived neurotrophic factor, cognition, static balance, and symptom scores. Brain Inj. 2018 2018/10/15;32(12):1556-65.

372. Tirassa P, Iannitelli A, Sornelli F, Cirulli F, Mazza M, Calza A, et al. Daily serum and salivary BDNF levels correlate with morning-evening personality type in women and are affected by light therapy. Rivista di psichiatria. 2012 Nov-Dec;47(6):527-34.

373. Susa TR, Brandt RD, Kangas KJ, Bammert CE, Ottem EN, Moore MT, et al. Elevated levels of serum, but not salivary, brain-derived neurotrophic factor following mild traumatic brain injury in collegiate athletes post return-to-play. Journal of Concussion. 2019 2019/01/01;3:2059700219894108.

374. Ikai S, Suzuki T, Uchida H, Saruta J, Tsukinoki K, Fujii Y, et al. Effects of weekly one-hour Hatha yoga therapy on resilience and stress levels in patients with schizophrenia-spectrum disorders: an eight-week randomized controlled trial. J Altern Complement Med. 2014 Nov;20(11):823-30.

375. Jasim H, Carlsson A, Hedenberg-Magnusson B, Ghafouri B, Ernberg M. Saliva as a medium to detect and measure biomarkers related to pain. Sci Rep. 2018 2018/02/19;8(1):3220.

376. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. Clin Chim Acta. 2007 2007/08/01/;383(1):30-40.

377. Esser D, Alvarez-Llamas G, de Vries MP, Weening D, Vonk RJ, Roelofsen H. Sample stability and protein composition of saliva: Implications for its use as a diagnostic fluid. Biomark Insights. 2008;3:25-7.

378. Walsh JJ, Tschakovsky ME. Exercise and circulating BDNF: Mechanisms of release and implications for the design of exercise interventions. Appl Physiol Nutr Metab. 2018 May 18:1-10.

379. Johnson DR, Nichol KL, Lipczynski K. Barriers to adult immunization. Am J Med. 2008 Jul;121(7 Suppl 2):S28-35.

380. Hang S. Assessment of peripheral BDNF levels over 30 days [Masters]. Digital Commons: Humboldt State University 2018.

381. Kjome RL, Granas AG, Nerhus K, Roraas TH, Sandberg S. The prevalence of self-monitoring of blood glucose and costs of glucometer strips in a nationwide cohort. Diabetes Technol Ther. 2010 Sep;12(9):701-5.

382. Sabat J, Gould S, Gillego E, Hariprashad A, Wiest C, Almonte S, et al. The use of finger-stick blood to assess lactate in critically ill surgical patients. Ann Med Surg (Lond). 2016;10:41-8.

383. Song SY, Lee SK, Eom KS, Investigators K. Analysis of Mortality and Epidemiology in 2617 Cases of Traumatic Brain Injury : Korean Neuro-Trauma Data Bank System 2010-2014. Journal of Korean Neurosurgical Society. 2016;59(5):485-91.

384. Voss P, Thomas ME, Cisneros-Franco JM, de Villers-Sidani É. Dynamic Brains and the Changing Rules of Neuroplasticity: Implications for Learning and Recovery. 2017 2017-October-04;8(1657).

385. Warraich Z, Kleim JA. Neural plasticity: the biological substrate for neurorehabilitation. PM R. 2010 Dec;2(12 Suppl 2):S208-19.

386. Notaras M, van den Buuse M. Brain-Derived Neurotrophic Factor (BDNF): Novel Insights into Regulation and Genetic Variation. Neuroscientist. 2019 Oct;25(5):434-54.

387. Chiaretti A, Piastra M, Polidori G, Di Rocco C, Caresta E, Antonelli A, et al. Correlation between neurotrophic factor expression and outcome of children with severe traumatic brain injury. Intensive Care Med. 2003 Aug;29(8):1329-38.

388. Chiaretti A, Antonelli A, Riccardi R, Genovese O, Pezzotti P, Di Rocco C, et al. Nerve growth factor expression correlates with severity and outcome of traumatic brain injury in children. Eur J Paediatr Neurol. 2008 May;12(3):195-204.

389. Failla MD, Conley YP, Wagner AK. Brain-derived neurotrophic factor (BDNF) in traumatic brain injury-related mortality: Interrelationships between genetics and acute systemic and central nervous system BDNF profiles. Neurorehabil Neural Repair. 2016 Jan;30(1):83-93.

390. Failla MD, Juengst SB, Arenth PM, Wagner AK. Preliminary associations between brain-derived neurotrophic factor, memory impairment, functional cognition, and depressive symptoms following severe TBI. Neurorehabil Neural Repair. 2016 Jun;30(5):419-30.

391. Simon D, Nascimento RIMd, Filho EMR, Bencke J, Regner A. Plasma brain-derived neurotrophic factor levels after severe traumatic brain injury. Brain Inj. 2016 2016/01/02;30(1):23-8.

392. Kalish H, Phillips TM. Analysis of neurotrophins in human serum by immunoaffinity capillary electrophoresis (ICE) following traumatic head injury. J Chromatogr B Analyt Technol Biomed Life Sci. 2010 Jan 15;878(2):194-200.

393. Jagoda AS, Bazarian JJ, Bruns JJ, Jr., Cantrill SV, Gean AD, Howard PK, et al. Clinical policy: neuroimaging and decisionmaking in adult mild traumatic brain injury in the acute setting. Ann Emerg Med. 2008 Dec;52(6):714-48.

394. Harri I, Grant LI, Noah DS, Anneli K, Antti B, Juha Ö, et al. Characterizing the type and location of intracranial abnormalities in mild traumatic brain injury. Journal of Neurosurgery JNS. 2018 01 Dec. 2018;129(6):1588-97.

395. Langdon S, Königs M, Adang EAMC, Goedhart E, Oosterlaan J. Subtypes of Sport-Related Concussion: a Systematic Review and Meta-cluster Analysis. Sports Med. 2020 2020/10/01;50(10):1829-42.

396. Bakdash JZ, Marusich LR. Repeated Measures Correlation. Front Psychol. 2017;8:456-.

397. He H-y, Tian J-l, Deng Y-q, Xiong X, Xu Y, Liao Y-m, et al. Association of brain-derived neurotrophic factor levels and depressive symptoms in young adults with acne vulgaris. BMC Psychiatry. 2019 2019/06/24;19(1):193.

398. Mora E, Portella MJ, Piñol-Ripoll G, López R, Cuadras D, Forcada I, et al. High BDNF serum levels are associated to good cognitive functioning in bipolar disorder. Eur Psychiatry. 2019;60:97-107.

399. Hori H, Yoshimura R, Katsuki A, Atake K, Igata R, Konishi Y, et al. Relationships between serum brain-derived neurotrophic factor, plasma catecholamine metabolites, cytokines, cognitive function and clinical symptoms in Japanese patients with chronic schizophrenia treated with atypical antipsychotic monotherapy. World J Biol Psychiatry. 2017 Aug;18(5):401-8.

400. Zhang Y, Fang X, Fan W, Tang W, Cai J, Song L, et al. Brain-derived neurotrophic factor as a biomarker for cognitive recovery in acute schizophrenia: 12-week results from a prospective longitudinal study. Psychopharmacology (Berl). 2018 Apr;235(4):1191-8.

401. Houlton J, Abumaria N, Hinkley SFR, Clarkson AN. Therapeutic Potential of Neurotrophins for Repair After Brain Injury: A Helping Hand From Biomaterials. 2019 2019-August-02;13(790).

402. Ganguly K, Poo MM. Activity-dependent neural plasticity from bench to bedside. Neuron. 2013 Oct 30;80(3):729-41.

403. Brown A, Weaver LC. The dark side of neuroplasticity. Exp Neurol. 2012;235(1):133-41.

404. Cramer SC, Sur M, Dobkin BH, O'Brien C, Sanger TD, Trojanowski JQ, et al. Harnessing neuroplasticity for clinical applications. Brain. 2011;134(6):1591-609.

405. Sufrinko AM, Mucha A, Covassin T, Marchetti G, Elbin RJ, Collins MW, et al. Sex Differences in Vestibular/Ocular and Neurocognitive Outcomes After Sport-Related Concussion. Clin J Sport Med. 2017 Mar;27(2):133-8.

406. Yorke AM, Smith L, Babcock M, Alsalaheen B. Validity and Reliability of the Vestibular/Ocular Motor Screening and Associations With Common Concussion Screening Tools. Sports Health. 2017;9(2):174-80.

407. Fehr SD, Nelson LD, Scharer KR, Traudt EA, Veenstra JM, Tarima SS, et al. Risk factors for prolonged symptoms of mild traumatic brain injury: A pediatric sports concussion clinic cohort. Clin J Sport Med. 2019;29(1).

408. RugbySmart. Recover and return. [cited; Available from: https://www.rugbysmart.co.nz/injuries/concussion/return/

409. Nelson LD, Temkin NR, Dikmen S, Barber J, Giacino JT, Yuh E, et al. Recovery After Mild Traumatic Brain Injury in Patients Presenting to US Level I Trauma Centers: A Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Study. JAMA Neurol. 2019 Sep 1;76(9):1049-59.

410. Voormolen DC, Zeldovich M, Haagsma JA, Polinder S, Friedrich S, Maas AIR, et al. Outcomes after Complicated and Uncomplicated Mild Traumatic Brain Injury at Three-and Six-Months Post-Injury: Results from the CENTER-TBI Study. J Clin Med. 2020;9(5):1525.

411. Corporation AC. Getting paid if you can't work - weekly compensation. 2021 [cited; Available from: https://www.acc.co.nz/im-injured/financial-support/weekly-compensation/#:~:text=We'll%20pay%20up%20to,get%20paid%20while%20you%20recover.

412. Ackaoui A. Treatment of anaphylaxis: EpiPen, Twinject, or another autoinjector? Canadian family physician Medecin de famille canadien. 2011;57(3):273-4.

413. Dalal R, Grujic D. Epinephrine. StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.

414. McLendon K, Sternard B. Anaphylaxis StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.

415. Mohs RC, Greig NH. Drug discovery and development: Role of basic biological research. Alzheimers Dement (N Y). 2017;3(4):651-7.

416. Metrics C. Quick view Brain Gauge Concussion Scoring. [cited; Available from: https://downloads.corticalmetrics.com/factsheets/concussionscore.pdf

417. Tommerdahl M, Favorov OV, Whitsel BL. Dynamic representations of the somatosensory cortex. Neurosci Biobehav Rev. 2010 Feb;34(2):160-70.

418. Stuart S, Parrington L, Martini D, Peterka R, Chesnutt J, King L. The Measurement of Eye Movements in Mild Traumatic Brain Injury: A Structured Review of an Emerging Area. 2020 2020-January-28;2(5).

419. McGeown JP, Zerpa C, Lees S, Niccoli S, Sanzo P. The Effects of Exercise on Cognitive Function, Balance, and Salivary Brain Derived Neurotrophic Factor in Healthy Individuals – A Pilot Study. Research in Neuroscience. 2016;5(1):15-23.

420. Lust CAC, Mountjoy M, Robinson LE, Oliver JM, Ma DWL. Sports-related concussions and subconcussive impacts in athletes: incidence, diagnosis, and the emerging role of EPA and DHA. Appl Physiol Nutr Metab. 2020 Aug;45(8):886-92.

421. Barrett EC, McBurney MI, Ciappio ED. ω-3 fatty acid supplementation as a potential therapeutic aid for the recovery from mild traumatic brain injury/concussion. Advances In Nutrition (Bethesda, Md). 2014;5(3):268-77.

422. Thielecke F, Blannin A. Omega-3 Fatty Acids for Sport Performance-Are They Equally Beneficial for Athletes and Amateurs? A Narrative Review. Nutrients. 2020 Nov 30;12(12).

423. Canhada S, Castro K, Perry IS, Luft VC. Omega-3 fatty acids' supplementation in Alzheimer's disease: A systematic review. Nutr Neurosci. 2018;21(8):529-38.

424. Di Pietro V, O'Halloran P, Watson CN, Begum G, Acharjee A, Yakoub KM, et al. Unique diagnostic signatures of concussion in the saliva of male athletes: the Study of Concussion in Rugby Union through MicroRNAs (SCRUM). Br J Sports Med. 2021 Mar 23.

425. Morris ZS, Wooding S, Grant J. The answer is 17 years, what is the question: understanding time lags in translational research. J R Soc Med. 2011;104(12):510-20.

426. Westfall JM, Mold J, Fagnan L. Practice-Based Research—"Blue Highways" on the NIH Roadmap. JAMA. 2007;297(4):403-6.

427. Balas EA, Boren SA. Managing clinical knowledge for health care improvement. 2000 2000.

428. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007 2007/05/01;39(2):175-91.

429. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009 Nov;41(4):1149-60.

430. Bujang MA, Sa'at N, Sidik TMITAB, Joo LC. Sample Size Guidelines for Logistic Regression from Observational Studies with Large Population: Emphasis on the Accuracy Between Statistics and Parameters Based on Real Life Clinical Data. The Malaysian journal of medical sciences : MJMS. 2018;25(4):122-30.

431. Figueroa RL, Zeng-Treitler Q, Kandula S, Ngo LH. Predicting sample size required for classification performance. BMC Med Inform Decis Mak. 2012;12:8-.

432. Hsieh FY. Sample size tables for logistic regression. Stat Med. 1989 Jul;8(7):795-802.

433. Narayanan A, Desai F, Stewart T, Duncan S, Mackay L. Application of Raw Accelerometer Data and Machine-Learning Techniques to Characterize Human Movement Behavior: A Systematic Scoping Review. Journal of physical activity & health. 2020 Mar 1;17(3):360-83.

434. Bujang M, Baharum N. A simplified guide to determination of sample size requirements for estimating the value of intraclass correlation coefficient: a review. Arch Orofacial Sci. 2017;12(1):1-11.

435. European Food Safety Authority. Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal. 2012;10(7):1-48.