Detecting and Ameliorating the Effects of Routine Sport-related Head Impacts on Brain Health

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Thesis submitted for the fulfilment of Doctor of Philosophy degree

December 2024

Declaration

I declare that this thesis is my own original work, with due acknowledgements provided where appropriate, produced under the guidance of my supervisory team, and has not been submitted to any other institution for an obtainment of a degree.

Date: 12th December 2024

Impact of COVID-19 on The Work Presented in This Thesis Statement

The Coronavirus pandemic placed the laboratory-based experiments on halt for 14 months. This significantly altered the initials plans that I had for my research. Most significantly, the incurred delay meant that I was forced to perform data collection for my laboratory-based studies in parallel. Under normal circumstance the results of experiment one (described in Chapter 4) would have informed the design and variables used for the experiment two (described in Chapters 4 and 5). Furthermore, after a 14-month delay caused by the ongoing Coronavirus pandemic, there were restrictions in place upon returning to the lab. These restrictions limited the number of staff members allowed on the premises and restricted contact between researchers and participants, impacting the decision to collect blood biomarker data.

To mitigate the constraints that the pandemic placed on my research I performed a scoping review to map the existing evidence of biofluid marker concentration following sport-related repetitive subconcussive head impacts (Chapter 2) in close collaboration with Professor Stefania Mondello (from University of Messina, Italy) who has extensive experience in the field of biofluid markers and neurotrauma. This review was conducted to assess whether biofluid markers are sensitive to repetitive subconcussive head impact induced brain changes and inform future research, including the research done as part of this thesis. Prior to the start of the review, we published the study protocol in BMJ Open (Lember et al., 2021). To ensure rigour of the review and to minimise bias, I performed the review in co-operation with a fellow PhD student (now graduated; Dr Michail Ntikas). We performed database searches together, whereas title/abstract and full-text screening and data extraction were performed separately, in parallel. Following each stage of the review we sought consensus to resolve any conflict. Following this procedure reduced any errors and bias in the review process. Data analysis was divided equally with both of us also engaged in checking each-other's results to ensure accurate and correct analysis. As a result, we share the authorship of the publication. In light of the scoping review, I also analysed blood biomarker data collected in our laboratory by Dr Thomas Di Virgilio during his doctoral research (2015-2018) examining brain-injury markers following a drill of soccer headers and boxing sparring (Chapter 3).

Acknowledgements

First and foremost, I would like to express my gratitude to my supervisors Dr Thomas Di Virgilio and Dr Magdalena Ietswaart, for their support, guidance and mentorship throughout the course of my PhD. Their expertise, patience, and unwavering encouragement were invaluable in helping me navigate the challenges of doing doctoral research. I would also like thank Professor Angus Hunter for his guidance and supervision during the first half of my PhD.

I would also like to thank the members of the Neuromuscular Journal Club, with a particular mention to Dr Lewis Macgregor, for their insightful feedback, suggestions, and constructive criticism that have shaped my research and improved the quality of my work. I am also grateful to Professor Stefania Mondello, Professor David Donaldson and Professor Lindsay Wilson for their advice, collaboration, expertise, and valuable contributions to my research.

A special thank you to the technicians, Catriona Bruce, Chris Grigson and especially Stephen Stewart, for providing technical support and expertise that were essential for the successful completion of my data collection. A heartfelt thank you also to the participants for their time and commitment, without whom this research would not have been possible.

I want to thank Storelli Sports Inc, University of Stirling Psychology Division, Brain Health Scotland and the Guarantors of Brain for the financial support, enabling me to focus on my research studies and personal development. I would hereby like to thank Stella Turner for all her administrative help.

I am grateful to my fellow students, Dr Robert Paval, Dr Ragul Selvamoorty, Dr Magda Mustile, Dr Judith Lowes, Dr Rosyl Somai, Dr Kirstin MacGregor, Dr Milena Banic, Emily Cunninham, Ali Muqtadir and Dr Michail Ntikas, for their camaraderie and support throughout this journey. Your friendship, knowledge and encouragement were a source of comfort and inspiration.

Last but not least, I would like to express my profound gratitude to my sister Liina Lember, mother Anne-Kai Toht, grandparents Aime and Ants Toht and my best friends Johanna Elisabeth Taalmann and Karin Kreekman Polat for their unwavering love, encouragement, and support. Your belief in me has been my greatest motivation and strength.

I have been incredibly lucky to be surrounded by so many great people whose knowledge, experience and support has helped me complete this journey. I am deeply grateful to you all. Thank you!

Publications and Presentations

Publications[1]

The Protocol for the Work Presented in Chapter 2 was Published Prior to the Work Being Conducted:

Lember, L.-M., Ntikas, M., Mondello, S., Wilson, L., Hunter, A., Di Virgilio, T., Santoro, E., & Ietswaart, M. (2021). Effects of sport-related repetitive subconcussive head impacts on biofluid markers: a scoping review protocol. *BMJ Open*, (Vol 11, Issue 6). https://doi.org/10.1136/bmjopen-2020-046452

Chapter 2 is Published:

Lember, L. M., Ntikas, M., Mondello, S., Wilson, L., Di Virgilio, T. G., Hunter, A. M., Kobeissy, F., Mechref, Y., Donaldson, D. I., & Ietswaart, M. (2024). The Use of Biofluid Markers to Evaluate the Consequences of Sport-Related Subconcussive Head Impact Exposure: A Scoping Review. *Sports Medicine - Open* (Vol. 10, Issue 1). https://doi.org/10.1186/s40798-023-00665-6

Presentations

PhD Plan Presentation:

Lember, L.-M., Di Virgilio, T., Donaldson, D.I., Wilson, L., Hunter, A., & Ietswaart, M. How much sport-related routine head impact is too much for the brain? A practical problem with a SINAPSE answer. 'Lightning talk' (poster pitches) and poster session, Scottish Imaging Network: A platform for scientific excellence (SINAPSE), Annual Scientific Meeting, virtual meeting (online), June 2020

Presentation of the Findings of Chapter 2:

Lember, L.-M., Ntikas, M., Mondello, S., Wilson, L., Hunter, A., Di Virgilio, T., Santoro, E., Donaldson, D.I., & Ietswaart, M. Do biofluid markers provide an objective measure of the effects of sport-related repetitive subconcussive head impact? Poster presentation, European College of Sport Science, 27th Annual Congress, Seville, Spain, August 2022

[1] Dr Michail Ntikas is joint first author.

General Abstract

Participation in sports has many physiological and psychological health benefits. Nonetheless, the benefits and safety of participation in contact sports has been under scrutiny as former boxers, soccer, rugby and American football players among others have been documented to develop adverse long-term brain health. Such consequences have raised concern about the safety of routine non-concussive head impacts that contact sport athletes are exposed to in vast amounts. Nonetheless, studying the acute effects of such routine impacts has proved difficult due to the subtleness of the brain's response to non-concussive, also referred to as subconcussive, impacts. These difficulties have led the field of research examining subconcussive impacts to a quest for sensitive measures. Concurrently, ongoing efforts are made in attempt to find ways to ameliorate the effects of subconcussive impacts through the use of protective equipment and by limiting the cumulative impact burden. This thesis was set out to investigate variables, including biofluid markers, electrophysiological changes, postural control and cognitive function, that may be sensitive to the effects of subconcussive impacts on the brain in order to find ways to mitigate subconcussive impact burden. The aims were to assess whether limiting the number of impacts and the use of headgear can prevent or reduce the acute effects of head impacts.

The first chapter of this thesis provides a general background about the effects of repetitive subconcussive head impacts (RSHI) on brain health and outlines the aims of the thesis. The subsequent two chapters (Chapter 2 and 3) examine the utilisation of biofluid markers of brain injury for detecting the effects of RSHI by investigating already existing research in the field. Chapter 2 (Lember et al., 2024) is the first review dedicated to systematically scoping the evidence of biofluid marker levels following RSHI exposure. The chapters conclude that although some markers demonstrate promising results in detecting the effects of RSHI, the findings are generally mixed and thus, the use of biofluid markers in this setting is currently premature. As such, experimental research in the following chapter uses cognitive, vestibular and electrophysiological measures that have previously shown sensitivity to the effects of RSHI in the field of subconcussive and/or concussive literature. Chapter 4 assesses whether reducing the number of head impacts (10 compared to 20) and the use of headgear ameliorates functional brain response to subconcussive impacts. The results demonstrate that subconcussive impacts did not affect any of the outcome measures, suggesting that the assessment methods were either not sensitive, or no brain changes occurred in response to the impacts. In light of the lack of detectable functional brain changes the following chapter (Chapter 5) examines whether the

use of headgear and physical characteristics such as (neck) strength are able to attenuate impact accelerations as a method for reducing cumulative impact burden. The results show that neither headgear nor physical characteristics affected heading induced head accelerations. The final chapter explores the methodological difficulties of studying the effects of RSHI with suggestions and considerations for future research. Chapter 6 also touches on alternative methods, such as jugular vein compression collar, for preventing or mitigating the effects of repetitive head impacts in contact sports.

General Abstract for the Repository (Maximum 300 Words)

Participation in contact sports has been under scrutiny as former contact sport athletes have been found at increased risk of adverse brain health, raising concern about the safety of routine head impacts in contact sports. Studying the acute effects of such impacts has proved difficult due to the subtleness of the brain's response to subconcussive impacts. The aim of this thesis was to investigate variables, that may be sensitive to the effects of subconcussive impacts in order to find ways to mitigate head impact burden.

The first chapter provides background information about the effects of repetitive subconcussive head impacts (RSHI) on brain health and outlines the aims of the thesis. Chapters 2 and 3 examine the use of biofluid markers for detecting the effects of RSHI by investigating already existing research. Chapter 2 (Lember et al., 2024) is the first scoping review dedicated to examining biofluid marker levels following RSHI exposure. The chapters conclude that although some markers demonstrate sensitivity to the effects of RSHI, the findings are generally mixed and the use of biofluid markers in this setting is currently premature. As such, Chapter 4 assesses whether reducing the number of head impacts and the use of headgear ameliorates functional brain response to subconcussive impacts using cognitive, vestibular and electrophysiological measures that have previously shown sensitivity to the effects of RSHI. The study found no heading related changes in any of the outcome measures, suggesting that the measures were either not sensitive, or no brain changes occurred in response to the impacts. Chapter 5 examines whether the use of headgear and (neck) strength can attenuate impact accelerations, finding that headgear nor strength affected heading induced head accelerations. The final chapter explores the methodological difficulties of studying the effects of RSHI with suggestions and considerations for future research.

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List of Abbreviations

aMT – active motor threshold

BBB – blood-brain barrier

BDNF – brain-derived neurotrophic factor

BESS – Balance Error Scoring System

CANTAB – Cambridge Neuropsychological Test Automated Battery

CHII – cumulative head impact index

Cho – choline

CNS – central nervous system

CSF – cerebrospinal fluid

CT – computed tomography

CTE – chronic traumatic encephalopathy

DHA – docosahexaenoic acid

DTI – diffusion tensor imaging

EEG – electroencephalogram

EMG – electromyography

EVs – extracellular vesicles

FA – Football Association

fMRI – functional magnetic resonance imaging

GABA – gamma-aminobutyric acid

GFAP – glial fibrillary acidic protein

GSH – glutathione

GVS – galvanic vestibular stimulation

ImPACT – Immediate Post-Concussion Assessment and Cognitive Testing

ISAK – The International Society for the Advancement of Kinanthropometry

KDT – Kind-Devick test

M1 – primary motor cortex

MEP – motor evoked potential

mI – myo-inositol

miRNAs – microRNAs

MRI – magnetic resonance imaging

MRS – magnetic resonance spectroscopy

MT – motor threshold

mTBI – mild traumatic brain injury

MVC – maximal voluntary contraction

NFL – National Football League

NfL – neurofilament light

NHS – National Health Service

NSE – neuron-specific enolase

NPC – near point convergence

PAL – paired associates learning

PECOS – population, exposure, comparator, outcomes and study design

PET – positron emission tomography

PLA – peak linear acceleration

PRA – peak rotational acceleration

PRISMA-ScR – preferred reporting items for systematic reviews and meta-analyses extension for scoping reviews

p-tau – phosphorylated tau

rMT – resting motor threshold

ROBINS-I – risk of bias in non-randomized studies of interventions

RSHI – repetitive subconcussive head impacts

S100B – S100 calcium-binding protein beta

SAC – Standardised Assessment of Concussion

SCAT6 – Sport Concussion Assessment Tool 6

SENIAM – Surface Electromyography for the Non-Invasive Assessment of Muscles

SOT – Sensory Organization Test

SP – silent period

SST – subconcussion-specific tool

SWM – spatial working memory

TBI – traumatic brain injury

TMS – transcranial magnetic stimulation

UCH-L1 – ubiquitin C-terminal hydrolase L1

UK – United Kingdom

VOMS – Vestibular Oculo-Motor Screening

WM – white matter

Chapter 1: General Introduction and Aims of the Thesis

1.1 Background

Participation in sports has many physical and psychological health benefits such as reduced risk of type two diabetes mellitus and cardiovascular disease, and improved self-esteem (McKee et al., 2014). Moreover, it has been suggested that team sport athletes are less likely to experience anxiety and depression than individual sport athletes (Pluhar et al., 2019). However, sport participation also carries the risk of injury. The risk-benefit ratio of participation in contact sports is being questioned since contact sport athletes are not only exposed to musculoskeletal injuries, but also receive direct impacts to the head (e.g., strikes to the head in boxing) and sustain acceleration forces to the brain through whiplash effect (e.g., falls and tackling). Besides the negative acute effects that exposure to accidental and routine head impacts may have on athletes, increasing evidence demonstrates adverse long-term brain health following exposure to repeated head impacts, where contact sport athletes have been found to suffer from behavioural, mood and cognitive impairments (Montenigro et al., 2014, 2017) which may be indicative symptoms of underlying pathologies. Especially in light of large retrospective cohort studies that have found former professional soccer (Mackay et al., 2019; Ueda et al., 2023), American football (Lehman et al., 2012) and rugby (Russell et al., 2022) players to have up to 3.5 times higher mortality from neurodegenerative disease than matched general population controls.

While traumatic brain injury (TBI) is a well-recognised risk factor for neurodegenerative disease development later in life (Maas et al., 2017; Wilson et al., 2017) there is growing attention and awareness surrounding the potential deleterious effects of cumulative repeated head impacts, both concussive and non-concussive (i.e., subconcussive) on brain health. The possibility of sport-related head impacts causing adverse long term brain health has raised concerns about the safety of contact sport participation since contact sport athletes can be exposed to a vast amount of head impacts; for example, American football players may experience around 1000 hits (albeit non-concussive) to the head in one season alone (Gysland et al., 2012), while one study estimated soccer players to perform up to 5400 headers (interquartile range: 276–1095) in one year alone (Lipton et al., 2013). Although the latter study estimated the impact exposure based on the self-reports of the participants and the number of headers has been observed lower in women's soccer (26-209 headers per season) (Kenny et al., 2024), the high

cumulative number of routine head impacts that athletes may be exposed to raises concern about the safety of routine sport-related head impacts on brain heath.

In 2019, it was estimated that 57 million people have dementia worldwide with the number projected to increase substantially over the next couple of decades (Nichols et al., 2022). The number of people with dementia in the United Kingdom (UK) is estimated at 850,000, costing approximately £34.8 billion to the economy each year (*Dementia: Applying All Our Health - GOV.UK, n.d.*). The risk of neurodegenerative diseases among contact sport athletes is therefore a potential concern for the National Health Service (NHS) in the UK considering that long-term exposure to the repetitive head impacts may lead to adverse long-term brain health, leading to increased healthcare costs associated with diagnosis, treatment and management. Moreover, with raising awareness the burden of consultations and assessments may also increase. The *Lancet* Commission 2024 report on dementia prevention, intervention, and care has highlighted 14 modifiable risk factors that are considered to substantially prevent or delay dementia (Livingston et al., 2024). One of those identified risk factors (among smoking, excessive alcohol consumption and physical inactivity) is head injury. Alongside the risks associated with sustaining a TBI, the report acknowledges that athletes participating in sports that involve frequent head contact and whiplash events may be at increased risk of dementia, and recommends the use of protective equipment, limiting head impacts, not playing acutely following TBI and potentially adapting rules to limit injury, highlighting that these actions are ought to be individual and a public health priority.

1.2 History and Chronic Traumatic Encephalopathy

The concept that repetitive head impacts can lead to long-term brain damage was first documented in boxers by an American pathologist H.S. Martland in 1928. At the time, boxers who were observed to suffer from cognitive and motor control disturbances, similar to Parkinson's disease, were called "punch drunk" (Martland, 1928). Later, "punch drunk" became known as "Dementia Pugilistica" (McKee et al., 2016). Today "Dementia Pugilistica" is referred to as chronic traumatic encephalopathy (CTE) and since its discovery this pathology has been diagnosed in various other contact sports athletes, including in American footballers, ice-hockey, rugby and soccer players (Lee et al., 2019; McKee et al., 2014; Omalu et al., 2005, 2006).

CTE is characterised by the accumulation of hyperphosphorylated tau (p-tau) in neurons and astroglia, subsequently forming neurofibrillary tangles at the depths of cortical sulci

that later spread to other areas of the brain, resulting in clinical symptoms that may affect behaviour, mood, cognitive function and motor control (Montenigro et al., 2014). The development of CTE has been considered unique to individuals with history of exposure to repeated head impacts, although it has been suggested that a single TBI may also lead to CTE pathology (Montenigro et al., 2014). Nonetheless, it has been reported that 16% of diagnosed CTE cases had no documented concussion history highlighting the risks of subconcussive impact exposure on long-term brain health (Stein et al., 2015). In addition, years of contact sport participation have been reported to predict CTE progression, while the number of concussions have not (Stein et al., 2015).

Currently, CTE can only be diagnosed post-mortem via a neuropathological examination (McKee et al., 2016) and the prevalence of CTE in people exposed to repetitive head impacts (such as contact sport athletes) or in the general population is not precisely known. A study of deceased American football players found CTE neuropathology in 87% of the convenience sample consisting of 202 former players, suggesting that the prevalence of CTE in contact sport athletes may be relatively high (Mez et al., 2017). However, CTE prevalence in the general population, or its contribution to the overall dementia count, is not well established and it is possible that many individuals with this pathology may never receive a diagnosis or be examined post-mortem. A neuropathological study screening 636 cases (consisting of neurodegenerative diseases and neurologically normal controls) from the Sydney Brain Bank for CTE found that only five cases (0.79%) had CTE pathology (of whom three had participated in contact sports) (McCann et al., 2022). A further eight cases almost met the criteria for CTE neuropathological change (two of whom had known background of contact sports). The authors acknowledged that despite efforts to gather information about the sporting career of the cases included in the study it was unclear whether and to which extent the cases had participated in contact sports. Noteworthily, all five diagnosed CTE cases also had a presence of a coexisting pathology (e.g., Alzheimer's disease).

Although evidence now suggests that CTE development is not unique to individuals with history of head impact exposure (Iverson et al., 2019), research demonstrating averse brain health following subconcussive impact exposure is continuously growing. Observations of adverse long-term brain health in contact sport athletes have led to studies investigating the acute effects of routine head impacts in contact sports on the brain to better understand how acute brain changes translate to the chronic effects observed following a ‘career’ of contact sport participation. Subconcussive head impacts are routine and repetitive in contact sports, and are

defined as impacts that do not result in clinically evident signs and symptoms of concussion (Mainwaring et al., 2018). The subtle nature of the effects of repetitive subconcussive head impacts (RSHI) on the brain is also one of the biggest challenges in the field of the RSHI research, and there is a continuous quest to find sensitive measures that can detect the effects of RSHI consistently. The current lack of sensitive measures inhibits the field from determining ways to prevent or reduce acute and long-term brain damage caused by subconcussive impacts. To date, research investigating the effects of RSHI on the brain has mostly employed measures that have been found to be affected by concussion. Those measures can be categorised into brain imaging, biofluid marker assessment, measures of motor control and cognitive testing. The following sections in this chapter will delve into studies on the aforementioned measures within the framework of RSHI, starting with possibly the most unbiased and precise approach, and concluding with the least sensitive method for evaluating brain changes.

1.3 Brain Imaging

Brain imaging allows the assessment of changes in brain structure (using computed tomography [CT], magnetic resonance imaging [MRI] and diffusion tensor imaging [DTI]), function (via functional MRI [fMRI], positron emission tomography [PET] and electroencephalogram [EEG]) (Hirsch et al., 2015) and metabolism (via magnetic resonance spectroscopy [MRS]) (Ntikas et al., 2022) following head impact exposure.

A CT study from 1992 scanning 338 active professional boxers found abnormal and borderline scans in 25 (7%) and 75 (22%) athletes, respectively (Jordan et al., 1992). The authors reported brain atrophy as the most common abnormality (n = 22), while focal lesions of low attenuation were found in three boxers. Noteworthily, EEG was also measured in 335 of the boxers and was found abnormal and borderline in 18 and 24 athletes, respectively (normal in 95% of the cases). Interestingly, EEG was normal in 92% of the boxers who had an abnormal CT scan, where only three boxers with abnormal CT scan also had an abnormal EEG. Nonetheless, Tysvaer et al. (1989) found higher incidence of abnormal EEG activity, as assessed by an experienced neurophysiologist blinded to the participant information, in former professional soccer players than in matched controls. Moreover, measuring event related potentials by combining cognitive testing with EEG may be a more sensitive measure of brain function than observations of resting state EEG. Furthermore, while the above findings were not directly linked to subconcussive events, structural brain changes have since also been documented in

American footballers (McAllister et al., 2014; Myer et al., 2016), ice-hockey (McAllister et al., 2014) and soccer players (Koerte et al., 2012; Lipton et al., 2013; Myer et al., 2019).

A cross-sectional study scanning 12 professional soccer players without history of symptomatic concussions and a control sample consisting of 11 swimmers, also without concussion history, found differences in white matter (WM) integrity between the groups (Koerte et al., 2012). Where the DTI acquired on a magnetic resonance scanner showed widespread increase in radial diffusivity in soccer players, suggesting possible demyelination. Myer et al. (2019) also found changes in mean axial and radial diffusivity in extensive WM areas following a season of high school female soccer (n = 24). Moreover, changes in the WM microstructure from pre to post season significantly correlated head impact exposure. Interestingly, Myer et al. (2019) reported that WM changes in diffusivity in female soccer players reversed towards baseline at three months off-season follow-up, suggesting that the brain may be able to repair any injuries from the impact exposure after a period without head impacts. Similarly, a prospective longitudinal study also found significant changes in mean, axial and radial diffusivity in the WM using DTI following a season of American football participation (n = 30), where changes in various brain regions significantly correlated with impact metrics (Myer et al., 2016). In addition, McAllister et al. (2014) also found a significant relationship between WM diffusivity in several brain areas (including corpus callosum, hippocampus, thalamus and amygdala) and head impact metrics (n = 80). The study also found that post-season change in mean diffusivity in corpus callosum was associated with reduced memory function and verbal learning.

Importantly, the emerging pattern from the imaging research is the association between brain changes and the impact dose. Furthermore, Lipton et al. (2013) carried out 3T diffusion-tensor magnetic resonance imaging and cognitive tests in 37 active amateur soccer players (eight females) finding abnormal WM microstructure and impaired cognitive performance that were associated with estimated number of headers in the prior year, but not with concussion history. Moreover, the authors found that heading was significantly associated with lower fractional anisotropy at three locations in the temporo-occipital WM, and threshold for changes varied with location, ranging from 885 to 1550 headers per year which may suggest that some brain areas are more susceptible to injuries and the time it takes for pathological symptoms to appear following injury varies across the brain.

Highlighting the consistency in structural brain changes following RSHI are perhaps the findings of a systematic review that reported 14 out of 16 studies demonstrating structural brain changes such as white mater diffusivity, decreased volume and cortical thinning following repetitive head impact exposure (Mainwaring et al., 2018). Notwithstanding, a study looking at acute brain changes via DTI and resting state fMRI scans before and after (within eight days post) a weekend of soccer games in youth players (approximately 13 years of age) found no changes in the neuroimaging (Chrisman et al., 2016). However, the study had a small sample size (15 players with head impact exposure) and the average number of head impacts that the players were exposed to was low (average of four impacts). Therefore, while capturing acute brain changes using neuroimaging may be challenging more research in this area is needed.

Adverse effects associated with RSHI have also been observed in brain function and neurochemistry. For example, Svaldi et al. (2017) found a significant reduction in cerebrovascular reactivity using fMRI in frontotemporal brain aspects in female soccer players (n = 14) that were associated with cumulative impact burden and persisted for four to five months post season. Moreover, a study scanning 11 former professional soccer players without history of clinically diagnosed concussion and 14 matched non-contact sport athletes via 3T MRS found a significant difference in choline (Cho) and myo-inositol (mI) in the soccer athletes compared to the control group; where increased Cho denotes membrane disruption that may be due to demyelination, diffuse axonal injury, astrocytosis or neuroinflammation and increased mI is thought to be caused by reactive astrocytosis (Koerte et al., 2015). The study also reported a significant correlation between the lifetime estimate of RSHI exposure and mI and glutathione (GSH). GSH is an antioxidant which increase is considered a neuroprotective response to oxidative stress caused by neuroinflammation (Koerte et al., 2015). Therefore, the findings of the study suggest neuroinflammation in the soccer players. Interestingly, the authors did not find differences in neurocognitive function or balance between the groups, suggesting that changes in neurochemistry precede neurocognitive changes.

Therefore, research examining brain changes following RSHI exposure using neuroimaging have found structural, functional and neurometabolic changes that have relatively consistently been associated with subconcussive impact exposure. Nonetheless, brain imaging can be expensive and time consuming and majority of the studies to date are either cross-sectional or longitudinal, where acute brain changes in an experimental setting using neuroimaging are lacking. An alternative brain imaging modality, that in comparison to the traditional neuroimaging is more cost and time effective, allows for the assessment of electrophysiological changes

in the brain through transcranial magnetic stimulation (TMS). The following section provides an overview of the use of TMS for the assessment of brain changes.

1.4 Transcranial Magnetic Stimulation

TMS is a non-invasive method of activating cortical neurons in a targeted brain area. This technique was developed in the 1980s and is based on the Faraday's principle of electromagnetic induction, where changing magnetic fields generate an electric current in a nearby conductor, in the case of TMS that is in the brain (Hupfeld et al., 2020; Zewdie & Kirton, 2016). TMS technique encompasses single-, paired-pulse and repetitive TMS, where the first two are generally used to study brain function and the latter is used for inducing changes in brain activity that last beyond the stimulation period (Klomjai et al., 2015).

When stimulating the primary motor cortex (M1), then the resultant response to the stimulation in the target muscle can be measured using surface electromyography (EMG). Applying TMS over M1 works by depolarizing cortical interneurons, triggering action potentials that lead to trans-synaptic activation of pyramidal cells which project on the descending corticospinal volleys (Hupfeld et al., 2020; Klomjai et al., 2015; Zewdie & Kirton, 2016). If the firing threshold is exceeded, then action potentials are triggered in spinal motor neurons resulting in muscle responses which can be detected using surface EMG as motor evoked potentials (MEPs) (Figure 1.1). MEPs reflect cortical and spinal excitability, mediated by a excitatory

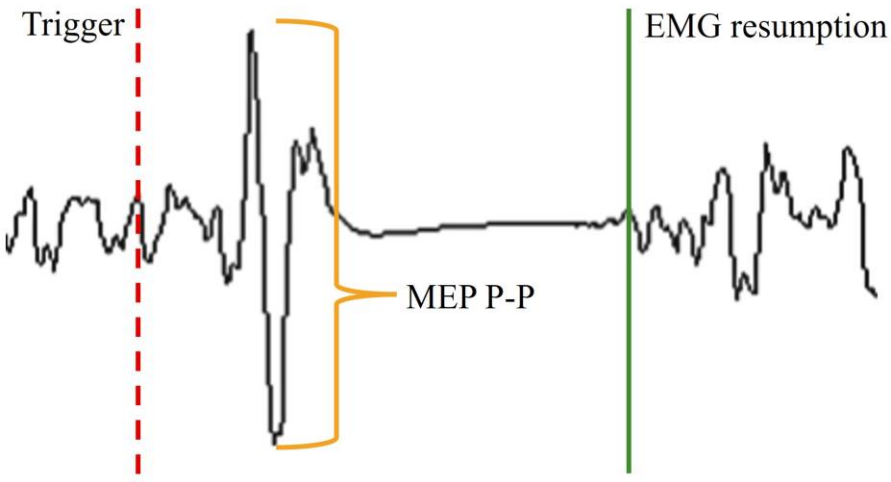


Figure 1.1. An example of the EMG silence denoting corticomotor inhibition (measured as the duration of the silent period). TMS trigger offset (marked in red) and the resumption of voluntary EMG activity (marked in green). Inhibition to excitability ratio is analysed by using MEP peak-to peak amplitude as a measure of excitation (marked in yellow).

neurotransmitter glutamate (Guerriero et al., 2015), and are measured as a peak-to-peak amplitude of the evoked potential where greater amplitude indicates greater excitation (Guerriero et al., 2015; Hupfeld et al., 2020). Motor threshold (MT) is the lowest stimulation intensity required to induce a MEP and can be measured at rest (rMT) or during contraction, known as active MT (aMT). The intensity of evoking a motor response is usually lower for aMT since voluntary contraction is suggested to increases the excitability of neurons (Zewdie & Kirton, 2016).

While MT and MEPs can be measured at rest, inhibition can only be recorded during muscle contraction. When stimulating M1 contralaterally using single pulse TMS, while recording surface EMG from the contracting target muscle, then a suppression in the EMG activity can be observed (Figure 1.1). The silence in the EMG activity occurs as a result of inhibitory circuits' activation and is known as cortical silent period (SP). SP is typically measured as a duration from the start or the end of the MEP, or sometimes from the TMS pulse to the resumption of voluntary EMG signal. Differences in how SP duration is defined however, makes it difficult to compare the results between studies. It is considered that both spinal and cortical mechanisms contribute to the SP duration where, cortical mechanisms are considered to account for majority (75%) of the SP duration, compared to the spinal structures, although it has been debated (Hupfeld et al., 2020). The beginning of the SP is attributed to the spinal mechanisms occurring through the Renshaw cell (inhibitory interneurons in the spinal cord) activation and motor neuron afterhyperpolarization (refractory period factor), while the latter part of SP is considered to occur due to the activation of cortical inhibitory interneurons (Hupfeld et al., 2020). SP duration is considered to reflect inhibition in M1, primarily mediated by gamma-aminobutyric acid (GABA), specifically $GABA_B$ receptors (Guerriero et al., 2015; Hupfeld et al., 2020). Pharmaceutical evidence supports this notion since SP duration has been found to elongate following administration of baclofen, a $GABA_B$ receptor agonist, in a patient with dystonia (Siebner et al., 1998). Moreover, prolonged SP duration has also been observed following ingestion of tiagabine, a GABA reuptake inhibitor that increases GABA availability (Werhahn et al., 1999).

In addition, assessing excitation to inhibition ratio (MEP:SP) allows the examination of intrahemispheric inhibition while controlling for cortical excitability, providing information about the balance between excitability and inhibition (Hupfeld et al., 2020). In healthy individuals, there is a balance between glutamate, the primary excitatory, and GABA, the primary inhibitory neurotransmitter of the central nervous system (CNS) (Guerriero et al., 2015). In

severe TBI pathophysiology, microdialysis studies have demonstrated acute release of glutamate into extracellular space resulting in excitotoxicity leading to neuronal injury, cell death and dysfunction of the surviving neurons (Guerriero et al., 2015). While research looking at intra and extracellular glutamate has demonstrated decreased glutamate levels six days following concussion in the motor cortex, but not in hippocampi or in dorsolateral prefrontal cortex (Henry et al., 2010). Suggesting that following injury glutamate may be depleted from the presynaptic vesicles, while extracellular glutamate from the synaptic cleft is taken up the neighbouring astrocytes and glutamate is rapidly converted into glutamine resulting in decrease in total glutamate levels (Guerriero et al., 2015). Furthermore, it has been suggested that GABA up-regulation following brain injury is a compensatory mechanism to protect the brain against glutamate excitotoxicity (Guerriero et al., 2015; Scott et al., 2020). Indeed, increased SP duration is most consistent TMS measure of concussion. A systematic review with meta-analysis found that SP duration was increased both acutely (up to 12 weeks) and post-acutely (12 weeks to two years) following concussion compared to the control group, demonstrating reduced net corticomotor excitability (Scott et al., 2020). Noteworthily, the authors reported no significant differences in the MT, MEP latency (time between stimulation and onset of MEP) or MEP amplitude data between concussed individuals or the controls acutely or post-acutely.

Increased SP duration has also been observed acutely following subconcussive impact exposure from soccer heading (Di Virgilio et al., 2016), sparring (Di Virgilio et al., 2019) and rugby tackling (McNabb et al., 2020). While Di Virgilio et al. (2016) assessed the effects of soccer heading without a control condition or group, the research examining the effects of sparring and tackling both found elongated SP only in the groups exposed to head impacts, suggesting that SP is a sensitive measure for detecting the effects of RSHI. Moreover, Di Virgilio (2016, 2019) found that SP duration returned to baseline within 24 hours, suggesting that prolonged corticomotor inhibition following a single bout of subconcussive impact exposure may be transient. What is more, no changes in excitability were observed following soccer heading (Di Virgilio et al., 2016). Therefore, it appears that similarly to concussion subconcussive impacts may induce a GABAergic response in M1, which may be neuroprotective against potential excitotoxicity, and this increase in inhibition can be measured with TMS.

1.5 Biofluid Markers of Brain Injury

Following TBI, proteins of brain injury are released into the CNS and can also be detected in the periphery. Biofluid markers can help establish whether brain injury has occurred

and provide information about the severity of the trauma and help monitor recovery (Zetterberg et al., 2013).

While measuring markers of brain injury from the cerebrospinal fluid (CSF) in the CNS may be considered optimal since CSF is in direct contact with the brain, the procedure of lumbar puncture for obtaining a CSF sample is considerably more difficult and invasive to perform compared to drawing a blood sample. Collecting a blood sample in contrast is a quick procedure; however, proteins may be present at lower concentrations in the periphery due to higher plasma volume (Zetterberg et al., 2013). It is thought that brain injury markers cross from the CNS to the blood through either trauma induced damage to the neurovascular unit, particularly to capillaries, allowing the biomarkers to cross the blood-brain barrier (BBB) or via the glymphatic system, where proteins are cleared from the CNS following injury via bulk fluid movement during waste clearance (Kawata et al., 2016). When measuring fluid biomarkers in the periphery it is important to consider whether the protein in question can originate from other sources in the periphery and whether the marker is affected by other factors such as exercise or musculoskeletal injury (Kawata et al., 2016).

Some of the most studied biofluid markers of brain injury include tau, neurofilament light (NfL), neuron specific enolase (NSE), S100 calcium-binding protein beta (S100B) and glial fibrillary acidic protein (GFAP) (Kawata et al., 2016; Zetterberg et al., 2013). Tau is a microtubule binding protein enhancing the viscoelastic properties of neurons and is abundant in thin non-myelinated axons of grey matter where it aids cytoskeletal shock absorption from mechanical strain (Kawata et al., 2016). NfL is also an axonal injury marker, present in large-calibre myelinated axons of the WM and the spinal cord (Zetterberg et al., 2013). It is hypothesised that axonal proteins, such as tau and NfL, are more sensitive to diffuse axonal injury (Whitehouse et al., 2022) where diffuse injury is caused by stretching and twisting from rapid rotation; whereas focal injury, resulting in hematomas and contusions, is common from direct impacts to the head (Kawata et al., 2016). NSE is considered a neuronal injury marker since it is predominantly found in neurons and is upregulated during axonal injury to maintain homeostasis (Kawata et al., 2016). S100B and GFAP are glial injury markers. S100B is an intracellular protein primarily expressed in astrocytes in the CNS but is also present in the periphery including in cardiomyocytes, adipocytes and pulmonary alveolar cells (Kawata et al., 2016). S100B increases following TBI proportionally to the severity of the injury, where TBI triggers inflammation, astrocyte activation and increased S100B production leading to the translocation of S100B to the extracellular matrix where is stimulates cellular damage and degeneration

(Kawata et al., 2016). Due to its presence in the peripheral nervous system cells, blood derived S100B has also been found to elevate in response to fasting, orthopaedic injury and exercise (Kawata et al., 2016), potentially limiting its utility as a marker of brain injury. GFAP-α is a structural protein expressed by astrocytes and is the most abundant isoform, while GFAP-β and -γ are present in Schwann cells and in the bone marrow in the PNS, respectively (Kawata et al., 2016). Increased levels of GFAP are considered indicative of astrocytic damage.

Biofluid markers are a sensitive measure of brain changes. For example, a case study of a 20-year-old amateur kickboxer with head trauma reported abnormal basal hormone levels, such as low total testosterone levels, suggestive of pituitary dysfunction while pituitary MRI result was normal (Tanriverdi et al., 2007). Moreover, another study found that higher baseline NfL levels in professional boxers were associated with lower baseline thalamus, hippocampus and corpus callosum volumes as seen from MRI and with lower psychomotor and processing speed in cognitive testing (Bernick et al., 2018). Some biofluid markers are now also used for clinical management of TBI. Namely, S100B levels have been used since 2013 in Scandinavian countries to assess which patients with head injury require a CT scan (Undén et al., 2013). Similarly the federal *Food and Drug Administration* in the USA approved GFAP and ubiquitin C-terminal hydrolase L1 (UCH-L1), a neuronal injury marker, in 2018 for the same purpose (FDA Authorizes Marketing of First Blood Test to Aid in the Evaluation of Concussion in Adults | FDA, n.d.).

Biofluid marker levels have also been investigated in association with acute, semi-acute and long-term exposure to RSHI. A panel of biofluid markers (NfL, t-tau, GFAP, S100B and albumin) were measured in CSF (S100B also measured in serum) 7–10 days following 10 (n = 10) or 20 (n = 13) soccer headers findings no significant changes in any of the markers (Zetterberg et al., 2007). While the same research group found significantly higher levels of CSF NfL, t-tau and GFAP in boxers (n = 14) seven to 10 days after a bout compared to three months after rest ($p < 0.01$ for all); no changes in p-tau, β-amyloid protein 1-40 ($A\beta_{1\text{-}40}$) and 1-42 ($A\beta_{1\text{-}42}$) were observed (Zetterberg et al., 2006). Moreover, NfL and GFAP levels were significantly higher ($p < 0.001$ and 0.05, respectively) in the boxers seven to 10 days following the boxing bout compared to the healthy controls (n = 10), with NfL levels remaining significantly higher ($p = 0.001$) in the boxers at three months post compared to the controls. Nonetheless, increases in astroglial injury marker S100B and neuronal injury marker NSE serum levels have been observed immediately following a soccer game, where the number of headers correlated in a dose-response manner with increases in S100B ($r = 0.43$; $p < 0.05$) (Stålnacke et al., 2004,

2006); however, neither of the studies employed a control group or condition and interestingly both found that NSE levels did not correlate with impacts. Significant increases in serum NfL have also been observed over the course of an American football season in starters (n = 11) but not in non-starters (n = 9) (Oliver et al., 2016). Furthermore, significant association between plasma t-tau levels and cumulative head impact index (CHII; r = 0.25, p = 0.01) has been reported in former National Football League players (n = 96), where CHII is a lifetime estimate of number of sport-related impacts to the head incorporating the athlete's career length, playing position and levels of play (Alosco et al., 2017). Nonetheless, tau levels were not significantly different between the former American football players and age-matched controls (n = 28).

Thus, biofluid markers have the potential to detect the effects of RSHI on the brain. However, it is presently unclear which proteins are sensitive to the effects of subconcussive impacts and what the temporal trend of various biofluid markers following RSHI exposure is. Kawata et al. (2016) suggested that tau peaks in serum wihtin one hour, S100B one to three hours, GFAP in around 12 hours and NSE within 24 hours following concussion. Zetterberg et al. (2007) sampled a panel of brain injury markers 7 to 10 days post soccer heading based on the biomarker kinetics following stroke. However, considering the subtle nature of RSHI effects on the brain, the presence of biomarkers in biofluids following subconcussive impacts is likely to be shorter lived than in stroke and in concussion.

1.6 Motor control

Motor control is typically used in sport-related concussion assessment. For example, the Sport Concussion Assessment Tool 6 (SCAT6) contains modified Balance Error Scoring System (BESS) as well as single-and dual-task tandem gait assessment (Echemendia et al., 2023). BESS consists of single leg, double leg and tandem stance trials lasting 20 seconds each with eyes closed, where the individuals' performance and errors are determined through visual assessment. Static balance assessment such as BESS and variations of the assessment are also commonly used in the field of RSHI research (Stephen et al., 2022). Generally, studies examining postural control following RSHI have displayed mixed findings.

Gysland et al. (2012) examined balance pre and post American football season (n = 46) using the Sensory Organization Test (SOT) where postural sway is measured using a force platform under six different conditions (normal vision, eyes closed and sway-referenced visual input on normal support surface and on sway-referenced support surface) and BESS completed on a firm and on a foam surface, reporting mixed findings. The number of years playing

American football was associated with significantly worse SOT score pre-to post-season. Surprisingly, however, higher number of head impacts and prior concussions were associated with significantly improved BESS scores post-season while, the higher cumulative head impact magnitude was predictive of worse BESS score as expected. Postural control has also been assessed acutely following RSHI exposure. Significant postural instability has been found 24 hours following a bout of 10 soccer headers (n = 8), but not following simulated heading (control group; n = 8) (Haran et al., 2013). The study measured postural control 1-, 24- and 48-hours following heading under six conditions where participants were standing on a posture platform that was manipulated (stationary versus dynamic) and were surrounded by a virtual environment that was either stationary, dark or rotating. Kinematic data were collected using a six-camera infrared motion analysis system. Interestingly, another study measuring vestibular function and walking stability before, immediately and 24 hours after 10 headers (n = 10) also found significant changes (Hwang et al., 2017). However, the changes in vestibular processing and mediolateral trunk orientation were observed immediately after the heading with performance returning to baseline within 24 hours suggesting that any changes in motor control after RSHI are transient. The study implemented (1) modified BESS, (2) standing postural control assessment while participants stood on a foam surface with eyes closed while receiving galvanic vestibular stimulation (GVS) with postural kinematics captured using a six-camera motion capture system and (3) a walking stability task where participants walked on a treadmill receiving visual feedback of trunk movement with the aim of maintaining trunk stability during gait. The study reported an improvement in BESS scores in the control group (n = 10), but not in the participants who performed heading, suggesting that RSHI exposure dampened the learning effect. Moreover, diminished response to GVS after heading suggests that RSHI disrupts vestibular processing.

In contrast, a study that assessed postural control (total sway and mean centre of pressure) a day before and immediately after 20 linear or rotational soccer headers, 20 simulated rotational headers (control group) and no intervention (control) (10 participants in each group), where participants stood on a on a firm and a foam surface placed on a force platform with eyes open or closed, reported no changes in postural control ($p > 0.2$; $\eta^2 > 0.04$) (Broglio et al., 2004). Di Virgilio et al. (2016) also assessed changes in postural control following a drill of 20 headers by measuring anterior-posterior and medial-lateral sway while participants (n = 19) stood on a circular dynamic platform, also finding no significant changes in postural stability. Moreover, one study assessed balance using a series of tasks (involving standing on a firm and

a foam surface with eyes open and closed, standing on one leg with eyes open and closed and lastly shifting centre of mass in various directions while keeping the base of support still) before and after shuttle runs (control; $n = 8$) and a bout of rugby tackling in ball carriers ($n = 9$) and tacklers ($n = 9$) finding no significant decrement associated with RSHI exposure (McNabb et al., 2020). In light of these findings, perhaps unsurprisingly, Di Virgilio et al. (2019) reported no changes in postural control following a sparring bout ($n = 20$) when assessing centre of pressure using a force platform where participants completed trials consisting of double and single support stand with eyes open and closed. However, the same study also measured motor control at the motor unit level using decomposed EMG, with the rationale that increased cortical inhibition from subconcussive impacts would dampen the neural drive to the muscles leading to reduced motor firing rate. Interestingly, the study found that low threshold motor units were activated later and high threshold units earlier following sparring. Although it is noteworthy that neither postural control nor motor unit activity was measured in the control group. Nonetheless, these findings are particularly relevant as athletes with impaired motor control, mediated by increased inhibition as also demonstrated by the elongation of SP duration in this study, may also be at greater risk of musculoskeletal injuries (Di Virgilio et al., 2019). Indeed, athletes have been found to be at increased risk of musculoskeletal injuries following concussion (Jildeh et al., 2022; McPherson et al., 2019). Taken together the above evidence suggest that more complex motor control assessment methods are likely to be more sensitive in detecting the effects of RSHI.

1.7 Cognitive Function

Neurocognitive assessment following brain injury both in clinical and research setting can help provide insight into whether and what brain areas are affected and identify the extent of the injury. Cognitive testing typically assesses brain function by measuring variables such as attention, processing speed, reaction time, verbal and visual memory. Similarly to motor control, widely used concussion assessments tools SCAT6, containing Standardised Assessment of Concussion (SAC), and Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) incorporate neurocognitive assessment. These assessments are also commonly used in the field of RSHI with the aim of identifying the effects of subconcussive impacts on the brain (Mainwaring et al., 2018; Stephen et al., 2022; Walter et al., 2022). Cognitive function has been studied in active and retired, amateur and professional athletes following RSHI exposure with mixed results.

A cross-sectional study comparing the performance of 33 amateur soccer players with 27 controls across a large panel of cognitive tests (assessing cognitive functions such as planning, mental speed, attention, memory, abstract reasoning, verbal fluency) found that soccer athletes had impaired planning (39% vs 13%; $p = 0.001$) and memory function (27% vs 7%; $p = 0.004$) compared to the controls (Matser et al., 1999). Whereas the number of concussions were inversely associated with attention, memory and visuoperceptual function. Noteworthily, not all aspects of cognitive function were affected by soccer participation (e.g., mental speed, abstract reasoning and verbal fluency). A subsequent study from the same authors investigated neurocognitive function in 84 active professional soccer players and reported that the estimated number of headers (median 500 headers, based on self-report) performed in the previous season was associated with significantly worse performance in focused attention and in visual and verbal memory, while prior number of concussions were related to worse sustained attention and visuoperceptual processing (Matser et al., 2001). Moreover, Witol and Webbe (2003) also assessed neurocognitive function in active amateur and professional soccer players ($n = 60$) in a cross-sectional study using a battery of cognitive tests assessing a range of cognitive functions and reported that athletes with higher estimated lifetime head impact exposure had poorer attention, concentration, cognitive flexibility and intellectual ability. Similarly, to Matser et al. (1999, 2001) findings, however, athletes' performance was not affected across all cognitive tests suggesting that not all cognitive functions or tasks are affected the same by exposure to head impacts. Interestingly, all three studies found that visuoperceptual functioning (i.e., Benton Facial Recognition Test) was not affected by soccer participation (or heading exposure) while it was affected by prior concussions in the two studies that examined this relationship (Matser et al., 1999, 2001).

While the above cross-sectional evidence concerns active athletes, reduced neuropsychological function has also been found in retired athletes and acutely following RSHI exposure. A study in 37 former Norwegian National Football Team players reported impaired attention, concentration, judgement and memory function in majority of the players (Tysvaer & Lochen, 1991) however, the study did not account for concussion history. Moreover, Montenigro et al. (2017) demonstrated a dose-response relationship between cumulative head impact exposure and the risk of cognitive impairment ($p < 0.0001$) in 93 former American Football players. While research undertaken within University of Stirling has demonstrated that a drill of 20 headers ($n = 19$) and sparring ($n = 20$) can cause acute and transient impairments in episodic and working memory assessed using Cambridge Automated

Neuropsychological Test Battery (CANTAB) Paired Associate Learning (PAL) and Spatial Working Memory (SWM) tasks, respectively (Di Virgilio et al., 2016, 2019). Interestingly, Reaction Time, Attention Switching Task (assessing executive function) and Rapid Visual Processing (sustained attention measure) were not affected by soccer heading (Di Virgilio et al., 2016).

Nonetheless, not all research has found cognitive deficits in athletes exposed to repetitive head impacts. A cross-sectional study in active Norwegian professional soccer players (n = 271; aged ~26 yr) found that neither lifetime heading exposure estimate nor concussion history were associated with neuropsychological test performance across a battery of tests assessing various aspects of cognitive function (e.g., attention, working memory and learning) (Straume-Naesheim et al., 2005). Similarly, Gysland et al. (2012) reported that the number and magnitude of head impacts observed during a season of American football, or concussion history, did not affect neuropsychological function (e.g., reaction time, visual and working memory) of the athletes (n = 46) from pre-to-post season. Moreover, a panel of neurocognitive measures (including working and episodic memory, reaction time, impulse control, executive function) and changes in neuroimaging (resting state fMRI and DTI) were assessed in a small sample (n = 15 with head impact exposure) of mixed sex youth soccer players with an average age of 13 years before and after a weekend of soccer matches (post measures completed within eight days) finding no changes in cognitive function or in brain imaging (Chrisman et al., 2016). The lack of changes observed in the study could be due to the small sample size, wide sampling window and a small average number of head impacts (average of four headers) sustained by the players. Nonetheless, a systematic review with meta-analysis (k = 17) also did not find support for heading being associated with adverse neurocognitive function, where measures of attention and concentration (k = 11), impulse control (k = 2), reaction time (k = 3), processing speed (k = 10) and verbal and visual memory (k = 16 and 11, respectively) were assessed (Kontos et al., 2017). Research investigating neurocognitive function in the field of RSHI has been criticised for lack of appropriate adjustment for multiple comparisons, where the use of multiple tests in the same study may have increased the chance of false positive findings (Ntikas et al., 2022).

Another systematic review concluded that neurocognitive assessment is not as sensitive for detecting the effects of RSHI on the brain as for example neuroimaging, potentially due to the brain's ability to compensate for changes through cognitive reserve (Mainwaring et al., 2018). Walter at al. (2022) suggested in their systematic review that neurocognitive assessment

in the field or RSHI alone may not be useful due to lack of sensitivity, however, when used in conjunction with other physiological assessments it may be beneficial. It can be difficult to detect and measure acute brain changes following RSHI due to the lack of sensitivity of the commonly used measures compared to the subtleness of the brain effects, highlighting the importance of using multiple modalities when investigating the effects of RSHI on the brain. Evidence from RSHI studies combining neuroimaging or biofluid markers with neuropsychological assessment has demonstrated promising results. A cross-sectional study by Lipton et al. (2013) reported reduced WM fractional anisotropy in 37 active amateur soccer players (discussed in the imaging section in the introduction) that was associated with reductions in memory function. Noteworthily, the impact threshold for neurocognitive changes was higher than for microstructural brain changes (1800 compared with 885 to 1550 headers per year) suggesting that neurophysiological changes precede neurocognitive deficits. Moreover, the study did not find an association between microstructural brain changes or cognitive function and concussion history. Interestingly, decrements in psychomotor speed, attention and executive function measured using comprehensive computer-administered battery of tests were not found. Worse neurocognitive performance has also been associated with levels of biofluid markers. Elevated levels of plasma exosomal tau in retired American football players (n = 78) have been associated with worse memory (p = 0.01) and psychomotor speed (p = 0.01) (Stern et al., 2016), while higher plasma NfL (but interestingly not tau) levels in professional fighters have been associated with worse psychomotor (r = -0.12; p = 0.02) and processing speed (r = -0.11 p = 0.04) in a large cohort study (Bernick et al., 2018).

Therefore, while the appeal of neurocognitive assessment is the ease of administration and affordability, making it an attractive measure, cognitive tests, especially ones that are designed and used for the assessment of TBI, may not be sensitive enough to detect the subtle effects of RSHI on the brain. Nonetheless, it has been suggested that computerised tests may be more sensitive to the effects of head trauma (Straume-Naesheim et al., 2005) and neuropsychological assessment may be beneficial when used in combination with other outcome measures. A further consideration is that cognitive tests may be susceptible to learning effect where individual performance improves with practice. Therefore, conducting familiarisation trials to mitigate any potential learning effects are an essential consideration especially when assessing subtle brain changes like in the case of RSHI. A study looking at the practice effect on cognitive test performance had participants complete a battery of cognitive tasks four times over a four-hour period with a 10-minute break between first and second and third and

fourth trial and a longer break of around hour and a half after the second trial and found that the biggest learning effect occurred between the first and second trial (Collie et al., 2003). This suggests that dual baseline testing, where disregarding the first trial can help minimise the learning effect.

1.8 Soccer Heading Paradigm

With the somewhat inconsistent findings from the RSHI research in mind and the potential effect of confounding variables that are present in the sporting setting (such as the effect of exercise [e.g., fatigue, hydration], potential musculoskeletal injuries, lack of control over impact exposure), and considering that even the most essential questions are yet to be answered in the field, it is potentially most beneficial at this stage to examine the effects of RSHI in a controlled environment. Laboratory results should later be implemented and re-assessed in the real-life environment to determine the ecological validity of findings and measures. Especially considering that the number and frequency of impacts completed in the lab-based studies may not be directly comparable to the real-world training or game scenarios.

Performing laboratory-based studies allows to assess the subtle effects of RSHI in isolation without the confounding variables 'mudding the water'. While the assessment methodologies are more convenient in the laboratory-based set-up, the challenge in the laboratory environment is the exposure to RSHI. Potentially the most feasible way, certainly the most adopted in this field of research, of mimicking real world RSHI exposure in the lab is through a soccer heading paradigm. Whereby, participants perform soccer headers, and the ball is either launched via a machine (Broglio et al., 2004; Di Virgilio et al., 2016; Dorminy et al., 2015; Haran et al., 2013; Nowak et al., 2020), thrown/dropped from above (Austin et al., 2021; Otto et al., 2000) or kicked (Naunheim et al., 2000; Zetterberg et al., 2007) a number of times. Bevilacqua et al. (2019) published an in vivo protocol of a recommended heading paradigm for studying the acute effects of RSHI on the brain. Their recommendation is to use a machine to dispense the ball, as it allows to control and manipulate the speed of the ball, the interval between and the number of impacts, and potentially also the impact location on the head.

Controlled heading research has produced a wide range of peak linear acceleration (PLA), from 13.1 to 50.7 g per impact (Austin et al., 2021; Di Virgilio et al., 2016; Dorminy et al., 2015; Nowak et al., 2020) which are comparable to impact accelerations observed in American Football (Duma et al., 2005; Mihalik et al., 2007; Naunheim et al., 2000), ice hockey (Mihalik et al., 2008; Naunheim et al., 2000) and rugby (King et al., 2015). While aspects such

as the accelerometer placement may affect the observed impact metrics, meriting caution over direct comparison between studies, the findings from RSHI done using the heading paradigm are likely to be generalisable to other contact sports. Furthermore, McNabb et al. (2021) research demonstrated that it is also possible to mimic more complex RSHI exposure in the laboratory setting by assessing participants before and after controlled rugby tackling. Unfortunately, however, the authors of the aforementioned study did not record head impact metrics.

1.9 Prevention Research

It is estimated that over 50 million people worldwide suffer a TBI each year with at least half of the world's population having one or more TBIs during their life (Maas et al. 2017). Concussions, also referred to as mild traumatic brain injuries (mTBIs), are considered to account for majority (70-90%) of the TBIs that occur, with traffic accidents being the most common cause (Maas et al. 2017). Sport-related concussions are also considered a frequent cause of TBIs, and it is likely that the prevalence of concussions is underestimated as many incidents are likely to go medically unreported (Chen et al., 2019; Maas et al., 2017).

Previous findings suggest that a wide range of impact magnitudes (~60-168 g in linear acceleration) can cause a concussion (Guskiewicz & Mihalik, 2011). A study using a fine element human head model, that includes anatomical brain structures and is validated using cadaveric ventricular and intracranial pressures data, reenacted head impacts from American football and reported that linear accelerations of 66, 82 and 106 g at the centre of gravity of the head have 25, 50 and 80% concussion probability, respectively (Zhang et al., 2004). Moreover, a systematic review with meta-analysis reported that the mean peak linear and rotational acceleration (PRA) associated with concussion in male athletes was 98.7 g and 5776.6 rads/s^2, respectively (Brennan et al., 2017). Also, it is noteworthy, that the latter analysis had a considerable sample size for concussions (PLA $n > 280$, PRA $n > 320$). Notably, PLA and PRA associated with sustaining a concussion has been reported much lower in females (43.0 ± 11.5g, 4030 ± 1435 rad/s^2; although these values are based on only four concussions; Wilcox et al., 2015).

However, simply because an impact does not result in concussive symptoms (diagnosis), it does not mean that the impact did not cause an acute brain response. Moreover, preventing concussions that are likely accidental, unlike subconcussive impacts that are routine and deliberate, may be more difficult than limiting subconcussive impacts. Additionally, while the risk of neurodegenerative disease is suggested to scale with the TBI severity (Maas et al. 2017), it has been suggested that cumulative subconcussive impact exposure is a stronger predictor

for adverse long-term brain health than concussion history in contact sport athletes (Montenigro et al., 2017). In addition to the research described before in this introduction, Montenigro et al. (2017) reported a clear dose-response relationship between cumulative head impact exposure and later-life behavioural dysfunction, depression, apathy, cognitive impairment and self-reported executive function. The authors reported that after a threshold, the risk of impairment increased with additional impacts, where the risk of depression, apathy, behavioural and executive dysregulation almost doubled with 2800 additional impacts above the threshold, and increasing head impact exposure from 6500 to over 12,000 increased the risk of cognitive impairment in athletes by 25-fold.

The easiest way to reduce the cumulative burden is by limiting the total number of impacts that an athlete sustains throughout their career. However, it is currently unclear whether there is also a dose-response tolerance for triggering acute brain changes that may add to the risk of long-term adverse brain health. Moreover, it is evident from the concussion research that the higher the impact magnitude, the greater the risk of injury. Therefore, it is potentially beneficial not only to limit the number of impacts, but also dampen the impact force. In soccer for example, multiple factors such as neck anthropometrics and strength (Caccese et al., 2018; Gutierrez et al., 2014), ball characteristics and travelling speed (Tierney et al., 2020), impact location and heading technique (Harriss et al., 2019) and the use of headgear (Naunheim et al., 2003; Tierney et al., 2008; Withnall et al., 2005) have been investigated in an attempt to provide recommendations for reducing heading induced impact force and subsequent risk of brain injury.

Generally, soccer is a unique sport due to people using their unprotected head to strike and direct the ball while other common contact sports such as boxing, rugby, American football, ice hockey implement the use of headgear or helmets to protect the players. However, heading in soccer has not always been part of the game. Football Association established the original guidelines in 1863 which were later (~1872) amended to prohibit the handling of the ball which lead to 'formation' of football heading, around 12 years after the original guidelines were introduced (Erkmen, 2009). While heading was initially considered ludicrous by the onlookers it is seen as a routine offensive and defensive element in soccer today (Erkmen, 2009). Today, some well-known players such as Wayne Rooney, Petr Čech and Cristian Chivu have been seen wearing headgear however, it is not common practice in soccer. Moreover, there is currently limited evidence of the benefits of using headgear for preventing or mitigating the effects of heading (Broglio et al., 2003; Elbin et al., 2015; Naunheim et al., 2003; Tierney et

al., 2008; Withnall et al., 2005) with considerable time gap since the last published research. Meanwhile various headgear models for soccer heading have become commercially available despite there being no proven benefits of these products. Nonetheless, soccer headgear has the potential to be protective and would be easy to implement, highlighting the need for more research especially into new and untested headgear models. Especially since athletes are known to sacrifice their health in pursuit of athletic achievements (Chen et al., 2019). It is important to consider that athletes are technically employees, who work in an environment where there appears to be a higher acceptance of occupational risk compared to other workplaces, whereas any risks to their health should be removed or mitigated (Chen et al., 2019).

1.10 Aims of the Thesis

As such, the general aims of this thesis are firstly, to find sensitive measures that can detect repetitive subconcussive impact induced acute brain changes consistently and reliably. This will be done first, by assessing whether and how RSHI affect biofluid markers of brain injury through performing a systematic scoping review and examining data previously collected in our laboratory with an added aim of informing subsequent research of this thesis. Further, using a soccer heading paradigm, where the effects of RSHI can be examined in isolation, changes in electrophysiological, cognitive and motor control measures will be examined to determine whether corticomotor inhibition, cognitive function, gait and motor unit recruitment strategies are affected and sensitive to the effects of subconcussive impacts.

Secondly, the objective of this thesis is to investigate potential protective measures to prevent and mitigate the effects of RSHI on the brain. This aim will be addressed primarily by investigating the acute dose-response relationship between RSHI and functional brain response, and by assessing whether the use of headgear can reduce impact magnitude and prevent acute brain changes.

Further details and rationale for each of the aims are provided in the thesis chapters.

Chapter 2 Preface

The scoping review has been published in *Sports Medicine – Open* (for information see *Publications* section on page 5). The publication is licensed under the *Creative Commons Attribution 4.0 International License*, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as appropriate credit to the original author(s) and the source is provided. The publication is presented in this chapter unchanged except for typesetting which has been modified to match the formatting of the rest of the thesis. Additionally, further information was added to section 2.4.2 in the discussion that was not part of the publication, this amendment is presented in a different font colour. For author details and contributions, abstract, key words, key points, funding information, declarations and supplementary materials (latter referred to as 'Additional file 1' in the chapter) please see publication. Abbreviations of the publication are incorporated into the *List of Abbreviations* in the beginning of the thesis (pages 17–19). The extracted data presented in this chapter are available at https://osf.io/kd4wn/.

Chapter 2: The Use of Biofluid Markers to Evaluate the Consequences of Sport-Related Subconcussive Head Impact Exposure: A Scoping Review

2.1 Introduction

A growing body of evidence demonstrates a link between participation in sports that have a high incidence of (head) impacts and long-term neurological impairment and/or neurodegenerative diseases (Chiò et al., 2005; Lehman et al., 2012; Ling et al., 2017; Mackay et al., 2019; Omalu et al., 2005, 2006; Russell et al., 2022; Ueda et al., 2023; Wilson et al., 2017). It is thought that as many as 10–20% of professional boxers suffer from chronic neuropsychiatric disorders (Förstl et al., 2010; Jordan, 2000; Jordan et al., 1992). Furthermore, an increased incidence of neurodegenerative diseases has been observed in ex-professional soccer (Mackay et al., 2019; Ueda et al., 2023), rugby (Russell et al., 2022), and National Football League players (Lehman et al., 2012) compared to the general population. Traumatic brain injury (TBI) is increasingly recognized as a risk factor for later developing neurodegenerative processes and diseases (Livingston et al., 2020; Maas et al., 2017; Wilson et al., 2017). Evidence for a link between contact sport and chronic traumatic encephalopathy (CTE) is also strengthening (Ling et al., 2017; Smith et al., 2019; Stein et al., 2015). Interestingly, years of contact sport exposure has been associated with CTE pathology regardless of the number of symptomatic TBIs such as sport-concussion (Stein et al., 2015). In fact, estimated total cumulative exposure to repetitive head impacts has been found to be a stronger predictor of later cognitive and neurobehavioral impairment than concussion history in American football players (Montenigro et al., 2017). The recently emerging picture is that routine exposure to repetitive head impacts may pose a significant risk to brain health, quite separate from (accidental) impact exposure resulting in TBI (e.g., sport-concussion). Routine impacts in sport are either direct hits to the head (such as soccer headers) or blows to the body (e.g., full-body collisions between players, which are frequent in sports such as rugby, ice hockey, and American football). In recent years, a prominent public debate has started regarding the safety of routine head impacts in contact sports (Ntikas et al., 2022). Such impacts are termed repetitive subconcussive head impacts (RSHI) and characterize the routine and repeated head impacts athletes sustain during contact sport participation that do not result in overt concussion symptoms (Lember et al., 2021). Different lines of enquiry are based on the idea that RSHI can trigger subclinical pathology and a complex cascade of molecular alterations (Myer et al., 2019).

There are two main reasons why the relationship between RSHI and pathological processes has been seemingly neglected until recently. One is that TBI, such as sport-concussion, is common in those sports that also expose participants to RSHI, meaning that the two sources of impact in sport are often conflated (a study challenge addressed in this review). Inevitably, the symptomatic source of impact (concussion) receives more attention with regard to consequences to brain health than the routine and 'normalized' source of impact that does not result in evident injury symptoms. The latter issue, lack of evident symptoms, is also the second main reason why RSHI may be under-researched. Until recently, measures to assess brain health consequences of RSHI appeared to lack sensitivity (Ntikas et al., 2022). While it is unclear what risk RSHI poses to brain health, there is a need for measures that are (1) sensitive, (2) specific, and (3) informative in revealing the effects of RSHI on the brain. Biofluid markers of brain injury have developed in recent years, and their use to detect RSHI-induced brain changes is an emerging field of research (Mainwaring et al., 2018; Walter et al., 2022). Biofluid markers of brain injury can potentially be an efficient and practical method for providing information about routine sport-related RSHI exposure effects on brain health.

Multiple international studies have provided evidence that biofluid markers are associated with brain damage after TBI and have the potential as an objective tool for diagnosis and outcome prediction (Czeiter et al., 2020; Helmrich et al., 2022; Korley et al., 2022; Mondello et al., 2016, 2021; Yue et al., 2019). The implementation of ultrasensitive assays has opened up possibilities to accurately and noninvasively detect subtle structural damage, and more recently, it has been shown that biomarkers can also be used to monitor progressive alterations in the brain, years after TBI (Newcombe et al., 2022). Furthermore, biomarker levels indicate axonal, neuronal and astroglial changes and injury, and their combination can reflect (and provide information on) molecular and cellular responses and underlying pathological mechanisms triggered by head trauma (Halford et al., 2017; Mondello et al., 2012; Zetterberg et al., 2013). As such, there is evident potential for the use of biomarkers to identify subtle RSHI-induced brain changes that may be undetectable based on clinical criteria or imaging assessment. Assessing the functionality of different biomarkers and their ability to detect the effects of RSHI on the brain is thus of great importance: these markers may aid understanding of RSHI-induced brain pathology and give an insight into the link between acute brain changes and chronic neurodegenerative sequelae. The biofluid marker evidence base specific to the effects of RSHI has, however, not yet been reviewed. The evaluation of the biomarkers in RSHI is complicated by

methodological and analytical variability among studies, including research designs, populations, settings, sampling times, analytical approaches, sources, and outcomes being assessed.

Therefore, we conducted a scoping review to identify and comprehensively map the number, features, and quality of studies that have explored the effects of RSHI on biomarker levels. Besides providing an overview of the existing and emerging evidence, we focused on defining methodological problems and identifying potential solutions and research gaps to inform and guide the design and analysis of future studies and research.

2.2 Methods

2.2.1 Protocol and Registration

This scoping review adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) (Tricco et al., 2018) guidelines. The review protocol has been published in BMJ Open (Lember et al., 2021).

2.2.2 Information Sources

The following seven electronic databases were searched from inception until March 2022: Cochrane Library, MEDLINE (EBSCO host), Scopus, SPORTDiscus, CINAHL Complete, PsycINFO, and OpenGrey. The following clinical trial registration platforms were also searched for relevant protocols and corresponding full-text publications: ClinicalTrials.gov and WHO International Clinical Trials Registry Platform. Key descriptors that included terms for subconcussive head impacts, biomarker, and contact sport (see Additional file 1: Table S1 for examples) were used for the search. The full search strategies are available in Additional file 1: Table S1. Reference lists of the included studies were also screened to identify additional records.

2.2.3 Study Selection and Eligibility Criteria

We used the web-based systematic review software Covidence (Covidence, Veritas Health Innovation, Melbourne, Australia; available at www.covidence.org) for the selection process. After the removal of duplicates, two reviewers (L-ML and MN) independently screened the titles and abstracts against the predetermined eligibility criteria, followed by full-text review of retained articles. Any disputes between reviewers were resolved through discussion and if necessary, by a third reviewer (SM).

We included studies that investigated biofluid markers, including brain injury markers such as S100 calcium-binding protein beta (S100B), ubiquitin C-terminal hydrolase L1 (UCH-L1), glial fibrillary acidic protein (GFAP), neurofilament light (NfL), tau, and microRNAs (miRNAs), cytokines, chemokines, and hormones, in blood (serum or plasma), cerebrospinal fluid (CSF), saliva or urine in athletes who were acutely or chronically exposed to sport-related RSHI. We excluded studies assessing biomarker concentrations following solely sports-related concussion or traumatic brain injury. Studies that assessed the effects of repetitive head impacts (both RSHI and concussions) were included. However, if those studies did not separate concussions from RSHI through either (1) exclusion of concussion cases or (2) analysis (covariate), then this was reflected in the bias and quality rating conducted as part of this review. Post-mortem and non-human examinations were also excluded. No restrictions were placed on methodological standards, analytical platforms, study design, and sample size. Studies were included regardless of geographic location and date of publication. We considered reports in the English, French, German, and Italian languages. Detailed inclusion criteria including the Population, Exposure, Comparator, Outcomes, and Study Design (PECOS) framework applied in this scoping review are available in Additional file 1: Table S2. A list of excluded articles with reasons for exclusion (e.g., duplication or redundant publication) during full-text screening is provided in Additional file 1 (Table S3).

2.2.4 Data Extraction and Results Categorization

Data were recorded independently by two reviewers using a standardized and piloted data collection form. Disagreements were discussed until consensus was reached, and, if necessary, a third reviewer was consulted for arbitration. Information about the study design, aim(s), population, RSHI definition, exposure to RSHI, and biofluid marker characteristics (including sampling time, source, analytical platform, and concentrations) were extracted. Studies were classified as either laboratory- or field-based, depending on whether the RSHI occurred in a controlled environment or in the field (such as during training, games, or matches). Further, studies were categorized as acute, semi-acute, or chronic. Studies were considered acute if changes in biomarker concentrations were assessed immediately following RSHI exposure (< 2 weeks) and semi-acute if changes were assessed following an extended rest period from RSHI (e.g., ≥ 2 weeks), or if the effects of accumulation of RSHI were assessed over a season. Studies that investigated the relationship between the history of contact sport participation (years of participation, total number of games or competitions in lifetime) and biofluid marker concentrations were considered to assess the chronic effects.

2.2.5 Risk of Bias and Quality Assessment of Included Studies

A modified version of the risk of bias in non-randomized studies of interventions (ROB-INS-I) tool (Sterne et al., 2016) was used to assess the methodological quality of all primary research publications by evaluating four domains: (1) confounding variables, (2) missing data, (3) measurement of outcomes, and (4) selection of reported results. Confounding variables were considered factors, other than RSHI, that could influence the concentration of the biofluid markers, such as exercise, history of concussion, peripheral injuries, neurological diseases, and so on.

In addition, to increase rigor and determine the quality of study reporting, a modified version of the Subconcussion-Specific Tool (SST) was utilized to assess the quality of the included studies (Comper et al., 2010; Mainwaring et al., 2018). Each study was assessed for the following six criteria: (1) Was there an attempt to define the term 'subconcussion'? (2) Was the number or magnitude of impacts reported (or used in the analysis)? (3) Were participants who sustained a concussion during the study controlled for or excluded from analyses? (4) Were participants with a history of concussion controlled for or excluded from the analyses? (5) Was the control group matched on two or more variables (e.g., history of concussion, sex, age, etc.)? (6) Did the study analyze sex differences, or acknowledge limitations associated with sampling only males or females? Studies were classified as category A, B, or C (i.e., high, medium, and low quality) depending on how many criteria were fulfilled. Category A studies met five or more criteria, B category studies three or four criteria, and C two or less. Question three was not relevant to cross-sectional studies assessing the chronic effects of RSHI in retired athletes and as such, for the purpose of classification, this criterion was considered achieved for these studies. Two of the review authors (L-ML, MN) independently assessed the studies for the risk of bias and quality. Disagreements were resolved through consensus and if necessary, arbitration by a third reviewer was sought.

2.2.6 Synthesis and Reporting of the Results

The search results are reported in a flow diagram detailing the review decision process. The synthesis of results includes a narrative and quantitative summary in text and the main characteristics of the included studies are presented in tables. The results are categorized and presented according to a priori defined categories and inductively developed categories (i.e., biofluid markers, the timing of sampling [acute, semi-acute or chronic], setting [laboratory or field], and sample source [blood, CSF or saliva]). Risk of bias graphs were generated using

robvis web-based software (McGuinness & Higgins, n.d.; available at https://mcguinlu.shinyapps.io/robvis/).

2.3 Results

2.3.1 Description of Studies

Our searches retrieved 7062 records from which 4135 titles and abstracts were screened following the removal of duplicates. One hundred and thirty-five full-text articles were assessed for eligibility and 79 articles were included in the review (see Figure 2.1; detailed information about the studies can be found in Additional file 1: Table S4). Inter-rater reliability in the study identification process was substantial for title/abstract screening and moderate for full-text review ($\kappa = 0.71$ and 0.60, respectively).

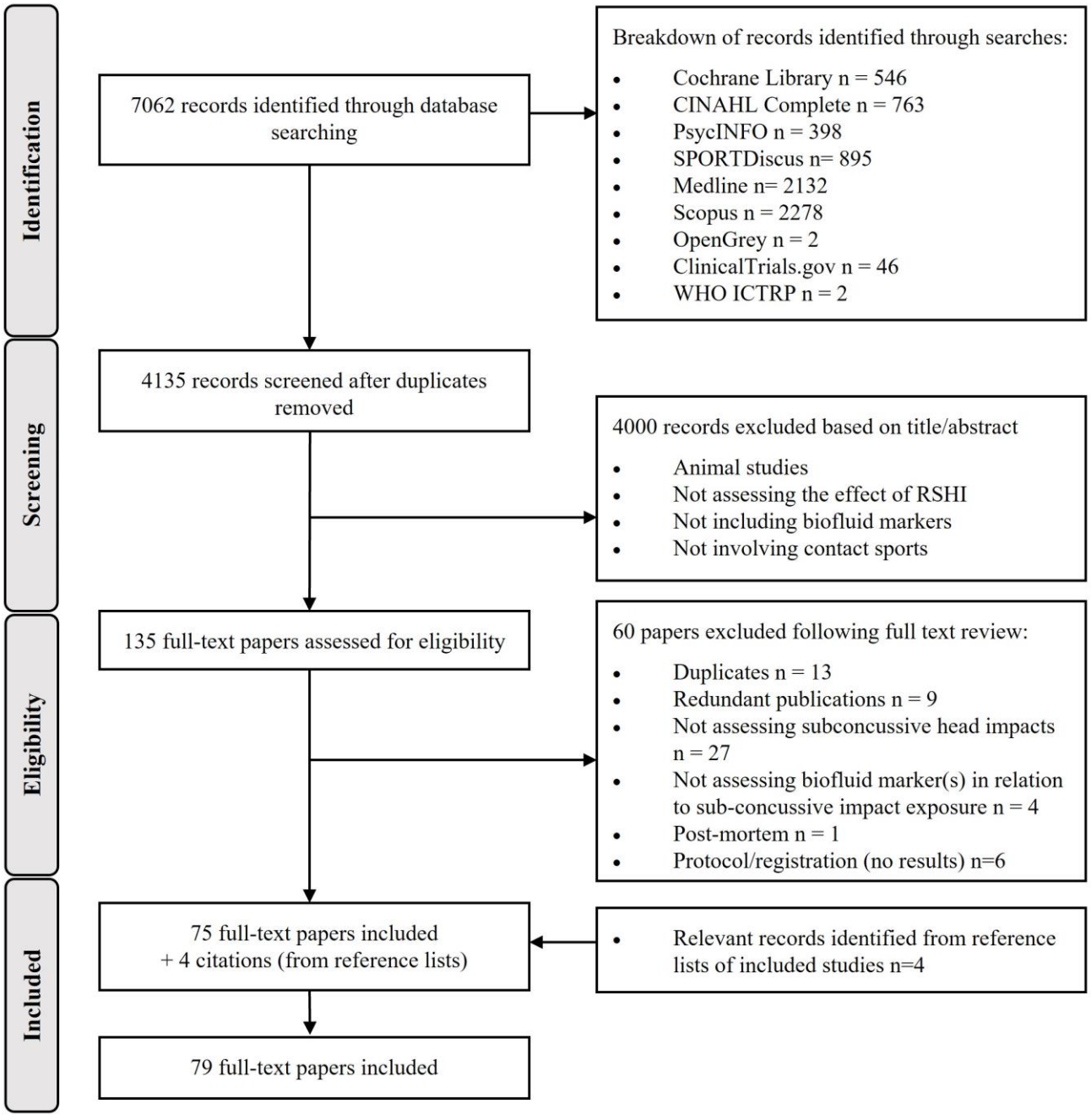


Figure 2.1. PRISMA flow diagram.

The earliest identified record was published in 1982 (Brayne et al., 1982) with the number of studies increasing remarkably in the last decade (Figure 2.2).

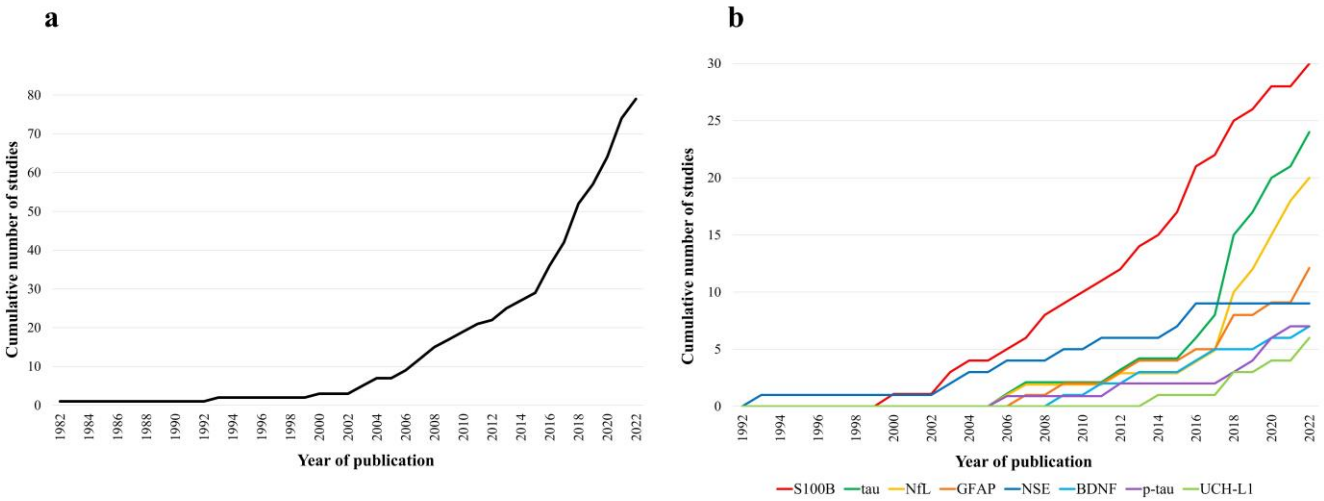


Figure 2.2. a Temporal trend of all the studies; b Temporal trend by biomarker.

The majority (~85%) of the studies employed an observational design with 44 cohort, 19 cross-sectional, and four case–control studies. Only 11 studies (~14%) employed an experimental design with seven of them being randomized. We identified just one case report relevant to this scoping review.

Forty-nine studies assessed markers acutely, 23 in the semi-acute-phase and 26 investigated long-term effects. Eighteen studies assessed a mix of acute, semi-acute, or chronic effects of RSHI exposure.

Further, 13 studies (~16%) were laboratory-based and 45 were field-based (~57%). A case report and the majority of the chronic studies were not considered laboratory- or field-based and were categorized as 'other' (22 out of 79, ~28% of studies).

Most studies (~53%) have been conducted on male athletes (42 out of 79). There were only two studies conducted on female participants (Antonio et al., 2021; Stålnacke et al., 2006) and 20 included a mixed population. Sex was not specified in 15 studies. Only three studies included exclusively individuals younger than 18 years (age range ~13-17; Joseph et al., 2019; Mussack et al., 2003; Zonner et al., 2019). Fifty-two studies employed either a control condition or had a control cohort.

Most studied markers were: S100B (30 studies), tau (24 studies, including 4 studies assessing tau in extracellular vesicles [EVs]), NfL (20 studies), GFAP (14 studies), NSE (9 studies), BDNF (7 studies), phosphorylated tau (p-tau) (7 studies), and UCH-L1 (6 studies) (see Table 2.1). Further, nine studies assessed the hormonal response to RSHI (~10%). All other

biofluid markers had fewer than five research publications available per marker; information about all markers is provided in Additional file 1: Table S4. The vast majority of the samples were venous i.e. from serum and/or plasma (72 studies), while some studies sampled from cerebrospinal fluid (six studies; Alosco et al., 2018; Muraoka et al., 2019; Neselius et al., 2012; Neselius, Zetterberg, Blennow, Marcusson, et al., 2013; Zetterberg et al., 2006, 2007), or from saliva (five studies; Hicks et al., 2021; Matuk et al., 2021; Pin et al., 2021; Soriano et al., 2022; Symons et al., 2020).

Table 2.1. Biomarker specific tables for selected biomarkers: S100B, tau, neurofilament light (NfL), glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), brain-derived neurotrophic factor (BDNF), and ubiquitin C-terminal hydrolase-L1 (UCH-L1).

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
S100B												
Arslan et al. (2010)	Acute	Cohort study	Field	Wrestling	15 male Greco-Roman wrestlers, median age (range) 19.0 (19–30); 16 male Free style wrestlers, aged 20.0 (19–26)	N/A	Wrestling competition (3 × 2 min)	Serum	Before and 20 min post	Significant findings	Serious	C (1)
Asken et al. (2018a)	Chronic	Observational cohort study	Other	Soccer, diving, wrestling, ice hockey, Am. football	415 (256 M, 159 F) collegiate athletes, aged 19.0 ± 1.2	N/A	Cumulative exposure to collision sports in years (and modified CHII)	Serum	Off-season	No significant findings	Moderate	B (4)
Bouvier et al. (2017)	Acute	Prospective cohort study	Field	Rugby	39 professional rugby players, aged 28.6 ± 4.0 (27 non-concussed, 5 concussed)	N/A	Rugby match (collisions)	Serum	3 basal levels during the season (> 48 h from competition) and within 2 and 36 h after a match	Significant findings	Moderate	B (4)
Di Battista et al. (2016)	Chronic	Cross-sectional study	Other	Ice hockey, football, rugby, lacrosse	41 (39 M, 2 F) collision sport athletes; aged (including all participants): M (n = 60) 19.5 ± 2.0, F (n = 27) 19.5 ± 1.8	46 (21 M, 25 F) non collision sport athletes (inadvertent contact: soccer, basketball)	Collision sport participation	Plasma	Before the start of varsity season	No significant findings	Moderate	B (4)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Dorminy et al. (2015)	Acute	Randomized controlled trial	Laboratory	Soccer	16 (10 M, 6 F), aged 20.4 ± 0.2	N/A	5 linear standing headers	Serum	Before and 1–1.5 h post	No significant findings	Serious	B (4)
Graham et al. (2011)	Acute	Retrospective cohort study	Field	Boxing	8 male amateur boxers, aged 17.6 ± 5.3 (PTH – punches to the head and body)	8 male amateur boxers, aged 19.1 ± 3.2 (PTB – punches to the body)	5 × 2-min boxing rounds	Serum	1 h before and after 5 min of cessation	Significant findings	Moderate	C (2)
Graham et al. (2015)	Acute	Cohort study	Field	Karate	12 males, aged 30.4 ± 6.7 (KTH – kicks to the head and body)	12 males, aged 28.2 ± 6.5 (KTB – kicks to the body)	4 × 3-min karate round	Serum	Before and immediately after	Significant findings	Moderate	C (1)
Hoffman et al. (2022)	Acute	Cohort study	Field	Am. football	15 Israel national football team players, aged 26.2 ± 5.3	N/A	Am. football match	Serum	1 week before, immediately (< 30 min) and 24 h post	No significant findings	Moderate	C (2)
Huibregtse et al. (2020b)	Acute	Randomized controlled trial	Laboratory	Soccer	37 (19 M, 18 F), median age (IQR) 21 (19–22)	31 (14 M, 17 F), median age (IQR) 21 (20–22)	10 linear headers; controls: 10 kicks	Plasma	Before and 0, 2 and 24 h post	No significant findings	Low	A (5)
Kawata (2016)	Semi-acute	Prospective longitudinal cohort study	Field	Am. football	22 male Division I collegiate footballers, aged 20.6 ± 1.5	N/A	Am. football season	Plasma	Pre- and post-season, and before and after 5 practices (1 non-contact, 4 full contact)	No significant findings	Moderate	A (6)
Kawata et al. (2017)	Acute	Prospective longitudinal cohort study	Field	Am. football	22 male Division I collegiate footballers, aged 20.6 ± 1.5	N/A	Pre-season Am. football practices	Plasma	Baseline, before and after 5 pre-season practices (1 non-contact, 4 full contact)	Significant findings (both in contact and no contact). Impacts	Moderate	A (5)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
										correlated with the increase		
Marchi et al. (2013)	Acute and semi-acute	Cohort study	Field	Am. football	Acute: 27 collegiate players, aged ~21 Semi-acute: 10 collegiate players	N/A	Am. football matches and season	Serum	Acute: baseline (prior to any football related activity), 24 h before, 1 and 24 h post Semi-acute: pre- and post-season	Acute: significant findings in players that had frequent head impacts. Increase correlated with head impact index Semi-acute: anti-S100B Ab increased in 5 out of 10 players	Serious	B (4)
Mussack et al. (2003)	Acute	Non-randomized experimental study	Laboratory	Soccer	61 male amateur players, median age (IQR) 15.3 (14.8–16.4)	58 male amateur players, median age (IQR) 15.9 (15.0–16.8); 81 mTBI controls: 20 CCT+ 41.8 (32.3–61.1), 61 CCT- 37.1 (27.6–53.5)	Controlled soccer heading aimed at the forehead performed for a median: 55 min; control: 61 min of exercise	Serum	Baseline and 1 and 6 h post	No significant findings. Significant findings in the mTBI group	Moderate	B (3)
Neselius et al. (2012)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) Olympic boxers, mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing bout	CSF	1–6 days post and after ≥ 14 days rest	Acute: significant findings Semi-acute: no	Moderate	C (2)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
										significant findings		
Neselius et al. (2013b)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) amateur boxers (competing at elite level), mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing	Serum	1–6 days post and after ≥ 14 days rest	No significant findings	Serious	C (2)
O'Connell et al. (2018)	Acute and semi-acute	Prospective longitudinal cohort study	Field	Rugby	38 professional male rugby players, aged 26.6 ± 4.4	15 rowers, median age (IQR) 22.0 (20.0–24.0)	Rugby training and games	Serum	Pre- and post-season, ≤ 2 h post-games Controls: pre- and post-80 min of training	Acute: significant findings Semi-acute: no significant findings	Moderate	C (1)
O'Keeffe et al. (2020)	Acute and semi-acute	Cohort study	Field	Rugby	8 rugby university team players, mean age (range) 22.1 (18–23); 11 male rugby school team players, mean age 17.4	27 non-contact sport athletes, median age (range) 28 (18–36); 26 healthy non-athlete controls, median age (range) 30 (18–40)	Rugby match (university team) and season (school and university team)	Plasma	University team: pre-season, ≤ 2 h post-match, 2 months post-season School team: pre- and post-season	Acute: significant findings Semi-acute: significant findings (decrease)	Moderate	B (3)
Otto et al. (2000)	Acute	Cohort study	Field, laboratory	Boxing, soccer	25 male amateur boxers: competitive fights $n = 10$, sparring fights $n = 15$ (13 with head protector), aged 17–40; heading:	35 male runners (sprinters, 10 and 25 km) aged 20–52. 12 male cyclists, aged 23–52	(1) 5 × 2-min competitive boxing rounds (2) 3 or 5 × 2-min sparring fights (3) 20 standing soccer	Serum	Before and ≤ 15 min post	Mixed findings (boxing: significant; soccer: not significant)	Serious	C (1)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					12 sportsmen, aged 20–52		headers (ball dropped from 7.5 m)					
Puvenna et al. (2014)	Acute	Cohort study	Field	Am. football	15 athletes	406 positive controls with mTBI and 465 negative controls	2 Am. football games	Serum	Baseline (day before) and post (< 1 h) (positive controls: < 6 h of injury)	Significant findings	Serious	B (3)
Rogatzki et al. (2016)	Acute	Cohort study	Field	Am. football	17 male Division III collegiate footballers, aged 19.5 ± 0.9	N/A	Am. football game	Serum	2 days before and 1 h post	Significant findings	Moderate	C (2)
Rogatzki et al. (2018)	Acute and semi-acute	Cohort study	Field	Am. football	16 male Division III collegiate footballers, age range 18 to 22	32 controls, age range 18–22 [control groups: resistance exercise *n* = 18 (10 M, 8 F); treadmill running *n* = 8 (5 M, 3 F); treadmill walking *n* = 6 (3 M, 3 F)]	Am. football game	Serum	Baseline (prior to training camp); before (day before, ≤ 30 min post-practice) and ≤ 30 min post-4 games Controls: immediately before and ≤ 30 min post	Acute: significant findings for experimental and 2 control groups Semi-acute: no significant findings Number of hits and plays correlated with S100B	Moderate	B (3)
Soriano et al. (2022)	Semi-acute	Cohort study	Field	Am. football	33 male collegiate players, aged 19.3 ± 1.4	N/A	Am. football season (games and training)	Serum	Mid-season, post-season and off-season (after a rest period)	No significant findings	Serious	B (4)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Stålnacke and Sojka (2008)	Acute	Randomized controlled trial	Laboratory	Soccer	10 male amateur players, aged 22 ± 8 (age for entire sample *n* = 19)	9 male amateur players	5 headers in 15–20 min (ball dropped from 18 m, velocity 63.6 km/h)	Serum	Before and 0.5, 2 and 4 h post	No significant findings	Moderate	B (3)
Stålnacke et al. (2003)	Acute	Cohort study	Field	Ice hockey	26 male elite ice hockey players, aged 28 ± 4	18 elite basketball players, aged 25 ± 4	Ice hockey game (body checkings, falls, collisions, boardings); basketball game (jumps, collisions, falls)	Serum	1–2 h before and ≤ 1 h post	Significant findings in all conditions	Serious	C (2)
Stålnacke et al. (2004)	Acute	Cohort study	Field	Soccer	28 male elite players, aged 26 ± 5	N/A	Headers, jumps, falls and collision during a competitive soccer game	Serum	1–5 h before and immediately post	Significant findings	Moderate	B (3)
Stålnacke et al. (2006)	Acute	Cohort study	Field	Soccer	44 female elite players, aged 23 ± 3	N/A	Headers, jumps, falls and collisions during a competitive soccer game	Serum	Before and immediately post	Significant findings (changes correlated with headers and jumps, collisions, and falls)	Moderate	B (3)
Straume-Naesheim et al. (2008)	Acute	Prospective cohort study	Field	Soccer	Professional soccer players: heading exercise *n* = 46, mean age 26.1;	Professional soccer players: high intensity exercise *n* = 48, 26.1; match	Heading exercise (90 min), head impacts (some	Serum	Baseline, 1 and 12 h post	Significant findings in all conditions	Serious	B (3)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					head impacts during a match *n* = 69, 28.1	control *n* = 56, 26.2	concussive) during match play Controls: 90 min exercise, match w/o head trauma					
Zetterberg et al. (2007)	Acute	Non-randomized experimental study	Laboratory	Soccer	23 male amateur soccer players, median age (range): 10 headers *n* = 10, 26 (19–32); 20 headers *n* = 13, 23 (20–28)	9 male non athletes, median age (range) 24 (22–27)	10 or 20 standing headers from a corner kick (kicked from 30 m)	CSF and serum	7–10 days post	No significant findings	Serious	B (3)
Zetterberg et al. (2009)	Chronic	Observational case–control study	Other	Boxing	44 male amateur boxers, median age (range) 19 (17–28)	23 healthy males w/o contact sport history, median age (range) 28 (19–50)	Boxing participation (boxing debut, boxing duration in yr, number of bouts)	Serum	After a 2-month period of nonparticipation in boxing	No significant findings	Moderate	C (1)
Zonner et al. (2019)	Acute and semi-acute	Longitudinal prospective cohort study	Field	Am. football	15 high school footballers, aged 16.4 ± 0.5	N/A	Am. football games and season	Serum	Semi-acute: pre- and post-season; acute: 4–5 h before and ≤ 1 h after 5 games	Acute: significant findings Semi-acute: no significant findings	Low	A (5)
Tau												
Alosco et al. (2017)	Chronic	Cross-sectional study	Other	Am. football	96 male symptomatic former	25 asymptomatic controls w/o contact	Am. football (NFL) career	Plasma	N/A	Mixed	Moderate	C (2)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					NFL players, aged 55.2 ± 7.9	sport history, aged 57.0 ± 6.6						
Alosco et al. (2018)	Chronic	Cross-sectional study	Other	Am. football	68 male symptomatic former NFL players, aged 54.4 ± 8.0	21 asymptomatic controls w/o contact sport history, aged 57.6 ± 7.1	Am. football (NFL) participation (CHII)	CSF	N/A	Tau: mixed P-tau: no significant findings	Moderate	C (2)
Asken et al. (2018a)	Chronic	Observational cohort study	Other	Soccer, diving, wrestling, ice hockey, Am. football	415 (256 M, 159 F) collegiate athletes, aged 19.0 ± 1.2	N/A	Cumulative exposure to collision sports in yr (and modified CHII)	Serum	Off-season	No significant findings	Moderate	B (4)
Bernick et al. (2018)	Acute, semi-acute, and chronic	Longitudinal cohort study	Other	Boxing, MMA	52 (50 M, 2 F) retired professional boxers, aged 48.0 ± 10.3 117 (110 M, 7 F) active professional boxers, aged 30.4 ± 6.9 169 (152 M, 17 F) active professional MMA fighters, aged 29.6 ± 4.8	79 (69 M, 10 F) controls w/o contact sport history, aged 30.8 ± 10.0	Fights and sparring (martial arts or boxing)	Plasma	Baseline and ≥ 2 measurements over 1.6 years (average) (range 1–5 years); active fighters: ≥ 45 days from a sanctioned fight	Acute and chronic: no significant findings Semi-acute: significant findings	Serious	C (1)
Di Battista et al. (2016)	Chronic	Cross-sectional study	Other	Ice hockey, football, rugby, lacrosse	41 (39 M, 2 F) collision sport athletes; aged (including all participants): M (*n* = 60) 19.5 ±	46 (21 M, 25 F) non collision sport athletes (inadvertent contact: soccer, basketball)	Collision sport participation	Plasma	Before the start of varsity season	Significant findings	Moderate	B (4)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					2.0, F (*n* = 27) 19.5 ± 1.8							
Hoffman et al. (2022)	Acute	Cohort study	Field	Am. Football	15 Israel national football team players, aged 26.2 ± 5.3 (range 18–35)	N/A	Am. football match	Serum	1 week before, immediately (< 30 min) and 24 h post	No significant findings	Moderate	C (2)
Joseph et al. (2019)	Acute and semi-acute	Prospective observational cohort study	Field	Am. football	16 male high-school varsity footballers, aged 16.9 ± 0.2 (pre- and post-season sample *n* = 12)	N/A	Am. football games, practices and season	Serum	Semi-acute: pre- and post-season; acute: 1–2 h post	Significant findings	Moderate	B (3)
Kawata et al. (2018a)	Semi-acute	Cohort study	Field	Ice hockey	8 male professional players (including 2 concussed athletes), aged 26.6 ± 1.6	N/A	Ice hockey season	Plasma	Pre- and post-season	No significant findings	Critical	C (1)
Kawata et al. (2018b)	Acute	Prospective longitudinal cohort study	Field	Am. football	23 male Division I collegiate footballers, aged 20.5 ± 1.3	N/A	Pre-season Am. football practices	Plasma	Pre-season baseline, immediately before and ≤ 1 h after 4 practices (1 non-contact)	Significant findings	Moderate	A (5)
Major et al. (2020)	Chronic	Cross-sectional study	Other	Au. football	81 (50 M, 31 F) amateur footballers (no-mTBI history *n* = 42; mTBI history *n* = 39), aged ~24	42 (23 M, 19 F) age-matched non-contact sport athletes	Au. football participation	Serum	Pre-season	Tau: no significant findings P-tau: no significant findings	Moderate	A (5)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Muraoka et al. (2019)	Chronic	Cross-sectional study	Other	Am. football	15 male symptomatic former NFL players, aged 56.3 ± 7.3	16 asymptomatic males w/o contact sport history, aged 57.1 ± 7.0	Am. football career	CSF	N/A	Tau: no significant findings P-tau: no significant findings	Moderate	B (3)
Muraoka et al. (2021)	Chronic	Cross-sectional study	Other	Am. football	27 male symptomatic former NFL players, aged 56.6 ± 7.6	25 asymptomatic males w/o contact sport history, aged 57.0 ± 6.6	Am. football career	Plasma	N/A	Tau: significant findings P-tau: significant findings	Moderate	B (3)
Neselius et al. (2012)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) Olympic boxers, mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing bout	CSF	1–6 days post and after ≥ 14 days rest	Tau: Acute: significant findings Semi-acute: no significant findings P-tau: no significant findings	Moderate	C (2)
Neselius et al. (2013b)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) amateur boxers (competing at elite level), mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing bout	Plasma	1–6 days post and after ≥ 14 days rest	Acute: significant findings Semi-acute: no significant findings	Serious	C (2)
Nowak et al. (2022)	Acute	Case–control study	Laboratory	Soccer	17 (6 M, 11 F) soccer players with ADHD, aged 20.2 ± 0.2; 17 (10 M, 6 F)	17 (7 M, 10 F) soccer players with ADHD, aged 20.5 ± 0.1	10 linear headers; controls: 10 kicks	Plasma	Baseline, 2 and 24 h post	No significant findings	Low	A (5)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					w/o ADHD, aged 21.1 ± 0.1							
Oliver et al. (2017)	Semi-acute	Longitudinal observational cohort study	Field	Am. football	19 (11 starters, 8 non-starters) Division I footballers, aged 20 ± 1	19 NCAA swimmers, aged 20 ± 1 (baseline sample only)	Am. football season	Plasma	T1: after 9 weeks of non-contact; T2: after training camp; T3: following pre-season camp (highest concentration of impacts); T4 through T8 mid-season, 36–48 h post-games	No significant findings	Moderate	B (3)
Oliver et al. (2019)	Semi-acute	Prospective longitudinal cross-sectional study	Field	Am. football	35 (20 starters, 15 non-starters) Division III footballers, aged 21 ± 1	N/A	Am. football season	Plasma	T1: after 14-weeks of non-contact; T2: end of camp (period with most impacts); T3: 72 h post-full-contact practice, T4 and T5: ~36 h following a game; T6 and T7: post-season	Significant findings (decrease)	Moderate	B (3)
Sandmo et al. (2020)	Acute and chronic	Prospective cohort study	Field	Soccer	Male premier league players: heading exercise group *n* = 47, head impacts during a match *n* = 35	Male premier league players: high intensity exercise *n* = 47	(1) heading exercise (90 min), (2) head impacts (some concussive) during match play	Serum	Baseline, 1 and 12 h post	No significant findings	Serious	B (2)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Soriano et al. (2022)	Semi-acute	Cohort study	Field	Am. football	33 collegiate athletes, aged 19.3 ± 1.4	N/A	Am. football season (games and training)	Serum	Mid-season, post-season and off-season (after a rest period)	Not detectable	Serious	B (4)
Stern et al. (2016)	Chronic	Case–control study	Other	Am. football	78 male symptomatic former NFL players, aged 54.5 ± 8.0	16 male asymptomatic non-contact sport athletes, 56.9 ± 7.2	Am. football career	Plasma	N/A	Significant findings	Moderate	C (2)
Symons et al. (2020)	Chronic	Cross-sectional study	Other	Au. football	95 (69 M, aged 23.3 ± 0.4; 26 F, aged 23.2 ± 0.9) amateur players	49 (28 M, aged 22.5 ± 0.4; 21 F, aged 23.1 ± 0.8) amateur basketball, tennis, cricket, track and field athletes	Au. football participation	Serum	N/A (pre-season)	Tau: significant findings P-tau: significant findings	Moderate	B (4)
Wallace et al. (2018)	Acute	Prospective controlled cohort study	Laboratory	Soccer	11 male collegiate players, aged 23.7 ± 3.9	N/A	40 headers; sham condition: contact with ball using hands, chest or thigh	Plasma	Immediately before and 1 h and 3 weeks post	No significant findings	Critical	C (2)
Zetterberg et al. (2006)	Acute and chronic	Longitudinal cohort study	Field	Boxing	14 (11 M, 3 F) amateur boxers, aged 22 ± 3.8	10 male non-athletic controls, aged 30 ± 6.3	Boxing bout	CSF	7–10 days post and after 3 months of rest	Tau: Acute: significant findings Chronic: no significant findings P-tau: no significant findings	Moderate	C (0)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Zetterberg et al. (2007)	Acute	Non-randomized experimental study	Laboratory	Soccer	23 male amateur soccer players, median age (range): 10 headers *n* = 10, 26 (19–32); 20 headers *n* = 13, 23 (20–28)	9 male non athletes, median age (range) 24 (22–27)	10 or 20 standing headers from a corner kick (kicked from 30 m)	CSF	7–10 days post	No significant findings	Serious	B (3)
NfL												
Antonio et al. (2021)	Chronic	Cross-sectional study	Other	Soccer	8 female Division II soccer players, aged 22 ± 6	17 female non-contact sport athletes, aged 25 ± 8	Soccer participation	Plasma	N/A	Significant findings	Serious	C (2)
Austin et al. (2021)	Acute	Randomized controlled trial	Laboratory	Soccer	36 males (12 in each heading group), aged 23.7 ± 4.8	8 males, aged 23.7 ± 4.8	10, 20 and 40 linear headers	Serum	Baseline, 6 h, 24 h, 7 days	No significant findings	Low	A (5)
Bernick et al. (2018)	Acute, semi-acute, and chronic	Longitudinal cohort study	Other	Boxing, MMA	52 (50 M, 2 F) retired professional boxers, aged 48.0 ± 10.3 117 (110 M, 7 F) active professional boxers, aged 30.4 ± 6.9 169 (152 M, 17 F) active professional MMA fighters, aged 29.6 ± 4.8	79 (69 M, 10 F) controls w/o contact sport history, aged 30.8 ± 10.0	Fights and sparring	Plasma	Baseline and ≥ 2 measurements over 1.6 years (average) (range 1–5 years); active fighters: ≥ 45 days from a sanctioned fight	Acute: significant findings (boxers) Semi-acute and chronic: not significant	Serious	C (1)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Heileson et al. (2021)	Semi-acute	Non-randomized controlled trial	Field	Am. football	66 male NCAA Am. football players	N/A	Am. football games and practices	Serum	Baseline: following > 14-week period of non-contact, after pre-season camp and throughout season	Significant findings	Moderate	C (2)
Joseph et al. (2019)	Acute and semi-acute	Prospective observational cohort study	Field	Am. football	16 male high-school varsity footballers, aged 16.9 ± 0.2 (pre- and post-season sample *n* = 12)	N/A	Am. football games, practices and season	Serum	Semi-acute: pre- and post-season; acute: 1–2 h post	No significant findings	Moderate	B (3)
Kawata et al. (2018a)	Semi-acute	Cohort study	Field	Ice hockey	8 male professional players (including 2 concussed athletes), aged 26.6 ± 1.6	N/A	Ice hockey season	Plasma	Pre- and post-season	Significant findings	Critical	C (1)
Major et al. (2020)	Chronic	Cross-sectional study	Other	Au. football	81 (50 M, 31 F) amateur footballers (no-mTBI history *n* = 42; mTBI history *n* = 39), aged ~24	42 (23 M, 19 F) age-matched non-contact sport athletes	Au. football participation	Serum	Pre-season	No significant findings	Moderate	A (5)
Neselius et al. (2012)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) Olympic boxers, mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing bout	CSF	1–6 days post and after ≥ 14 days rest	Significant findings	Moderate	C (2)
Nowak et al. (2022)	Acute	Case–control study	Laboratory	Soccer	17 (6 M, 11 F) soccer players with ADHD,	17 (7 M, 10 F) soccer players	10 linear headers; controls: 10 kicks	Plasma	Baseline, 2 and 24 h post	Significant findings (in	Low	A (5)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					aged 20.2 ± 0.2; 17 (10 M, 6 F) w/o ADHD, aged 21.1 ± 0.1	with ADHD, aged 20.5 ± 0.1				w/o ADHD group)		
Oliver et al. (2016)	Semi-acute	Observational cohort study	Field	Am. football	116 Division I American footballers (baseline), aged 20 ± 1 (of whom 19 were sampled over the season; 9 non-starters, 11 starters)	19 male NCAA Division I swimmers, aged 20 ± 1 (baseline sample only)	Am. football season	Serum	T1: after 9 weeks of non-contact; T2: after training camp; T3: following pre-season camp (highest concentration of impacts); T4 through T8 mid-season, 36–48 h post-games	Significant findings	Moderate	C (2)
Oliver et al. (2019)	Semi-acute	Prospective longitudinal cross-sectional study	Field	Am. football	35 (20 starters, 15 non-starters) Division III footballers, aged 21 ± 1	N/A	Am. football season	Serum	T1: after 14-weeks of non-contact; T2: end of camp (period with most impacts); T3: 72 h post-full-contact practice, T4 and T5: ~36 h following a game; T6 and T7: post-season	Significant findings	Moderate	B (3)
Rubin et al. (2019)	Acute	Cohort study	Field	Am. football	18 Division I college footballers, median age (IQR) 20.5 (20–22)	N/A	Am. football pre-season practices	Plasma	Baseline: 2 months prior to any practices; < 1 h before and < 1 h post-practices	Significant findings	Moderate	A (6)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Sandmo et al. (2020)	Acute and chronic	Prospective cohort study	Field	Soccer	Male premier league players: heading exercise group *n* = 47, head impacts during a match *n* = 35	Male premier league players: high intensity exercise *n* = 47	(1) heading exercise (90 min), (2) head impacts (some concussive) during match play	Serum	Baseline, 1 and 12 h post	No significant findings	Serious	B (2)
Shahim et al. (2017)	Acute and chronic	Prospective cohort study	Field	Boxing	14 (11 M, 3 F) amateur boxers, median age (IQR) 21.5 (20–26)	14 healthy non-athletic controls, 23.5 (23–26); 12 gymnasts, 19 (18–22)	Boxing bout	Serum	7–10 days post and after 3 months of rest	Significant findings	Serious	C (1)
Soriano et al. (2022)	Semi-acute	Cohort study	Field	Am. football	33 collegiate athletes, aged 19.3 ± 1.4	N/A	Am. football season (games and training)	Serum	Mid-season, post-season and off-season (after a rest period)	No significant findings	Serious	B (4)
Symons et al. (2020)	Chronic	Cross-sectional study	Other	Au. football	95 (69 M, aged 23.3 ± 0.4; 26 F, aged 23.2 ± 0.9) amateur players	49 (28 M, aged 22.5 ± 0.4; 21 F, aged 23.1 ± 0.8) amateur basketball, tennis, cricket, track and field athletes	Au. football participation	Serum	N/A (pre-season)	No significant findings	Moderate	B (4)
Wallace et al. (2018)	Acute	Prospective controlled cohort study	Laboratory	Soccer	11 male collegiate players, aged 23.7 ± 3.9	N/A	40 headers; sham condition: contact with ball using hands, chest or thigh	Serum	Immediately before and 1 h and 3 weeks post	Mixed	Critical	C (2)
Wirsching et al. (2019)	Acute	Randomized controlled trial	Laboratory	Soccer	18 (7 M, 11F), aged 20.3 ± 1.5	16 (6 M, 10F), aged 21.2 ± 1.4	10 soccer headers; controls: 10 kicks	Plasma	Before and 0, 2 and 24 h post	Significant findings	Low	A (5)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Zetterberg et al. (2006)	Acute and chronic	Longitudinal cohort study	Field	Boxing	14 (11 M, 3F) amateur boxers, aged 22 ± 3.8	10 male non-athletic controls, aged 30 ± 6.3	Boxing bout	CSF	7–10 days post and after 3 months of rest	Significant findings	Moderate	C (0)
Zetterberg et al. (2007)	Acute	Non-randomized experimental study	Laboratory	Soccer	23 male amateur soccer players, median age (range): 10 headers *n* = 10, 26 (19–32); 20 headers *n* = 13, 23 (20–28)	9 male non athletes, median age (range) 24 (22–27)	10 or 20 standing headers from a corner kick (kicked from 30 m)	CSF	7–10 days post	Not detectable	Serious	B (3)
GFAP												
Asken et al. (2018a)	Chronic	Observational cohort study	Other	Soccer, diving, wrestling, ice hockey, Am. football	415 (256 M, 159 F) collegiate athletes, aged 19.0 ± 1.2	N/A	Cumulative exposure to collision sports in yr (and modified CHII)	Serum	Off-season	No significant findings	Moderate	B (4)
DiBattista et al. (2016)	Chronic	Cross-sectional study	Other	Ice hockey, football, rugby, lacrosse	41 (39 M, 2 F) collision sport athletes; aged (including all participants): M (*n* = 60) 19.5 ± 2.0, F (*n* = 27) 19.5 ± 1.8	46 (21 M, 25 F) non collision sport athletes (inadvertent contact: soccer, basketball)	Collision sport participation	Plasma	Before the start of varsity season	No significant findings	Moderate	B (4)
Hoffman et al. (2022)	Acute	Cohort study	Field	Am. football	15 Israel national football team players, aged 26.2 ± 5.3	N/A	Am. football match	Serum	1 week before, immediately (< 30 min) and 24 h post	No significant findings	Moderate	C (2)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Joseph et al. (2019)	Acute and semi-acute	Prospective observational cohort study	Field	Am. football	16 male high-school varsity footballers, aged 16.9 ± 0.2 (pre- and post-season testing *n* = 12)	N/A	Am. football games, practices and season	Serum	Semi-acute: pre- and post-season; acute: 1–2 h post	No significant findings	Moderate	B (3)
Kawata et al. (2018a)	Semi-acute	Cohort study	Field	Ice hockey	8 male professional players (including 2 concussed athletes), aged 26.6 ± 1.6	N/A	Ice hockey season	Plasma	Pre- and post-season	No significant findings	Critical	C (1)
Major et al. (2020)	Chronic	Cross-sectional study	Other	Au. football	81 (50 M, 31 F) amateur footballers (no-mTBI history *n* = 42; mTBI history *n* = 39), aged ~24	42 (23 M, 19 F) age-matched non-contact sport athletes	Au. football participation	Serum	Pre-season	No significant findings	Moderate	A (5)
Neselius et al. (2012)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) Olympic boxers, mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing bout	CSF	1–6 days post and after ≥ 14 days rest	Significant findings	Moderate	C (2)
Neselius et al. (2013b)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) amateur boxers (competing at elite level), mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing bout	Serum	1–6 days post and after ≥ 14 days rest	GFAP not detectable	Serious	C (2)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
Nowak et al. (2022)	Acute	Case–control study	Laboratory	Soccer	17 (6 M, 11 F) soccer players with ADHD, aged 20.2 ± 0.2; 17 (10 M, 6 F) w/o ADHD, aged 21.1 ± 0.1	17 (7 M, 10 F) soccer players with ADHD, aged 20.5 ± 0.1	10 linear headers; controls: 10 kicks	Plasma	Baseline, 2 and 24 h post	Significant findings (ADHD cohort only)	Low	A (5)
O'Keeffe et al. (2020)	Acute and semi-acute	Cohort study	Field	Rugby	8 rugby university team players, mean age (range) 22.1 (18–23); 11 male rugby school team players, mean age 17.4	27 non-contact sport athletes, median age (range) 28 (18–36); 26 healthy non-athlete controls, median age (range) 30 (18–40)	Rugby match (university team) and season (school and university team)	Plasma	University team: pre-season, ≤ 2 h post-match, 2 months post-season School team: pre- and post-season	GFAP not detectable	Moderate	B (3)
Soriano et al. (2022)	Semi-acute	Cohort study	Field	Am. football	33 collegiate athletes, aged 19.3 ± 1.4	N/A	Am. football season (games and training)	Serum	Mid-season, post-season and off-season (after a rest period)	Significant findings	Serious	B (4)
Zetterberg et al. (2006)	Acute and chronic	Longitudinal cohort study	Field	Boxing	14 (11 M, 3 F) amateur boxers, aged 22 ± 3.8	10 male non-athletic controls, aged 30 ± 6.3	Boxing bout	CSF	7–10 days post and after 3 months of rest	Acute: significant findings Chronic: no significant findings	Moderate	C (0)
Zetterberg et al. (2007)	Acute	Non-randomized experimental study	Laboratory	Soccer	23 male amateur soccer players, median age (range): 10 headers n = 10, 26 (19–32); 20	9 male non athletes, median age (range) 24 (22–27)	10 or 20 standing headers from a corner kick (kicked from 30 m)	CSF	7–10 days post	No significant findings	Serious	B (3)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					headers *n* = 13, 23 (20–28)							
Zetterberg et al. (2009)	Chronic	Observational case–control study	Other	Boxing	44 male amateur boxers, median age (range) 19 (17–28)	23 healthy males w/o contact sport history, median age (range) 28 (19–50)	Boxing participation (boxing debut, boxing duration in yr, number of bouts)	Serum	After a 2-month period of nonparticipation in boxing	GFAP not detectable	Moderate	C (1)
NSE												
DiBattista et al. (2016)	Chronic	Cross-sectional study	Other	Ice hockey, football, rugby, lacrosse	41 (39 M, 2F) collision sport athletes; aged (including all participants): M (*n* = 60) aged 19.5 ± 2.0, F (*n* = 27) aged 19.5 ± 1.8	46 (21 M, 25F) non collision sport athletes (inadvertent contact: soccer, basketball)	Collision sport participation	Plasma	Before the start of varsity season	No significant findings	Moderate	B (4)
Graham et al. (2011)	Acute	Retrospective cohort study	Field	Boxing	8 male amateur boxers, aged 17.6 ± 5.3 (PTH – punches to the head and body)	8 male amateur boxers, aged 19.1 ± 3.2 (PTB – punches to the body)	5 × 2-min boxing rounds	Serum	1 h before and after 5 min of cessation	Significant findings	Moderate	C (2)
Graham et al. (2015)	Acute	Cohort study	Field	Karate	12 males, aged 30.4 ± 6.7 (KTH – kicks to the head and body)	12 males, aged 28.2 ± 6.5 (KTB – kicks to the body)	4 × 3-min karate round	Serum	Before and immediately after	Significant findings	Moderate	C (1)
Horner et al. (1993)	Acute	Cohort study	Field	Boxing	8 male Olympic boxers, age range 18–28	17 male amateur oarsmen, age range 18–23	3 × 3-min boxing rounds;	Serum	Before and after	Significant findings	Moderate	C (1)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
							controls: 6-min ergometer test					
Rogatzki et al. (2016)	Acute	Cohort study	Field	Am. football	17 male Division III collegiate footballers, aged 19.5 ± 0.9	N/A	Am. football game	Serum	2 days before and 1 h post	Significant findings	Moderate	C (2)
Stålnacke et al. (2003)	Acute	Cohort study	Field	Ice hockey	26 male elite ice hockey players, aged 28 ± 4	18 elite basketball players, aged 25 ± 4	Ice hockey game (body checkings, falls, collisions, boardings); basketball game (jumps, collisions, falls)	Serum	1–2 h before and within 1 h post	No significant findings	Serious	C (2)
Stålnacke et al. (2004)	Acute	Cohort study	Field	Soccer	28 male elite players, aged 26 ± 5	N/A	Headers, jumps, falls and collision during a competitive soccer game	Serum	1–5 h before and immediately post	Significant findings	Moderate	B (3)
Stålnacke et al. (2006)	Acute	Cohort study	Field	Soccer	44 female elite players, aged 23 ± 3	N/A	Headers, jumps, falls and collisions during a competitive soccer game	Serum	Before and immediately post	Significant findings	Moderate	B (3)
Zetterberg et al. (2009)	Chronic	Observational case–control study	Other	Boxing	44 male amateur boxers, median age (range) 19 (17–28)	23 healthy males w/o contact sport history, median age	Boxing participation (boxing debut, boxing	Serum	After a 2-month period of nonparticipation in boxing	Significant findings	Moderate	C (1)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
						(range) 28 (19–50)	duration (yr), number of bouts)					
BDNF												
Bamaç et al. (2011)	Acute	Non-randomized experimental study	Laboratory	Soccer	17 male professional soccer players, aged 24.6 ± 4.4	N/A	15 jumping soccer headers; headed from a corner kick	Serum	Before and after	Significant findings	Serious	B (4)
DiBattista et al. (2016)	Chronic	Cross-sectional study	Other	Ice hockey, football, rugby, lacrosse	41 (39 M, 2 F); age for all sample: M (*n* = 60) 19.5 ± 2.0, F (*n* = 27) 19.5 ± 1.8	46 (21 M, 25 F) non collision sport athletes (inadvertent contact: soccer, basketball)	Collision sport participation	Plasma	Before the start of varsity season	No significant findings	Moderate	B (4)
Hoffman et al. (2022)	Acute	Cohort study	Field	Am. football	15 Israel national football team players, aged 26.2 ± 5.3	N/A	Am. football match	Serum	1 week before, immediately (< 30 min) and 24 h post	Significant findings	Moderate	C (2)
Neselius et al. (2013b)	Acute and semi-acute	Prospective cohort study	Field	Boxing	30 (28 M, 2 F) amateur boxers (competing at elite level), mean age (range) 22 (17–34)	25 (20 M, 5 F) healthy controls, mean age (range) 22 (17–30)	Boxing bout	Serum	1–6 days post and after ≥ 14 days rest	No significant findings	Serious	C (2)
O'Keeffe et al. (2020)	Acute and semi-acute	Cohort study	Field	Rugby	8 rugby university team players, mean age (range) 22.1 (18–23); 11 male rugby school team	27 non-contact sport athletes, median age (range) 28 (18–36); 26 healthy non-athlete controls, median	Rugby match (university team) and season (school and university team)	Plasma	University team: pre-season, ≤ 2 h post-match, 2 months post-season School team: pre- and post-season	Acute: no effects Semi-acute: significant findings	Moderate	B (3)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					players, mean age 17.4	age (range) 30 (18–40)						
Oztasyonar (2017)	Acute	Cohort study	Field	Boxing, Tae Kwon Do	20 male boxers, aged 20.15 ± 1.52; 20 male Tae Kwon Do fighters, aged 20.60 ± 1.65	20 male runners, aged 19.87 ± 1.60; 20 sedentary participants, aged 20.40 ± 1.85	Boxing: 3 × 3-min rounds; Tae Kwon Do: 2 × 3-min rounds; controls: running	Serum	Immediately before and after	Significant findings	Moderate	C (1)
Zetterberg et al. (2009)	Chronic	Observational case–control study	Other	Boxing	44 male amateur boxers, median age (range) 19 (17–28)	23 healthy males w/o contact sport history, median age (range) 28 (19–50)	Boxing participation (boxing debut, boxing duration in yr, number of bouts)	Serum	After a 2-month period of nonparticipation in boxing	No significant findings	Moderate	C (1)
UCH-L1												
Asken et al. (2018a)	Chronic	Observational cohort study	Other	Soccer, diving, wrestling, ice hockey, Am. football	415 (256 M, 159 F) collegiate athletes, aged 19.0 ± 1.2	N/A	Cumulative exposure to collision sports in yr (and modified CHII)	Serum	Off-season	No significant findings	Moderate	B (4)
Joseph et al. (2019)	Acute and semi-acute	Prospective observational cohort study	Field	Am. football	16 male high-school varsity footballers, aged 16.9 ± 0.2 (pre- and post-season testing n = 12)	N/A	Am. football games, practices and season	Serum	Semi-acute: pre- and post-season; acute: 1–2 h post	Significant findings	Moderate	B (3)
Major et al. (2020)	Chronic	Cross-sectional study	Other	Au. football	81 (50 M, 31 F) amateur footballers (no-mTBI history n	42 (23 M, 19 F) age-matched non-contact sport athletes	Au. rules football participation	Serum	N/A	No significant findings	Moderate	A (5)

Reference	Study type	Design	Setting	Sport	Athlete group	Control group	Exposure	Source	Sample times	Findings	Bias	QA
					= 42; mTBI history *n* = 39), aged ~24							
Nowak et al. (2022)	Acute	Case–control study	Laboratory	Soccer	17 (6 M, 11 F) soccer players with ADHD, aged 20.2 ± 0.2; 17 (10 M, 6 F) w/o ADHD, aged 21.1 ± 0.1	17 (7 M, 10 F) soccer players with ADHD, aged 20.5 ± 0.1	10 linear headers; control: 10 kicks	Plasma	Baseline, 2 and 24 h post	Significant findings (for ADHD cohort only at 24 h post)	Low	A (5)
Puvenna et al. (2014)	Acute	Cohort study	Field	Am. football	15 athletes	406 positive controls with mTBI and 465 negative controls	2 Am. football games	Serum	Baseline (day before) and post (< 1 h) (positive controls: < 6 h of injury)	Significant findings. No correlation with head hits	Serious	B (3)
Soriano et al. (2022)	Semi-acute	Cohort study	Field	Am. football	33 male collegiate players, aged 19.3 ± 1.4	N/A	Am. football season (games and training)	Serum	Mid-season, post-season and off-season (after a rest period)	UCH-L1 levels not detectable (for majority of samples)	Serious	B (4)

Am. football – American football, Au football – Australian Rules football, CHII – cumulative head impact index, MMA – mixed martial arts, NCAA – National Collegiate Athletic Association, NFL – National Football League

American football was the most studied sport with 26 studies, followed by soccer with 21, and boxing with 18 (including 2 kickboxing) studies (Figure 2.3).

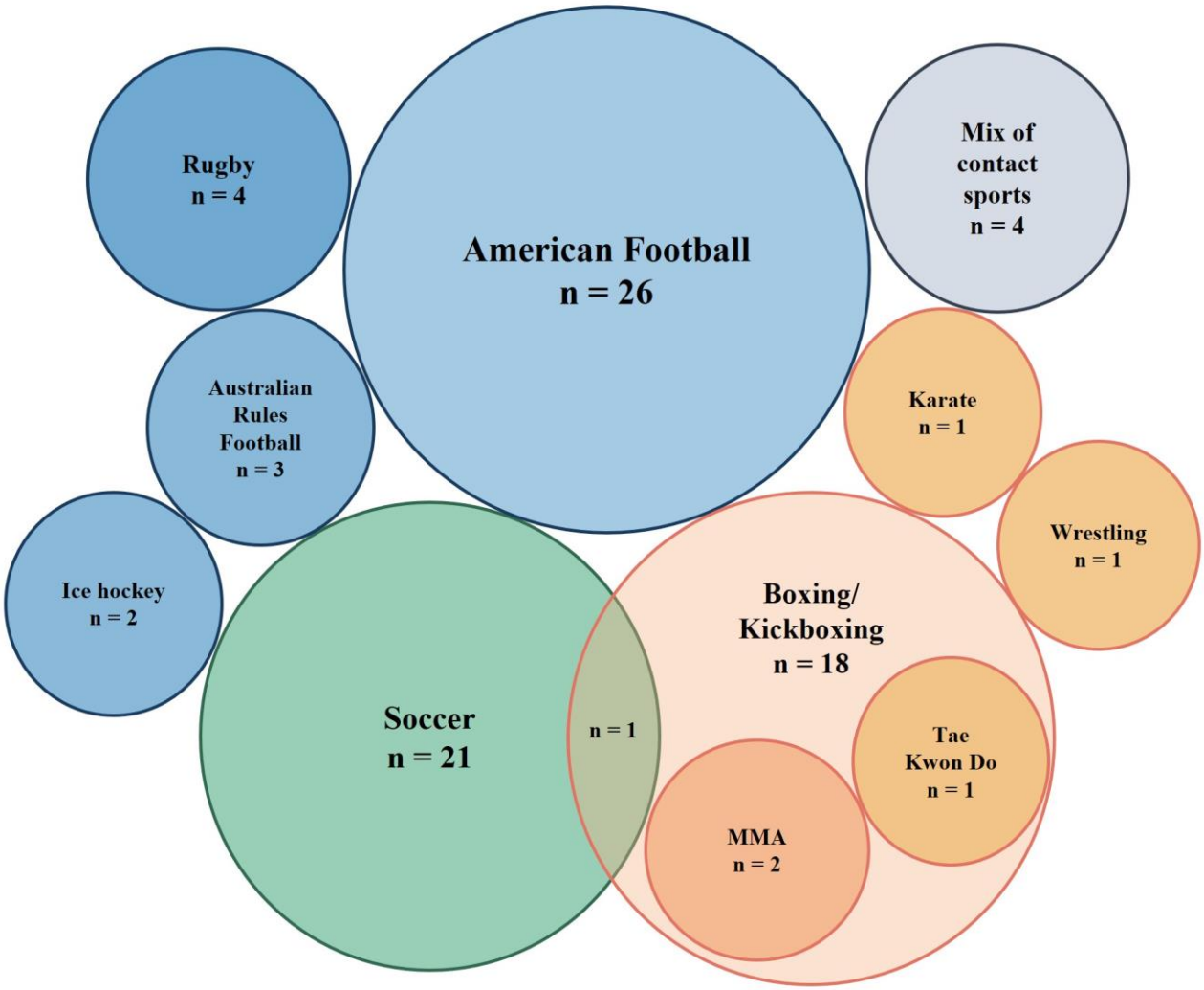


Figure 2.3. Number of articles per sport (MMA – mixed martial arts).

Fifteen research reports (~19%) provided a definition for subconcussive head impacts (definitions provided in Additional file 1: Table S5). Forty-seven studies (~60%) quantified or estimated RSHI exposure. Of the acute and semi-acute studies, 12 employed the use of accelerometers to document impact (see Additional file 1: Table S6 for impact information). Five of the 12 studies assessed impact data from soccer heading and six studies assessed RSHI metrics in American football. Where reported, peak average (or median) linear acceleration per impact ranged from 13.3 to 114.7 g.

Thirty (~38%) of the studies included an additional outcome measure other than biofluid marker(s) to assess the effects of RSHI. Commonly used measures included brain imaging

(in 9 studies) and neurocognitive tests, motor control, and/or concussion symptom assessment (in 26 studies); five studies had a multimodal approach integrating brain imaging and neurocognitive tests, motor control, and/or concussion symptom assessment.

2.3.2 Methodological Quality of Evidence

Based on our analysis of the risk of bias, two studies (~2.5%) were scored as critical, 20 (~25%) as serious, 49 (~62%) as moderate, and only 8 (~10%) of the studies received a low risk of bias rating. Most studies that received moderate or higher risk of bias did so due to failing to control for confounding variables (Figure 2.4). The findings of all identified studies are considered in this review to fully scope the body of evidence.

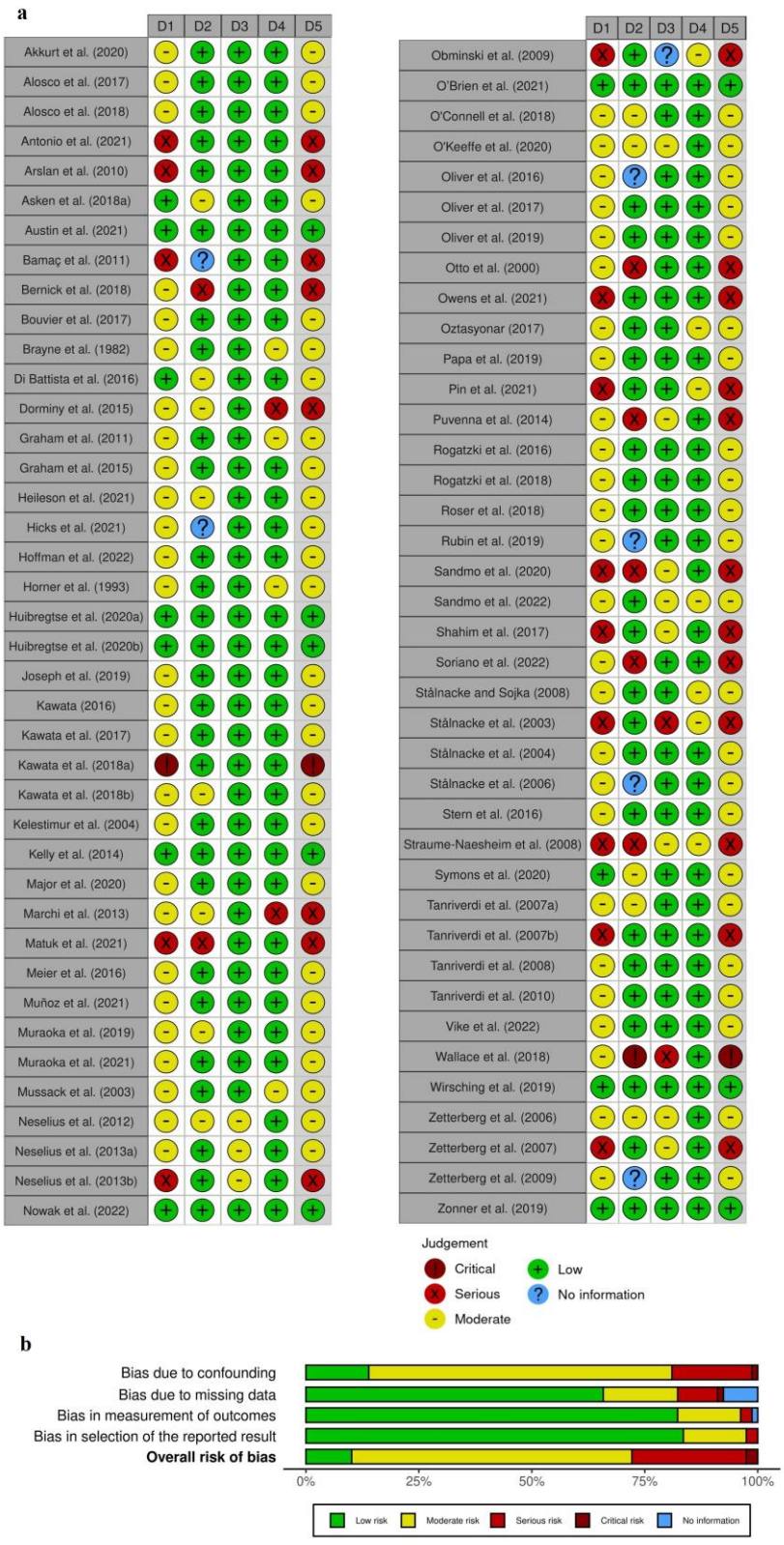


Figure 2.4. a Review authors' rating for individual risk of bias domains and the overall score for each study; b An applicability concerns graph summarizing the pooled risk of bias score for each domain as a percentage. D1: Bias due to confounding; D2: Bias due to missing data; D3: Bias in measurement of outcomes; D4: Bias in selection of the reported results; D5: Overall bias result.

Specific subconcussion methodological quality assessment results are displayed in Table 2.2; ~46% of studies received a category C (n = 36), ~40.5% category B (n = 32), and ~14% category A (n = 11) rating meeting almost all of the criteria with regard to subconcussion methodological quality. The most common unmet criteria were a failure to provide a definition for RSHI or account for sex differences.

Table 2.2. Quality assessment outcomes for individual studies using a modified version of the Subconcussion-Specific Tool.

Reference	1	2	3	4	5	6	Category	Score
Akkurt et al. (2020)	No	Yes	Yes	Yes	Yes	No	B	4
Alosco et al. (2017)	No	Yes	Yes	No	No	No	C	2
Alosco et al. (2018)	No	Yes	Yes	No	No	No	C	2
Antonio et al. (2021)	No	No	Yes	No	Yes	No	C	2
Arslan et al. (2010)	No	No	No	No	Yes	No	C	1
Asken et al. (2018a)	No	No	Yes	Yes	Yes	Yes	B	4
Austin et al. (2021)	No	Yes	Yes	Yes	Yes	Yes	A	5
Bamaç et al. (2011)	No	Yes	Yes	Yes	Yes	No	B	4
Bernick et al. (2018)	No	No	No	No	Yes	No	C	1
Bouvier et al. (2017)	No	Yes	Yes	No	Yes	Yes	B	4
Brayne et al. (1982)	No	Yes	No	No	Yes	No	C	2
Di Battista et al. (2016)	No	No	Yes	Yes	Yes	Yes	B	4
Dorminy et al. (2015)	No	Yes	Yes	Yes	Yes	No	B	4
Graham et al. (2011)	No	Yes	No	No	Yes	No	C	2
Graham et al. (2015)	No	No	No	No	Yes	No	C	1
Heileson et al. (2021)	No	No	Yes	No	Yes	No	C	2
Hicks et al. (2021)	No	Yes	Yes	No	Yes	No	B	3
Hoffman et al. (2022)	No	No	Yes	No	Yes	No	C	2
Horner et al. (1993)	No	No	No	No	Yes	No	C	1
Huibregtse et al. (2020a)	No	Yes	Yes	Yes	Yes	No	B	4
Huibregtse et al. (2020b)	Yes	Yes	Yes	Yes	Yes	No	A	5
Joseph et al. (2019)	No	Yes	Yes	No	Yes	No	B	3
Kawata (2016)	Yes	Yes	Yes	Yes	Yes	Yes	A	6
Kawata et al. (2017)	Yes	Yes	Yes	Yes	Yes	No	A	5
Kawata et al. (2018a)	No	No	No	No	Yes	No	C	1
Kawata et al. (2018b)	Yes	Yes	Yes	Yes	Yes	No	A	5
Kelestimur et al. (2004)	No	No	Yes	No	Yes	No	C	2
Kelly et al. (2014)	No	No	Yes	Yes	Yes	No	B	3
Major et al. (2020)	Yes	No	Yes	Yes	Yes	Yes	A	5
Marchi et al. (2013)	No	Yes	Yes	Yes	Yes	No	B	4
Matuk et al. (2021)	No	No	No	No	No	Yes	C	1
Meier et al. (2016)	No	No	Yes	Yes	Yes	No	B	3
Muñoz et al. (2021)	No	Yes	Yes	Yes	No	No	B	3
Muraoka et al. (2019)	No	Yes	Yes	No	Yes	No	B	3

Muraoka et al. (2021)	No	Yes	Yes	No	Yes	No	B	3
Mussack et al. (2003)	No	No	Yes	Yes	Yes	No	B	3
Neselius et al. (2012)	No	No	No	Yes	Yes	No	C	2
Neselius et al. (2013a)	No	No	No	Yes	Yes	No	C	2
Neselius et al. (2013b)	No	No	No	Yes	Yes	No	C	2
Nowak et al. (2022)	Yes	Yes	Yes	Yes	Yes	Yes	A	5
Obminski et al. (2009)	No	No	No	No	No	No	C	0
O'Brien et al. (2021)	No	No	Yes	Yes	Yes	Yes	B	4
O'Connell et al. (2018)	No	No	Yes	No	No	No	C	1
O'Keeffe et al. (2020)	Yes	No	No	Yes	Yes	No	B	3
Oliver et al. (2016)	No	No	Yes	No	Yes	No	C	2
Oliver et al. (2017)	Yes	No	Yes	No	Yes	No	B	3
Oliver et al. (2019)	Yes	No	Yes	No	Yes	No	B	3
Otto et al. (2000)	No	Yes	No	No	No	No	C	1
Owens et al. (2021)	No	Yes	No	No	Yes	No	C	2
Oztasyonar (2017)	No	No	No	No	Yes	No	C	1
Papa et al. (2019)	No	No	No	Yes	No	Yes	C	2
Pin et al. (2021)	Yes	Yes	Yes	Yes	Yes	Yes	A	6
Puvenna et al. (2014)	Yes	Yes	Yes	No	No	No	B	3
Rogatzki et al. (2016)	No	No	Yes	No	Yes	No	C	2
Rogatzki et al. (2018)	No	Yes	Yes	No	Yes	No	B	3
Roser et al. (2018)	No	No	Yes	No	Yes	No	C	2
Rubin et al. (2019)	Yes	Yes	Yes	Yes	Yes	Yes	A	6
Sandmo et al. (2020)	No	Yes	Yes	No	Yes	No	B	3
Sandmo et al. (2022)	No	Yes	Yes	No	Yes	No	B	3
Shahim et al. (2017)	No	No	No	No	Yes	No	C	1
Soriano et al. (2022)	Yes	No	Yes	Yes	Yes	No	B	4
Stålnacke and Sojka (2008)	No	Yes	Yes	No	Yes	No	B	3
Stålnacke et al. (2003)	No	Yes	No	No	Yes	No	C	2
Stålnacke et al. (2004)	No	Yes	Yes	No	Yes	No	B	3
Stålnacke et al. (2006)	No	Yes	No	No	Yes	Yes	B	3
Stern et al. (2016)	No	No	Yes	No	Yes	No	C	2
Straume-Naesheim et al. (2008)	No	Yes	Yes	No	Yes	No	B	3
Symons et al. (2020)	No	No	Yes	Yes	Yes	Yes	B	4
Tanriverdi et al. (2007a)	No	No	Yes	No	Yes	No	C	2
Tanriverdi et al. (2007b)	No	No	No	No	Yes	No	C	1
Tanriverdi et al. (2008)	No	No	Yes	No	Yes	No	C	2
Tanriverdi et al. (2010)	No	No	Yes	No	Yes	No	C	2
Vike et al. (2022)	No	Yes	No	Yes	Yes	No	B	3
Wallace et al. (2018)	No	Yes	No	No	Yes	No	C	2
Wirsching et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	A	5
Zetterberg et al. (2006)	No	No	No	No	No	No	C	0
Zetterberg et al. (2007)	No	Yes	Yes	No	Yes	No	B	3
Zetterberg et al. (2009)	No	No	Yes	No	No	No	C	1
Zonner et al. (2019)	Yes	Yes	Yes	Yes	Yes	No	A	5

Category A: met five or more criteria, Category B: met three or four criteria and Category C: met two or less criteria. Domains assessed: (1) Was there an attempt to define the term 'sub-concussion'? (2) Was the number or magnitude of impacts reported? (note that if impacts were recorded however not reported but impact data were included in the analysis, then the criterion was considered met) (3) Were subjects who sustained a concussion during the study controlled for or excluded from analyses? (4) Were subjects with a history of concussion controlled for or excluded from the analyses? (5) Was the control group matched on two or more variables (e.g., history of concussion, history of contact sport participation, age etc.)? (6) Did the study analyse sex differences, or acknowledge limitations associated with sampling only males or females?

2.3.3 Summaries for the Most Studied Biofluid Markers

2.3.3.1 S100 Calcium-Binding Protein Beta (S100B)

Glial injury marker S100B was the most examined protein with 30 studies (Table 2.1), with a median study sample size of 29 (range 8 to 415) contact sport athletes. Twenty-four studies assessed the acute effects of RSHI on S100B concentrations in blood (Arslan et al., 2010; Bouvier et al., 2017; Dorminy et al., 2015; Graham et al., 2011, 2015; Hoffman et al., 2022; Huibregtse, Nowak, et al., 2020; Kawata et al., 2017; Marchi et al., 2013; Mussack et al., 2003; Neselius, Zetterberg, Blennow, Randall, et al., 2013; O'Connell et al., 2018; O'Keeffe et al., 2020; Otto et al., 2000; Puvenna et al., 2014; Rogatzki et al., 2016, 2018; Stålnacke et al., 2003, 2004, 2006; Stålnacke & Sojka, 2008; Straume-Naesheim et al., 2008; Zetterberg et al., 2007; Zonner et al., 2019), of which 17 studies found a significant increase in S100B within two hours of RSHI exposure (range 1.3–5.3-fold, 26%–431% increase; Arslan et al., 2010; Bouvier et al., 2017; Graham et al., 2011, 2015; Kawata et al., 2017; Marchi et al., 2013; O'Connell et al., 2018; O'Keeffe et al., 2020; Otto et al., 2000; Puvenna et al., 2014; Rogatzki et al., 2016, 2018; Stålnacke et al., 2003, 2004, 2006; Straume-Naesheim et al., 2008; Zonner et al., 2019). All 17 studies were field based, where the effect of physical activity could not be eliminated. Eight of the 17 studies employed a control group or condition investigating the effect of exercise and/or peripheral injuries on S100B levels (Graham et al., 2011, 2015; Kawata et al., 2017; O'Connell et al., 2018; Otto et al., 2000; Rogatzki et al., 2018; Stålnacke et al., 2003; Straume-Naesheim et al., 2008). Critically, in six of the eight studies, a significant increase in S100B was observed also in the control group or control condition (Kawata et al., 2017; O'Connell et al., 2018; Otto et al., 2000; Rogatzki et al., 2018; Stålnacke et al., 2003; Straume-Naesheim et al., 2008). Overall, S100B increased 1.3–1.8-fold (26–78% increase) following exercise alone (O'Connell et al., 2018; Rogatzki et al., 2018; Straume-Naesheim et al., 2008). Laboratory-based studies investigating the effects of soccer heading, where physical

activity was controlled, reported no effect of RSHI on S100B (Dorminy et al., 2015; Huibregtse, Nowak, et al., 2020; Mussack et al., 2003; Otto et al., 2000; Stålnacke & Sojka, 2008; Zetterberg et al., 2007).

Increases in S100B were found to be significantly correlated with impact metrics, with studies reporting correlation coefficients ranging from 0.43 to 0.66. However, one study reported a correlation between increases in S100B and the number of jumps in a basketball game ($r = 0.71$; Stålnacke et al., 2003).

Two studies measured S100B in CSF following exposure to RSHI. One study reported significantly (~1.2-fold) higher S100B concentrations in CSF (but not in serum) 1–6 days after a boxing bout compared to the controls (Neselius et al., 2012; Neselius, Zetterberg, Blennow, Randall, et al., 2013). In the other study, S100B levels in CSF and serum were not significantly higher compared to the control group 7–10 days after controlled soccer heading (Zetterberg et al., 2007). Overall, S100B appears to increase following RSHI only if accompanied by physical exertion and the marker is sensitive to the effect of exercise regardless of head impacts.

None of the nine studies that assessed the semi-acute effects of RSHI found a significant increase in S100B (Kawata, 2016; Marchi et al., 2013; Neselius et al., 2012; Neselius, Zetterberg, Blennow, Randall, et al., 2013; O'Connell et al., 2018; O'Keeffe et al., 2020; Rogatzki et al., 2018; Soriano et al., 2022; Zonner et al., 2019). Also, no relationship between prior contact sport exposure and S100B was found in the three studies that investigated the chronic effects of RSHI in active contact sport athletes following a period of rest (~2–6 months) from contact sport participation (Asken, Bauer, Dekosky, et al., 2018; Battista et al., 2016; Zetterberg et al., 2009). Overall, semi-acute and chronic RSHI exposure does not appear to cause elevations in S100B levels.

2.3.3.2 Tau

Twenty-four studies examining the effects of RSHI on tau were identified (all 24 studies assessed total tau levels), with a median study sample size of 32 (range 8 to 415) contact sport athletes. Information about study type and design, type of exposure, and participant characteristics can be found in Table 2.1. Of these, 11 studies examined the acute effects of RSHI on tau concentrations; six studies had a moderate or low risk of bias, four of which reported significant tau increases after impacts incurred in boxing (Neselius et al., 2012; Zetterberg et al., 2006) and American football (Joseph et al., 2019; Kawata, Rubin, et al., 2018), the other two did not report significant findings after soccer heading (Nowak et al., 2022) and an American football

match (Hoffman et al., 2022). Eight studies investigated the semi-acute effects of RSHI yielding mixed findings. Four of the studies found no significant differences in tau concentrations (Kawata, Mitsuhashi, et al., 2018; Neselius et al., 2012; Neselius, Zetterberg, Blennow, Randall, et al., 2013; Oliver et al., 2017), while two found significant increases (Bernick et al., 2018; Joseph et al., 2019) (albeit one of these studies found yearly increases only in active mixed martial arts fighters but not in boxers; Bernick et al., 2018) and another a significant decrease (Oliver et al., 2019) (one study failed to detect tau in serum; Soriano et al., 2022). Twelve of the 24 studies examined if RSHI causes chronic tau increases, of which six did not find significant differences (Asken, Bauer, Dekosky, et al., 2018; Bernick et al., 2018; Major et al., 2020; Muraoka et al., 2019; Sandmo et al., 2020; Zetterberg et al., 2006), while four studies found increased tau levels (Battista et al., 2016; Muraoka et al., 2021; Stern et al., 2016; Symons et al., 2020). Furthermore, two studies found a correlation between RSHI career exposure and tau concentrations, although the concentrations were not significantly different to those of controls (Alosco et al., 2017, 2018). Therefore, although tau is one of the markers currently receiving the most attention (see Figure 2.2), its utility in evidencing the effects of RSHI in contact sport is uncertain.

Seven of the aforementioned 24 studies also examined p-tau. Two investigated the acute effects of RSHI on p-tau, finding no significant differences (Neselius et al., 2012; Zetterberg et al., 2006). One study (Neselius et al., 2012) also investigated the semi-acute effects, again reporting no significant results. The chronic sequelae of RSHI on p-tau concentrations in active and former athletes were investigated in six studies, with four of them reporting no significant effects in American (Alosco et al., 2018; Muraoka et al., 2019) and Australian Rules football players (Major et al., 2020), or boxers (Zetterberg et al., 2006), while two studies reported significant 1.2–1.8-fold increases in former and active American football players (Muraoka et al., 2021; Symons et al., 2020). All seven studies had a moderate risk of bias. Overall, the utility of p-tau in evidencing the effects of RSHI in contact sports is uncertain.

2.3.3.3 Neurofilament Light (NfL)

Twenty studies examining the effects of RSHI on NfL concentrations were identified, with a median study sample size of 32 (range 8 to 338) contact sport athletes. Information about study type and design, type of exposure, and participant characteristics can be found in Table 2.1. Twelve studies investigated the acute effects of RSHI on NfL concentration, with eight of them reporting a significant increase (boxing $n = 4$, soccer heading $n = 3$, American football n

= 1) (Bernick et al., 2018; Neselius et al., 2012; Nowak et al., 2022; Rubin et al., 2019; Shahim et al., 2017; Wallace et al., 2018; Wirsching et al., 2019; Zetterberg et al., 2006). NfL levels increased ~1.2–1.9-fold when sampled from serum compared to baseline levels or controls (Bernick et al., 2018; Nowak et al., 2022; Shahim et al., 2017; Wallace et al., 2018; Wirsching et al., 2019) and 4.1-fold in CSF (Zetterberg et al., 2006). The earliest increase was observed ~1 h post-RSHI exposure (Rubin et al., 2019; Wallace et al., 2018), with the majority of the studies finding a significant increase at ≥ 24 h (Bernick et al., 2018; Neselius et al., 2012; Nowak et al., 2022; Shahim et al., 2017; Wirsching et al., 2019; Zetterberg et al., 2006). Two of the eight studies sampled NfL from CSF (Neselius et al., 2012; Zetterberg et al., 2006), with one study demonstrating that increases in CSF NfL concentration were positively correlated with serum NfL levels (Shahim et al., 2017; Zetterberg et al., 2006). Five of the acute studies also suggested a dose–response relationship between impact exposure (severity and/or quantity) and NfL levels (Bernick et al., 2018; Neselius et al., 2012; Rubin et al., 2019; Shahim et al., 2017; Zetterberg et al., 2006). Five of the seven studies identified as having a low or moderate risk of bias reported significant effects. NfL levels were not detectable in one of the 12 studies (Zetterberg et al., 2007).

Significant increases were also reported in five of the eight studies that examined the semi-acute effects of RSHI in American football (Heileson et al., 2021; Oliver et al., 2016, 2019), boxing (Neselius et al., 2012), and ice hockey (Kawata, Mitsuhashi, et al., 2018). Seven studies investigated whether chronic RSHI exposure results in elevated NfL levels, with three of them reporting significantly higher NfL levels than in controls (~2 times higher; Antonio et al., 2021; Shahim et al., 2017; Zetterberg et al., 2006). However, none of the three studies investigated the relationship between lifetime exposure to RSHI and NfL levels, and all three studies were conducted in active contact sport athletes. Therefore, of the 'up-and-coming' biomarkers (see Figure 2.2), NfL appears as one of the most promising in demonstrating the effects of RSHI on the brain, irrespective of the sport.

2.3.3.4 Glial Fibrillary Acidic Protein (GFAP)

Fourteen studies assessing the effects of RSHI on GFAP were identified with a median study sample size of 30 (range 8 to 415) contact sport athletes (see Table 2.1 for details on study type and design, type of exposure, and participant characteristics). Eight studies investigated the acute effects (Hoffman et al., 2022; Joseph et al., 2019; Neselius et al., 2012; Neselius, Zetterberg, Blennow, Randall, et al., 2013; Nowak et al., 2022; O'Keeffe et al., 2020;

Zetterberg et al., 2006, 2007) (three in CSF; Neselius et al., 2012; Zetterberg et al., 2006, 2007), of which three studies found significant increases (1.3-2-fold) in GFAP levels following a boxing bout (in CSF; Neselius et al., 2012; Zetterberg et al., 2006) and soccer heading (in plasma; Nowak et al., 2022). GFAP was not detectable (in serum/plasma) in two of the studies (Neselius, Zetterberg, Blennow, Randall, et al., 2013; O'Keeffe et al., 2020).

Six studies assessed the semi-acute effects of RSHI by measuring GFAP concentrations (Joseph et al., 2019; Kawata, Mitsuhashi, et al., 2018; Neselius et al., 2012; Neselius, Zetterberg, Blennow, Randall, et al., 2013; O'Keeffe et al., 2020; Soriano et al., 2022) (one in CSF; Neselius et al., 2012), with only one study reporting a significant increase (Soriano et al., 2022). All five chronic studies (carried out in active athletes) found no effect of RSHI on GFAP levels (Asken, Bauer, Dekosky, et al., 2018; Battista et al., 2016; Major et al., 2020; Zetterberg et al., 2006, 2009). GFAP was not detectable (in serum/plasma) in three studies: two assessing semi-acute (Neselius, Zetterberg, Blennow, Randall, et al., 2013; O'Keeffe et al., 2020) and one assessing chronic effects (Zetterberg et al., 2009).

With regard to methodological constraints, the limits of detection for the assays failing to detect GFAP levels were 150 and 780 ng/L. Overall, GFAP appears not to be affected by RSHI; however, this conclusion is subject to limited evidence.

2.3.3.5 Neuron-Specific Enolase (NSE)

Nine studies investigating the effects of RSHI on NSE concentrations were identified, with a median study sample size of 26 (range 8–44) contact sport athletes (Table 2.1). Seven studies assessed the acute effects, of which all studies with moderate risk of bias (n = 6) reported significant findings, with soccer games causing a 1.1-2-fold increase in NSE (Stålnacke et al., 2004, 2006), American football a 1.9-fold increase (Rogatzki et al., 2016), and boxing a 1.6–2.5-fold increase (Graham et al., 2011; Horner et al., 1993). The two studies examining the chronic effects yielded mixed results (Battista et al., 2016; Zetterberg et al., 2009). Therefore, higher quality studies including NSE to examine the effects of RSHI showed promise, demonstrating the acute effects of head impact in sport.

2.3.3.6 Brain-Derived Neurotrophic Factor (BDNF)

Seven studies assessing the effects of RSHI on BDNF were found, with a median study sample size of 30 (range 15 to 44) contact sport athletes (Table 2.1). The acute effects were assessed in five studies, yielding mixed results (Bamaç et al., 2011; Hoffman et al., 2022;

Neselius, Zetterberg, Blennow, Randall, et al., 2013; O'Keeffe et al., 2020; Oztasyonar, 2017). BDNF was found to increase after boxing and taekwondo training (Oztasyonar, 2017) and an American football game (Hoffman et al., 2022), but not after a rugby match (O'Keeffe et al., 2020), in studies with a moderate risk of bias. The two studies that were identified as having a serious risk of bias showed increased BDNF after soccer heading (Bamaç et al., 2011) but no effects after a boxing bout (Neselius, Zetterberg, Blennow, Randall, et al., 2013). Two studies investigated the semi-acute effects (Neselius, Zetterberg, Blennow, Randall, et al., 2013; O'Keeffe et al., 2020), with one revealing increased BDNF concentrations after a rugby season (O'Keeffe et al., 2020), and two studies investigated the chronic effects without finding evidence of BDNF alterations (Battista et al., 2016; Zetterberg et al., 2009). Therefore, BDNF as a measure appears to reveal little about the effect of RSHI in sport.

2.3.3.7 Ubiquitin C-Terminal Hydrolase L1 (UCH-L1)

Six studies used UCH-L1 (Asken, Bauer, Dekosky, et al., 2018; Joseph et al., 2019; Major et al., 2020; Nowak et al., 2022; Puvenna et al., 2014; Soriano et al., 2022) to investigate the effects of RSHI on athletes' brain health (see Table 2.1 for details), with the median sample size of 34 (range 15 to 415) contact sport athletes. Three studies reported a significant increase in UCH-L1 levels acutely following RSHI exposure (Joseph et al., 2019; Nowak et al., 2022; Puvenna et al., 2014). Two studies also assessed UCH-L1 concentrations in semi-acute and two in chronic settings. One of the semi-acute studies found a significant increase in UCH-L1 concentrations following a season of American football (Joseph et al., 2019), whereas the majority of the samples were not quantifiable in the other study (Soriano et al., 2022). Neither of the studies assessing the chronic effects of RSHI found increased UCH-L1 levels (Asken, Bauer, Dekosky, et al., 2018; Major et al., 2020). Therefore, UCH-L1 appears to be increased acutely but not chronically following RSHI exposure; however, the evidence thus far is limited.

2.3.3.8 Hormonal Studies

Nine studies investigated the effects of RSHI on the hormonal response (see Additional file 1: Table S7), with the median sample size of 22 (range 11 to 68) contact sport athletes. One case study reported the acute and semi-acute effects of RSHI on hormone levels in a kickboxer (Tanriverdi, Unluhizarci, Selcuklu, et al., 2007), and eight studies reported the chronic effects (Akkurt et al., 2020; Kelestimur et al., 2004; Kelly et al., 2014; Obmiński et al., 2009; Roser et al., 2018; Tanriverdi et al., 2008, 2010; Tanriverdi, Unluhizarci, Coksevim, et al., 2007). Five studies that examined the chronic effects of RSHI in boxing and American football revealed

growth hormone secretory deficiencies (Kelestimur et al., 2004; Kelly et al., 2014; Tanriverdi et al., 2008; Tanriverdi, Unluhizarci, Coksevim, et al., 2007), anti-hypothalamus and anti-pituitary antibodies presence (Tanriverdi et al., 2010), insulin-like growth factor 1 (Kelestimur et al., 2004; Tanriverdi, Unluhizarci, Coksevim, et al., 2007) and adrenocorticotropic hormone (Tanriverdi et al., 2008; Tanriverdi, Unluhizarci, Coksevim, et al., 2007) deficiency, and hypogonadism (Kelly et al., 2014). RSHI exposure in soccer players revealed no long-term effects on hormonal responses (Akkurt et al., 2020; Roser et al., 2018). Overall, sustained exposure to RSHI appears to increase the risk of pituitary dysfunction in contact sport athletes.

2.4 Discussion

This scoping review provides a broad overview of the currently available evidence on the effects of RSHI on biofluid marker levels. We identified 79 studies, with research in this field demonstrating exponential growth (Figure 2.2). This review sheds light on a significant body of evidence not previously identified, i.e., two previous systematic reviews on the same topic identified just five relevant papers each (Mainwaring et al., 2018; Walter et al., 2022). The discrepancy in the number of relevant articles identified in the current and prior reviews is perhaps caused by the latter either not focusing solely on biofluid markers, thus including fewer biomarker-specific keywords in their search strategy (Mainwaring et al., 2018; Walter et al., 2022), or focusing on specific study designs (Walter et al., 2022).

The findings of our review demonstrate that acute effects of RSHI have been studied most (n = 49), while the number of studies assessing biofluid marker levels following semi-acute (n = 23) and chronic (n = 26) RSHI exposure are similar. Our inclusion criteria allowed us to identify a large panel of biofluid markers linked to traumatic brain injury, such as axonal damage, compromised blood–brain-barrier integrity and neurodegeneration. Although there were several interesting candidate biomarkers with fewer than five studies available, making marker-specific conclusions was not feasible due to methodological differences such as sampling times. Therefore, this review focused on detecting patterns in the most studied biofluid markers.

S100B, an astrocyte-enriched Ca^{2+}-binding protein that helps regulate intracellular calcium concentrations (Azar et al., 2017), was the most extensively studied biomarker. However, its utility for the purpose of examining RSHI effects in contact sports is questionable due to its extracerebral presence (S100B is also present in other tissues such as chondrocytes, adipocytes, and bone marrow cells; Azar et al., 2017). Indeed, we noted significant increases in S100B in

the control group or control condition of several studies included in this review where exercise was involved (Kawata et al., 2017; O'Connell et al., 2018; Otto et al., 2000; Rogatzki et al., 2018; Stålnacke et al., 2003; Straume-Naesheim et al., 2008). This is unsurprising considering that previous evidence has demonstrated S100B increases in athletes participating in noncontact sports without RSHI (Hasselblatt et al., 2004). Consequently, although S100B demonstrated a dose–response relationship with impact metrics, this marker may not be suited for assessing the effects of RSHI in a sporting setting due to also being affected by exercise alone. Based on our findings, S100B shows very limited, if any, utility in detecting RSHI-induced changes in semi-acute and chronic settings.

GFAP, a cytoskeletal protein almost exclusively present in astrocytes (Petzold, 2015), demonstrated no effects in the majority (~67%) of the studies investigating the acute effects of RSHI. Previously, GFAP has been shown to differentiate mild brain injury (Glasgow Coma Scale score 13–15 with clear MRI scans) from healthy control data (Yue et al., 2019). Semi-acute and chronic levels of GFAP did not appear to be affected by RSHI exposure.

The axonal injury marker NfL (Zetterberg et al., 2013) is, perhaps, the most promising of all the studied markers in demonstrating elevated levels acutely following RSHI exposure. Importantly, its levels appear to increase in a dose–response manner (Bernick et al., 2018; Neselius et al., 2012; Rubin et al., 2019; Zetterberg et al., 2006). NfL also demonstrated some promise in evidencing the semi-acute effects of RSHI. In contrast, tau, the second most studied marker in RSHI research (and abundant in thin unmyelinated cortical interneurons; Zetterberg et al., 2013) yielded mixed findings across acute, semi-acute, and chronic settings. Biofluid marker concentrations are known to scale to the severity of brain injury (Whitehouse et al., 2022), and as such, it is possible that some studies did not find significant effects because the quantity and severity of the RSHI did not result in injury, whereas some studies may simply have failed to detect changes in marker concentrations due to methodologies surrounding assays and sampling times.

The most frequently studied neuronal injury markers, NSE and UCH-L1, had limited numbers of studies of RSHI available, and no conclusions could be drawn.

2.4.1 Sampling Source and Time

The majority of the studies sampled biofluid markers from venous blood (n = 72), with only six studies assessing the concentrations in CSF and four in saliva. More studies sampling biomarkers from both blood and CSF are necessary to ensure that the changes in blood reflect

changes in the central nervous system (CNS). This is particularly important for markers that are not specific to the CNS. Although the current research is not at a stage where blood or saliva samples can be reliably associated with brain alterations caused by RSHI exposure, the end goal in this field of research should be the identification of biofluid markers that can be sampled efficiently and non-invasively for the routine monitoring of athletes' brain health.

In this review, we were unable to identify the most appropriate marker-specific sampling times following RSHI exposure. This was due to the mixed findings reported, the heterogeneity of the included studies, and the fact that most studies provided little, if any, justification surrounding sampling time choices. The time course of RSHI effects and how it relates to the changes in the levels of different biofluid markers is currently unclear. Critically, more research is needed, as discussed below.

2.4.2 Quality and Limitations of the Identified Studies

A further aim of this scoping review was to assess the quality of the available evidence and identify research gaps in order to guide future research. We identified limitations in the following three main categories: (1) lack of appropriate control of confounding variables, (2) lack of impact monitoring, and (3) representativeness of the sampled populations. Similar concerns have been highlighted before in the field of RSHI in general (Mainwaring et al., 2018; Stephen et al., 2022; Walter et al., 2022).

We found that only ~10% of the studies could be considered to have a low risk of bias and that the primary domain increasing the bias was controlling for confounding variables. Common confounding variables that were not controlled include prior concussions, concussions occurring during the study, and the effect of exercise. Furthermore, 27 studies (34%) did not employ a control condition or a control group. It is imperative that future research utilizes control groups, or conditions to control for the effect of exercise, to ensure that changes in biomarker concentrations are not driven by confounding variables.

Another important limitation of the current evidence was the lack of monitoring and quantification of RSHI exposure. Strikingly, around 40% of the studies did not quantify or estimate RSHI exposure. Moreover, only 12 studies employed accelerometers to document impact. The sports where accelerometers were utilized most in the context of RSHI were American football (n = 6) and soccer (n = 5). There were no studies measuring impact magnitude in boxing, despite it being the third most studied sport. The number of studies assuming, rather than measuring, the occurrence of RSHI is concerning, especially since without data on impact

metrics it is not possible to examine the dose–response relationship between impact exposure and brain changes. Furthermore, only 15 studies (~19%) provided a definition for RSHI. Not characterizing RSHI is an issue, especially if investigators do not separate RHSI from concussive impacts in research.

The studied samples were not fully representative of the population of interest, especially in respect to sex and age, hampering the generalizability of the results. There was limited evidence documenting the effects of RSHI exposure in females using biofluid markers. The majority of the studies ($n = 42$) were carried out in a male-only cohort, whereas there were only two female-only studies. Noteworthily, both female-only studies reported elevated levels of biofluid markers. One of the studies assessed the chronic effects of RSHI in Division II soccer players, finding significantly higher plasma NfL levels in the soccer players compared to non-contact sport female athletes (Antonio et al., 2021). However, these results are based on a small sample size (8 soccer players and 17 controls), and the study received a serious risk of bias rating. The second study found a significant increase in serum S100B and NSE levels in 44 female elite players acutely following a competitive soccer game, although the study did not control for the effect or exercise (Stålnacke et al., 2006). There were also 20 studies that used a mixed-sex approach and only 14 compared or acknowledged sex differences. Concerningly, sex was not specified in 15 studies. We also identified only three studies done in a juvenile-only cohort (~13–17-year-olds), with retired contact sport athletes also being understudied, as the majority of the studies assessing chronic effects of RSHI were conducted in active contact sport athletes.

2.4.3 Strengths and Limitations

The strengths of the current review are the adherence to an a priori-developed and published review protocol, following the PRISMA-ScR guidelines, and most importantly, the comprehensive search strategy used. The latter has reduced the risk of overlooking relevant research conducted in the field of RSHI and biofluid markers, and has enabled us to provide a full overview of the research done in this field from its inception until this review—an overview that was not available until now.

We acknowledge that this review has limitations. The generalizability of our findings is limited to sport-specific effects and may not be true for RSHI occurring in other settings (e.g., military, domestic abuse, etc.). Furthermore, concussion studies that employed a control group of contact sport athletes (where it was not clear whether RSHI had occurred) were not

included in this review. As such, potentially relevant research may have been excluded from the current review; however, we believe that any such studies would have added little value for the purpose of this review due to the ambiguity surrounding the occurrence of RSHI.

2.4.4 Future Research

Our analysis showed that many of the studies included in this review are highly variable and present issues in the study design, quality, and analysis, resulting in biased reporting. This review demonstrated that most of the current research does not define RSHI or quantify impact exposure (Table 2.2); this prevents studies from drawing firm conclusions and consequently hinders the advancement of the field. Therefore, future studies should ensure that RSHI is clearly defined and distinguishable from concussive impacts. Furthermore, all future studies of RSHI should aim to quantify the impacts, for example, by using sensors.

One of the most common confounding variables identified in this review was the effect of exercise. Notably, S100B was found to be increased in the control groups/conditions of several studies included in this review where exercise was involved (Kawata et al., 2017; O'Connell et al., 2018; Otto et al., 2000; Rogatzki et al., 2018; Stålnacke et al., 2003; Straume-Naesheim et al., 2008). Furthermore, physical exertion and its duration are known to affect serum levels of GFAP and UCH-L1 (Bazarian et al., 2022). Therefore, future biofluid marker RSHI studies need to control for the effects of exercise.

Few studies included in this review were found to examine females and juveniles, while studies including both male and female athletes did not always consider the role of sex. The influence of sex on neurobiology and neurophysiology is largely recognized, and several lines of evidence confirm sex differences in biomarker levels that must be accounted for (Asken, Bauer, DeKosky, et al., 2018; Koerte et al., 2020; Mondello et al., 2020). Therefore, future studies in RSHI and biofluid markers should consider sex differences. Age is also an important covariate that should be controlled. Indeed, studies of juvenile cohorts are limited, and a major knowledge gap remains with regard to how age influences biomarker levels.

One factor that can reduce the heterogeneity of the studies in the field could also be the way the methodological aspects of sampling are standardized and reported. The uncertainty around the best time to sample following RSHI is an urgent pre-analytical factor that needs to be resolved. Furthermore, methods of sampling blood biomarkers are subject to substantial variation with respect to blood collection, choice and preparation of serum or plasma, storage of samples, and the analytical platforms used (Mcdonald et al., 2021). Lack of standardization

of such pre-analytical variables often makes it impossible to compare results from different laboratories, and potentially adds to noise within studies. In agreement with Mcdonald et al. (2021), we observed that aspects of these processes were often inconsistently documented and note that addressing such variation remains a key issue for future work.

With regard to the current uncertainty about optimal sampling time following exposure to RSHI, marker-specific factors such as half-life should be considered. While it is presently unknown when to sample following RSHI, it may be possible to initially use the marker-specific temporal trends following concussion as a frame of reference. We note, however, that critical RSHI-specific information will rely on researching individual markers' RSHI-specific response and temporal profile, where possible, sampling at multiple timepoints following RSHI exposure to define the RSHI-specific response and temporal profile of individual markers. Evidence from studies in TBI suggests that temporal profiles of biomarkers are important, and specifically that late biomarker elevation may signal progressive neurological disease (Newcombe et al., 2022). Long-term longitudinal studies in RSHI are needed to address this issue.

There is a need for novel markers capable of providing insight into the pathobiology and pathogenetic mechanisms and demonstrating the link with neurodegeneration (e.g., CTE) (Alosco & Stern, 2019). The assessment of circulating levels of p-tau181 and p-tau217 (Asken et al., 2022), and markers reflecting changes in baseline cerebral physiology and metabolism (Mondello et al., 2022; Peng et al., 2022), would be instrumental for the accurate characterization of cerebral health and are therefore a critical avenue for future investigation. Both with regard to novel markers and established markers, we need to understand the mechanisms and understand the link with neurodegeneration. Multimodal studies have a critical role to play, however, in this review, we identified a limited number of studies using multiple methods to date. Therefore, it is recommended that future studies combine biofluid markers with other methods that can reveal the mechanisms of pathology following RSHI exposure, such as combining neuro-imaging and sensitive and informative measures of cognition and motor control (Ntikas et al., 2022), and that imaging methods are multi-modal (e.g., Champagne et al., 2020; Koerte et al., 2022).

2.5 Conclusion

In this first review dedicated to systematically scoping the evidence of biofluid marker levels following RSHI exposure, a considerable number of studies were identified.

Nevertheless, biofluid marker RSHI research was found to be in its early stages. Presently, the field is overwhelmingly heterogeneous, and the available studies suffer from specific methodological weaknesses. Through systematic scoping of the current evidence, however, we could determine specific ways in which the quality of future studies can be improved. Improving the quality of future research is necessary to assess the utility of under-explored markers as well as those markers that currently appear to show promise. In the meantime, despite the limitations and quality of the current evidence base, the fact that increased levels of brain injury markers were found in biofluids following RSHI exposure warrants caution over the safety of routine RSHI exposure.

Chapter 3: Blood-derived Brain Injury Marker Levels Acutely Following Exposure to Repetitive Subconcussive Head Impacts

3.1 Introduction

In light of the findings presented in the previous chapter we decided to assess blood biomarker data of glial (S100B, GFAP) and neuronal (NfL, UCH-L1, tau, p-tau181) injury collected in our laboratory acutely following subconcussive head impacts. Data presented in this chapter have not been previously disseminated. The blood samples were collected by TDV together with other outcome variables for which data have been published (Di Virgilio et al., 2016, 2019).

The scoping review highlighted that only a small number of studies (n = 13) assessing biofluid markers acutely following subconcussive impacts have been conducted in a controlled environment, where potential confounding variables such as the effect of exercise have been controlled. All laboratory studies included in the review implemented a soccer heading paradigm and most commonly assessed biofluid marker levels following 10 headers (n = 7; Austin et al., 2021; Huibregtse, Ejima, et al., 2020; Huibregtse, Nowak, et al., 2020; Muñoz et al., 2021; Nowak et al., 2022; Wirsching et al., 2019; Zetterberg et al., 2007); however, the number of headers ranged from 5 to 40 across the studies. Data collected in our laboratory implemented a heading paradigm consisting of 20 headers in the first experiment (Di Virgilio et al., 2016). The second experiment measured blood biomarkers following a three 3-minute sparring routine where the number of head impacts was not predetermined however, the number or force of the impacts were not recorded (Di Virgilio et al., 2019). Impact quantity and magnitude were documented in the soccer study which may enable a comparison with the laboratory studies identified in the scoping review. Data presented here may add coherence to the inconclusive findings described in the previous chapter.

Despite the comprehensiveness of the scoping review, we were unable to draw marker specific conclusions for most part due heterogeneous and limited evidence. The most studied marker, S100B was the only protein with sufficient evidence to make conclusions about its utility for assessing the acute effects of sport-related subconcussive impacts. Regrettably, it appears that S100B is not suitable for detecting the effects of sport-related subconcussive impact exposure due to its sensitivity to the effect of exercise. Of the 24 studies assessing S100B acutely following subconcussive head impacts only six studies were laboratory-based studies, where S100B levels were measured at 0, 0.5, 1, 2, 4, 6, 24 hours and 7–10 days post following

soccer heading across studies (Table 2.1; Dorminy et al., 2015; Huibregtse, Mussack et al., 2003; Otto et al., 2000; Stålnacke & Sojka, 2008; Zetterberg et al., 2007). S100B levels were not elevated in any of the six laboratory-based studies, while significant changes were observed in approximately 70% of the field studies, where the effect of physical activity could not be eradicated. The sample sizes in the lab and field studies were similar, ranging between 10-61 and 8-46 participants in the RSHI exposure group, respectively (with the mean sample size being 27 and 25, respectively; Table 2.1). It was the comparison between the field and laboratory-based experiments that allowed these conclusions to be formed, highlighting the importance of having data from controlled research settings, where serial sampling has been implemented.

The other glial injury marker, GFAP had limited number of acute studies available (n = 8) and overall displayed mixed findings. For example, one of the two laboratory-based studies found GFAP significantly increased following 10 soccer headers, but only in participants with ADHD (Nowak et al., 2022). Similarly, mixed results were presented for the most studied neuronal injury marker tau. Around 45% of the acute studies found significant increases in tau following RSHI however, all three laboratory-based studies reported no significant changes in tau levels following 10, 20 or 40 soccer headers sampled at 1, 2, 24 hours, within 7–10 days and 3 weeks post (Table 2.1; Nowak et al., 2022; Wallace et al., 2018; Zetterberg et al., 2007). Moreover, there were only two studies assessing p-tau181 acutely following RSHI in boxing (both from CSF), neither finding significant effects (Neselius et al., 2012; Zetterberg et al., 2006). However, the earliest that p-tau181 was sampled was a day after the head impacts had occurred, leaving a large time gap not yet investigated. UCH-L1 also had too few studies (n = 3) assessing the acute effects of subconcussive impacts available for any conclusions to be drawn (with only one laboratory experiment). Nonetheless, all studies reported a significant increase in UCH-L1 levels following RSHI (Joseph et al., 2019; Nowak et al., 2022; Puvenna et al., 2014) calling attention to examining this marker further. NfL was considered the most promising marker for demonstrating the acute effects of RSHI with over 65% of the studies demonstrating significant increases in the marker level, including three (Nowak et al., 2022; Wallace et al., 2018; Wirsching et al., 2019) of the five (Austin et al., 2021; Nowak et al., 2022; Wallace et al., 2018; Wirsching et al., 2019; Zetterberg et al., 2007) laboratory studies done.

The anticipation is that the data presented in this chapter will contribute to existing research identified in Chapter 2, reinforcing the emerging picture and facilitating the development of new insights. Based on the findings from the scoping review the expectations were that

S100B levels will not be affected by soccer heading in the laboratory setting but would be increased following sparring where head impacts were accompanied by physical activity. Further, we postulated that NfL and UCH-L1 levels may be increased following soccer heading and sparring. Due to the mixed findings observed for GFAP, tau and p-tau in the previous chapter, we did not expect those markers to be sensitive to the effects of subconcussive impacts.

3.2 Methodology

3.2.1 Participant Characteristics and Ethical Approval

Twenty-three amateur football players (5 females; aged 22 ± 3 years; height 175.4 ± 10.2 cm; mass 72.9 ± 8.3 kg) were recruited for the football heading study (referred to as experiment one) of whom 19 (5 females) were included in the study. Three participants withdrew from the study for personal reasons and one participant was excluded due to the absence of EMG response to TMS. A mixture of amateur boxers and Muay Thai athletes (n = 23; 3 females; aged 22 ± 1.7 years; height 178 ± 8.4 cm; mass 76 ± 7.5 kg) were recruited for the sparring study (referred to as experiment two) of whom 20 (2 females) were included in the study. Two participants were excluded from the study due to their medication and one participant withdrew following a syncopal episode during baseline testing.

The following exclusion criteria applied for both experiments: (1) history of brain injury resulting in loss of consciousness; (2) history of a neurological condition; (3) concussion in the last year; (4) family history of epilepsy; (5) current use of psychoactive drugs. Both experiments had received approval from the local Research Ethics Committee and conformed to the Declaration of Helsinki guidelines. All participants provided written informed consent prior to participation.

3.2.2 Study Design

Both experiments employed a repeated measures design where the participants were sampled for blood at baseline and following exposure to repeated subconcussive head impacts. In experiment one participants were sampled immediately, 24 h, 48 h and 2 weeks following a drill of football heading and in experiment two the participants were sampled immediately and 24 h after boxing sparring. Participants were instructed to refrain from consuming alcohol, caffeine, smoking and vigorous exercise for 24 hours before each session. Participants attended the testing facilities after an overnight fast and were provided with a standardized breakfast which consisted of 30 g of cornflakes with 150 ml of semi-skimmed milk.

3.2.3 Procedures and Apparatus

3.2.3.1 Subconcussive Impact Exposure Procedure

3.2.3.1.1 Experiment One

Participants were instructed to perform 20 rotational headers, redirecting the ball perpendicularly to its original trajectory, similar to a corner kick scenario. The heading procedure was completed in 10 minutes. A standard size 5 football (circumference: 70 cm; mass: 400 g; pressure 8 psi) was projected from 6 meters at an average speed of 38.7 ± 2.1 km per hour using a ball delivery machine (JUGS Sports, Tualatin, Oregon, USA). Ball speed was determined for each participant depending on their perceived ability to head the ball and was set within the range of 30 to 50 km per hour. Majority of the balls that can be headed travel at velocities below 65 km per hour (Erkmen, 2009). For example, an average ball speed of 63 km per hour has been observed during simulated play, where the headed balls were kicked from a distance of ~27 meters and the ball velocity was measured using a hand-held radar gun ~3 meters before the impact (Naunheim et al., 2000). In contrast, lower average ball speeds of 22.5 km per hour have been observed for throw-in balls that were headed from a 5 meter distance from the sideline (Peek et al., 2021). Participants wore a custom-built accelerometer, positioned approximately over the occipital protuberance using a headband, recording the peak linear acceleration of each impact.

3.2.3.1.2 Experiment Two

Participants completed three sets of three-minute sparring with a two-minute rest between each set. Participants were instructed to mimic sparring in their normal training sessions and provided their own boxing equipment and partner. The impact sensors encountered a technical problem, and no impact data are available.

3.2.3.2 Biochemical Analyses

Blood samples were collected through venipuncture into 6 ml BD Vacutainer serum tubes containing Silica (clot activator). Tubes were inverted several times and allowed to coagulate at room temperature for 60 minutes. The tubes were then centrifuged (Heraeus Multrifuge 3SR Plus Centrifuge, Thermo Scientific, Massachusetts, USA) at 4°C for 16 minutes at 3500 revolutions per minute to sperate serum. Serum was then aliquoted into two 1.5 ml microtubes and stored at −80°C until transported to Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, University of Gothenburg (Mölndal, Sweden) for analysis.

Serum was analysed for the following markers: S100B, GFAP, NfL, (total) tau, p-tau at position threonine 181 (p-tau181) and UCH-L1.

S100B was measured using Electro-chemiluminescence immunoassay (ECLIA) (Roche Diagnostics Scandinavia AB, Bromma, Sweden) as per manufacturer's instructions (limit of detection: 0.015 µg/L; limit of quantification: 0.02 µg/L). GFAP, NfL, UCH-L1 and tau were measured using Simoa Human Neurology 4-Plex A assay (N4PA) and HD-X analyser (Quanterix, Billerica, Massachusetts, USA) following manufacturer's guidelines and p-tau181 was analysed using in house Simoa assay developed at University of Gothenburg (described in Benussi et al., 2020). Operators were blinded to the participant and trial information.

3.2.4 Statistical Analysis

Data for the experiments were analysed separately. Data was assessed for outliers using boxplots and extreme outliers were winsorized by replacing the extreme outlier values with a value equal to mean + 3 × *SD* (Field, 2012). Combination of visual inspection (histograms and normal Q-Q plots), normality testing (Shapiro-Wilk test) and assessment of skewness and kurtosis (skewness < 2 and kurtosis [excess] < 4 indicating normal distribution) were used to determine if the data were normally distributed (Kim, 2013). Changes in biofluid marker concentrations were assessed through repeated measures ANOVAs where $p \leq 0.05$ denoted statistically significant changes in the biofluid marker level. Partial eta squared (η_p^2: 0.0099 = small; 0.0588 = medium; 0.1379 = large; Richardson, 2011) was used to quantify effect sizes. When assumption of sphericity was violated Greenhouse-Geisser correction was used. Bonferroni correction was applied to post-hoc pairwise comparisons to reduce false-positive findings (type I errors). Data are reported as mean ± *SD* unless stated otherwise. Statistical tests were carried out using IBM SPSS (version 28.0.0.0, SPSS Inc., Chicago, USA).

3.3 Results

3.3.1 Experiment One

There were data available for 18 participants for S100B, GFAP, NfL, UCH-L1 and tau, and for 19 participants for p-tau181. There was no significant difference between the timepoints for S100B [$F(4,68) = 1.265$, $p = 0.292$, $\eta^2 = 0.069$], GFAP [$F(4,68) = 2.409$, $p = 0.058$, $\eta^2 = 0.124$], UCH-L1 [$F(4,68) = 1.486$, $p = 0.216$, $\eta^2 = 0.080$], tau [$F(1.685,28.640) = 0.187$, $p = 0.793$, $\eta^2 = 0.011$] and p-tau181 [$F(4,72) = 1.809$, $p = 0.136$, $\eta^2 = 0.091$] levels (Figure 3.1 [a, c, g, i, k] and Table A.1). However, there was a significant effect of time for NfL concentration,

$F(4,68) = 6.047$, $p < 0.001$, $\eta^2 = 0.262$ (Figure 3.1 [e] and Table A.1). Post hoc analysis showed that NfL levels significantly increased from immediately post to 24 hours post [mean difference: 1.133 pg/mL (95% CI, 0.321 to 1.946), $p = 0.003$] and to 48 hours post [mean difference: 1.008 pg/mL (95% CI, 0.025 to 1.991), $p = 0.042$] football heading. Further, NfL levels significantly decreased from 24 hours post to 2 weeks post [mean difference: 1.199 pg/mL (95% CI, 0.238 to 2.160), $p = 0.009$]. None of the markers differed significantly from baseline at any of the timepoints.

3.3.2 Experiment Two

There were data available for 15 participants for S100B and for 16 participants for GFAP, NfL, UCH-L1, tau and p-tau181. There was a significant effect of time for S100B concentration, $F(1.298,18.165) = 24.607$, $p < 0.001$, $\eta^2 = 0.637$ (Figure 3.1 [b] and Table A.1). Post hoc analysis revealed that S100B concentration was significantly increased from baseline to immediately post [mean difference: 0.039 ug/L (95% CI, 0.019 to 0.059), $p < 0.001$] and significantly decreased from immediately post to 24 hours post [mean difference: 0.036 ug/L (95% CI, 0.017 to 0.055), $p < 0.001$]. S100B levels were not significantly different between baseline and 24 hours post [mean difference: 0.003 ug/L (95% CI, -0.006 to 0.011), $p = 1.000$].

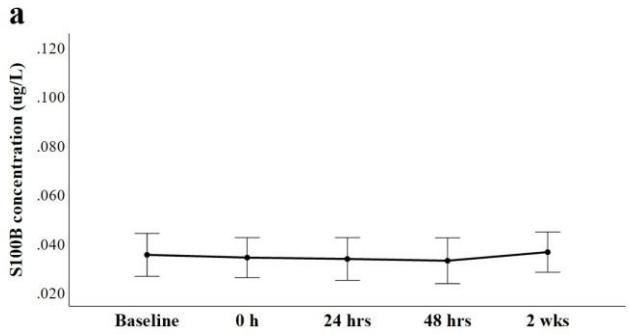
There were no significant changes in GFAP, $F(1.253,18.794) = 1.831$, $p = 0.193$, $\eta^2 = 0.109$, or in NfL levels, $F(2,30) = 0.736$, $p = 0.487$, $\eta^2 = 0.047$ between the timepoints (Figure 3.1 [d, f] and Table A.1).

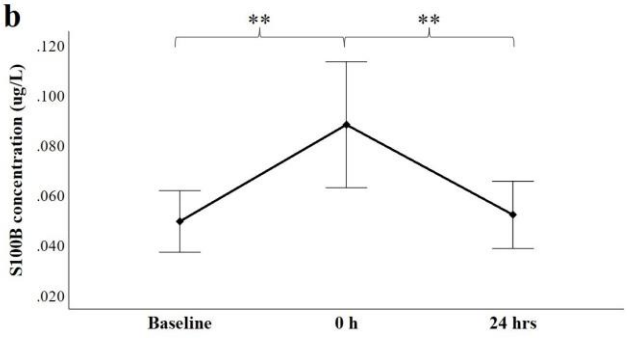
There was a significant effect of time for UCH-L1 concentration, $F(2,30) = 6.370$, $p = 0.005$, $\eta^2 = 0.298$ (Figure 3.1 [h] and Table A.1). Post hoc analysis showed that UCH-L1 concentration significantly increased from immediately post to 24 hours post [mean difference: 13.890 pg/mL (95% CI, 1.604 to 26.176), $p = 0.025$]. UCH-L1 levels were not significantly different between baseline and immediately post [mean difference: 9.983 pg/mL (95% CI, -2.149 to 22.116), $p = 0.128$] or between baseline and 24 hours post [mean difference: 3.907 pg/mL (95% CI, -3.342 to 11.155), $p = 0.501$].

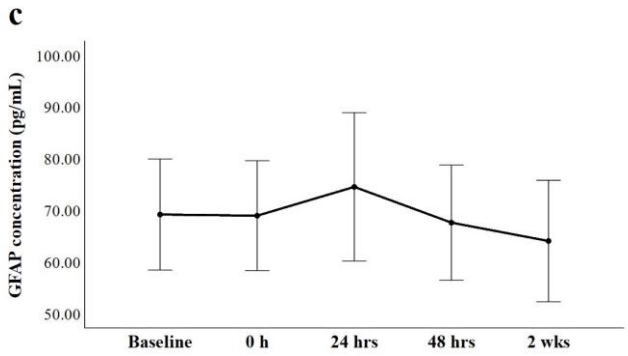
There was also a significant effect of time for tau concentration, $F(2,30) = 10.276$, $p < 0.001$, $\eta^2 = 0.407$ (Figure 3.1 [j] and Table A.1). Post hoc analysis revealed that tau was significantly increased from baseline to immediately post [mean difference: 2.301 pg/mL (95% CI, 0.916 to 3.685), $p = 0.001$]. Tau levels were not significantly different between baseline and 24 hours post [mean difference: 0.888 pg/mL (95% CI, -0.265 to 2.040), $p = 0.167$] or between

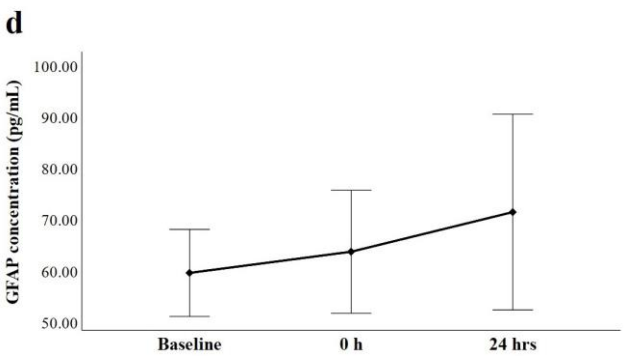
immediately post and 24 hours post [mean difference: 1.413 pg/mL (95% CI, -0.155 to 2.981), $p = 0.085$].

Further, there was a significant effect of time for p-tau181 concentration, $F(2,30) = 15.702$, $p < 0.001$, $\eta^2 = 0.511$ (Figure 3.1 [l] and Table A.1). Post hoc analysis showed that p-tau181 significantly increased from baseline to immediately post [mean difference: 2.944 pg/mL (95% CI, 1.285 to 4.603), $p < 0.001$] and significantly decreased from immediately post to 24 hours post [mean difference: 2.689 pg/mL (95% CI, 1.120 to 4.259), $p = 0.001$]. P-tau181 levels were not significantly different between baseline and 24 hours post [mean difference: 0.255 pg/mL (95% CI, -1.215 to 1.726), $p = 1.000$].


a
S100B concentration (ug/L)
.120
.100
.080
.060
.040
.020
Baseline
0 h
24 hrs
48 hrs
2 wks


b
**
**
S100B concentration (ug/L)
.120
.100
.080
.060
.040
.020
Baseline
0 h
24 hrs


c
GFAP concentration (pg/mL)
100.00
90.00
80.00
70.00
60.00
50.00
Baseline
0 h
24 hrs
48 hrs
2 wks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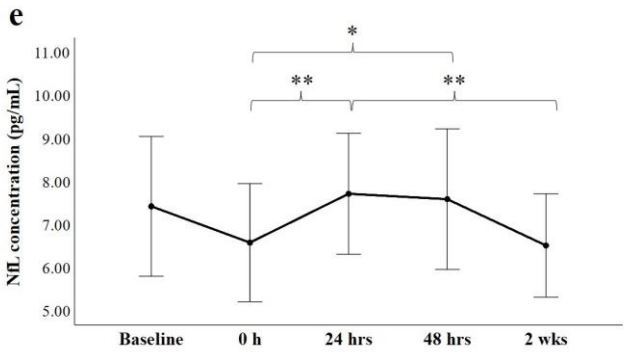
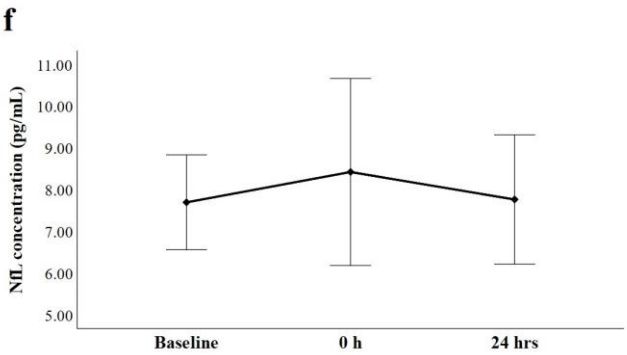
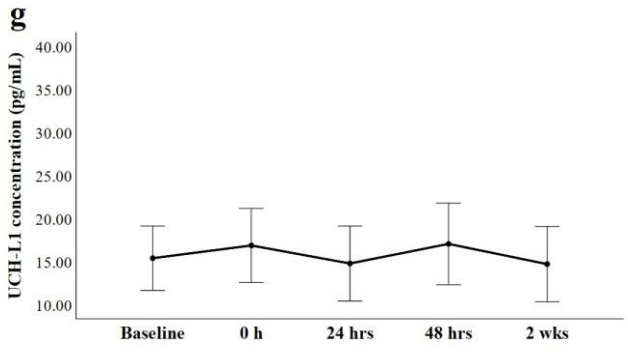
GFAP concentration (pg/mL)
100.00
90.00
80.00
70.00
60.00
50.00
Baseline
0 h
24 hrs


e
*
**
**
NfL concentration (pg/mL)
11.00
10.00
9.00
8.00
7.00
6.00
5.00
Baseline
0 h
24 hrs
48 hrs
2 wks


f
NfL concentration (pg/mL)
11.00
10.00
9.00
8.00
7.00
6.00
5.00
Baseline
0 h
24 hrs


g
UCH-L1 concentration (pg/mL)
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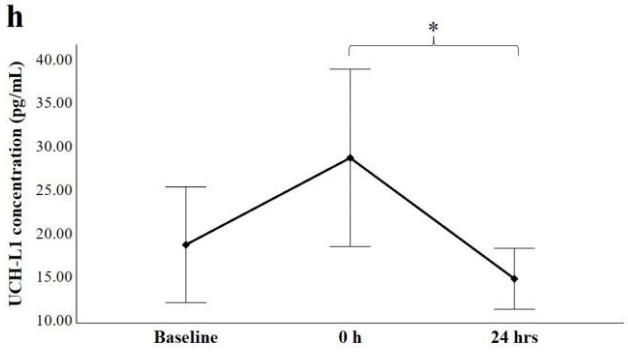
35.00
30.00
25.00
20.00
15.00
10.00
Baseline
0 h
24 hrs
48 hrs
2 wks


h
*
UCH-L1 concentration (pg/mL)
40.00
35.00
30.00
25.00
20.00
15.00
10.00
Baseline
0 h
24 hrs

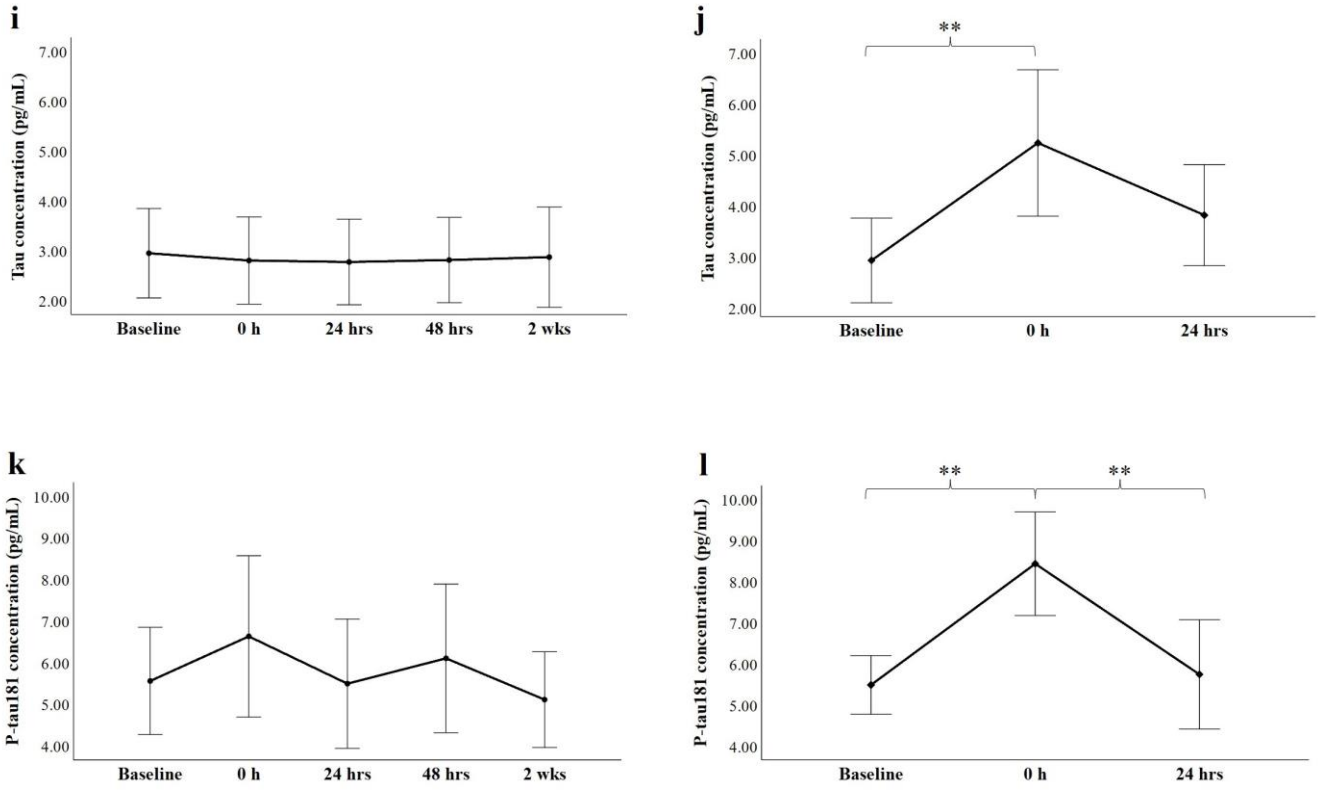


Figure 3.1. Average blood biomarker levels across time with 95% CI for experiment one (a, c, e, g, i, k) and two (b, d, f, h, j, l). *p < 0.05 and **p < 0.01.

3.4 Discussion

Blood biomarker data presented in this chapter were analysed with the aim to add coherence to the findings discussed in the scoping review and help determine the utility of these markers for detecting the effects of sport-related subconcussive impacts. Data for two glial (S100B, GFAP) and four neuronal (NfL, UCH-L1, tau, p-tau181) injury markers were collected prior to and following soccer heading and sparring in boxing. We expected S100B levels not to be affected by soccer heading in a laboratory setting where the effect of exercise was controlled but increase following sparring where participants displayed signs of physical exertion. We hypothesised that NfL and UCH-L1 concentration would significantly increase in both studies. In addition, we sought to determine whether GFAP, tau and p-tau181 levels would be heightened following heading and sparring. We found that S100B, tau and p-tau181 were the only markers that displayed a significant increase from baseline following head impacts. These increased were only observed following sparring and not after soccer heading. Significant changes were also observed in NfL levels in the soccer study and UCH-L1 concentration in the

sparring experiment. However, the fluctuations in these marker levels were not related to baseline. Finally, no significant changes were observed in GFAP levels.

S100B levels increased approximately 1.8-fold from baseline immediately after sparring and returned to baseline levels at 24 hours post. Increases ranging from 1.3–5.3-fold in blood S100B levels were observed acutely in the field studies included in the scoping review (Arslan et al., 2010; Bouvier et al., 2017; Graham et al., 2011, 2015; Kawata et al., 2017; Marchi et al., 2013; O'Connell et al., 2018; O'Keeffe et al., 2020; Otto et al., 2000; Puvenna et al., 2014; Rogatzki et al., 2016, 2018; Stålnacke et al., 2003, 2004, 2006; Straume-Naesheim et al., 2008; Zonner et al., 2019). Noteworthily, the scoping review results demonstrate that S100B displays similar increases to the sparring study results following physical exertion alone. It is therefore possible that the changes observed in the sparring study here were caused by the physical activity from sparring considering that S100B levels remained stable in the soccer study. However, since there is no head impact or physical exertion data available for the sparring experiment, it is not possible to determine whether the observed effects were due to exercise.

Tau and p-tau181 also significantly increased following sparring (1.8- and 1.5-fold, respectively), but not following soccer heading. While S100B is present in extracerebral sources, tau is primarily present in neuronal axons (Kawata et al., 2016) which may suggest that the observed changes in the biofluid markers were impact, rather than exercise related. Nonetheless, tau and p-tau181 levels have been observed to increase 1.4-fold following exercise alone (Cente et al., 2023). Tau increased acutely following RSHI exposure in approximately 45% of the studies (n = 5) included in the scoping review (Chapter 2) all of which were field studies involving physical activity (Joseph et al., 2019; Kawata, Rubin, et al., 2018; Neselius et al., 2012; Neselius, Zetterberg, Blennow, Randall, et al., 2013; Zetterberg et al., 2006), whereas three studies assessing tau following controlled soccer heading found no significant changes (Nowak et al., 2022; Wallace et al., 2018; Zetterberg et al., 2007) suggesting that increases in tau may be exercise dependent. It is also possible that participants sustainer a higher number of impacts and of greater force to the head during the sparring bout compared to the soccer paradigm in the current experiments, causing the biofluid markers to significantly increase in the boxing, but not in the soccer experiment. The average peak linear acceleration (PLA) reported by Di Virgilio (2016) for soccer heading study is 13.1 g. A separate study measured impacts occurring from sparring and reported a median PLA of 19.7 and 16.0 g for men and women, respectively (Jansen et al., 2021). Nonetheless, these differences in impact metrics

may be caused by other factors such as the placement of the accelerometers and may not be representative of actual differences between these activities. Especially, considering that the impact values reported by Di Virgilio (2016) are lower (~13 vs > 30 g) than described in other studies where a similar heading paradigm has been implemented (i.e., using a machine to dispense the ball at a similar speed; Huibregtse, Nowak, et al., 2020; Nowak et al., 2022; Wirsching et al., 2019). Therefore, soccer headers may result in similar (Harriss et al., 2019), if not higher, impacts as reported for sparring by Jansen et al. (2021). Without impact data from sparring, it is not possible to determine the cause of the different findings between the soccer and boxing experiments in the current study. Nonetheless, data presented in this chapter adds to the existing evidence base. In particular, this is the first time p-tau181 levels have been assessed within 24 hours of RSHI exposure. We identified only two studies assessing phosphorylated tau acutely following RSHI when conducting the scoping review; both of these studies assessed p-tau from CSF 1–6 days (Neselius et al., 2012) and 1–7 days (Zetterberg et al., 2006) following a boxing bout without finding any significant changes. The results of this chapter suggest that p-tau181 may be acutely elevated in response to the subconcussive impacts and prior research potentially missed the time window where p-tau levels peak in response to RSHI exposure. Moreover, data from the sparring study demonstrates that any increases in the biofluid markers return to baseline within 24 hours, suggesting that any increases in biofluid markers following subconcussive impacts are likely short-lived.

Significant changes in NfL and UCH-L1 were also observed however, these changes were not related to the baseline levels. These findings may suggest that subtle changes in biofluid marker levels due to subconcussive head impacts may be at risk of being masked by the variability in the protein levels. For example, significant intrapersonal semidiurnal differences have been found in serum GFAP levels, with levels increasing between 9 am and 12 pm ($p < 0.00001$) and decreasing from 12 pm to 9 pm ($p < 0.001$; $n = 32$) (Christensen et al., 2022). While, NfL levels for example have been found to be significantly different within healthy individuals between different days in a study where samples were drawn at the same time each day ($p = 0.02$; $n = 33$) (Hviid et al., 2022). Noteworthily, such variations are not well documented for all biofluid markers. In the case of subconcussive impacts, the expected changes in marker levels are very subtle as proteins are released into the extracellular space proportionally to the amount of injured nervous tissue (Hviid et al., 2022). Therefore, care should be taken to ensure that changes in marker levels caused by injured tissue are not masked or confounded by biological fluctuations in the marker levels.

Finally, GFAP was the only marker for which significant changes were not observed. Form the studies included in the scoping review assessing the acute effects of GFAP, approximately 38% did not find significant effects, while another 25% were not able to detect GFAP levels. In addition, one study demonstrated mixed findings reporting increased GFAP levels only in participants with ADHD (Nowak et al., 2022). However, there were two studies that found a significant increase in GFAP levels within 1–14 days following a boxing bout (Neselius et al., 2012; Zetterberg et al., 2006). Interestingly, when GFAP was sampled within the same timeframe from CSF following soccer heading then the results were not significant (Zetterberg et al., 2007). Therefore, it is possible that this marker is only sensitive to higher impact load. In any case, it appears that this marker may not be best suited for detecting the effects of RSHI based on the evidence available thus far.

3.5 Conclusion

Results from experiments described in this chapter further highlight the need for recording impacts and controlling for physical activity in sport-related subconcussive impact research as not doing so prevents the accurate interpretation of the research findings. Furthermore, the data from the studies described here demonstrate the importance of controlling and accounting for biological variability in the marker levels, especially considering the subtle effects of subconcussive impacts. Currently the window in which biofluid markers peak following subconcussive head impacts is not known. Therefore, it is possible that the current findings were affected by the sampling times and potentially missed the peak effects of RSHI. As such, future research should consider sampling between immediately post and 24 hours post to ensure that the window where biofluid markers may peak following subconcussive impact exposure is captured. Finally, the data from the sparring study demonstrated significant increases in three of the six biofluid markers suggesting that subconcussive impacts may cause brain injury markers to elevate as a consequence of injured tissue. Nonetheless, the contrasting findings from the heading and sparring experiments demonstrate that biofluid markers are not sensitive enough to consistently detect the effects of RSHI and any increases may be exercise dependent.

Chapter 4: The Quest for Strategies to Prevent Acute Brain Changes Following Repetitive Subconcussive Head Impacts

4.1 Introduction

Previous chapters (Chapter 2 and 3) investigated the levels of biofluid markers of brain injury following subconcussive head impacts and concluded that even though biofluid markers show promise, they are not sensitive enough to consistently detect the effects of subconcussive head impacts on the brain. As such we conducted the two studies described in this chapter using a range of different measures assessing corticomotor inhibition, motor control and cognitive function, as they have previously shown promise in detecting the effects of repetitive head impacts on the brain.

Research undertaken at the University of Stirling looking into the acute effects of RSHI has shown that 20 soccer headers and three bouts of sparring in boxing significantly decreased memory function and increased corticomotor inhibition (Di Virgilio et al., 2016, 2019). Similar findings of increased corticomotor inhibition have also been observed following rugby tackling (McNabb et al., 2020). Therefore, despite very limited evidence, corticomotor inhibition may prove a sensitive and reliable measure for detecting the effects of RSHI. Measures of cognitive and vestibular function are commonly employed within RSHI research (Mainwaring et al., 2018; Stephen et al., 2022); however, like with biofluid markers, the findings tend to be mixed raising questions about the sensitivity of these measures. Nonetheless, while static balance appears unaffected by RSHI, reduced dynamic postural control (Stephen et al. 2022) and altered motor unit recruitment strategies (Di Virgilio et al. 2019) have been associated with RSHI exposure and thus show potential as sensitive measures of motor control. Furthermore, a systematic review examining gait function following concussion concluded that gait is acutely affected after mild TBI and suggested the use of dual task or complex gait assessment as those appeared to show more promise even outside the acute timeframe (Fino et al., 2018). In addition, a systematic review with meta-analysis reported that gait velocity, measured during dual-gait assessment, can distinguish between concussed and non-concussed individuals acutely following mild TBI (Lee et al., 2013). Higher stride time variability during dual gait assessment has also been associated with greater self-reported symptomatic head trauma in retired professional American football players (n = 66; r^2 = 0.20, p = 0.003) (Manor et al., 2020). Nonetheless, to date there is no research examining temporal gait parameters acutely following RSHI exposure. Based on initial findings, the present research utilised measures of corticomotor

inhibition, gait, motor unit recruitment strategies and cognitive function to study the effects of RSHI.

Chapter 1 described various studies assessing the effects of long-term exposure to subconcussive head impacts on brain health that demonstrated a dose-response relationship between impact exposure and neuropsychological and -physiological changes. For example, Montenigro et al. (2017) demonstrated a dose-response relationship between cumulative head impact exposure and the risk of impaired cognitive function in former American Football players. Lipton et al. (2013) found abnormal white matter microstructure and impaired cognitive function in soccer players, which were associated with the number of soccer headers performed in the prior year. The acute dose-response relationship between RSHI and brain changes, however, is not clear. Previous attempts to investigate the acute dose-response relationship have examined the release of biofluid markers following 10 and 20 headers (Zetterberg et al., 2007), and after 10, 20 and 40 headers (Austin et al., 2021) without finding any changes in brain injury marker concentrations. Nonetheless, acute changes to brain function have been observed following 10 (Haran et al. 2013; Nowak et al. 2020) and 20 headers (Di Virgilio et al. 2016) when assessing measures such as balance, neuro-ophthalmologic and cognitive function. It is noteworthy that regardless of the limited research on the acute dose-response relationship between impact exposure and brain effects, the Football Association (FA) has published guidelines recommending limiting the number of headers performed per week (The Football Association, 2020) to address the long-term risk associated with heading. Assessing short-term dose-response relationships can provide valuable insight into preventing acute brain changes that may contribute to long-term neurological changes. Therefore, research investigating the acute dose response relationship between impact exposure and functional brain response using sensitive measures is needed. As such, one of the aims of this chapter is to determine whether limiting the number of head impacts in training and during games can be utilised as a potential preventative method for protecting brain health.

Limiting the acute dose of subconcussive head impacts is one potential strategy for preventing acute changes in brain function. However, despite the FA recommendations to limit the number of impact exposure per week, there is no mechanism in place for enforcing these guidelines, and it may be difficult to effectively regulate impact exposure in real life. Headgear is commonly used in contact sports like rugby and American football as a protective strategy, while it is still common practice for soccer players to control the movement of the ball using their unprotected head. Despite the potential benefits of implementing headgear in soccer there

is limited research investigating its effectiveness. Only three studies (Elbin et al., 2015; R. T. Tierney et al., 2008; Withnall et al., 2005) to date have used human participants to assess the effectiveness of soccer headgear for heading. Remaining research has used a force platform (Broglio et al., 2003) or a head form (Naunheim et al., 2003) instead. Moreover, the results from the available evidence are unclear on whether headgear can attenuate the effects of heading. Two of the four studies to date suggest that headgear may be ineffective at attenuating impact forces from heading (Naunheim et al., 2003; Withnall et al., 2005) while the findings of Tierney et al. (2008) are inexplicit. Namely, the latter study found that there were no significant sex differences in linear head acceleration when heading was performed without headgear, whereas women sustained significantly higher (32% and 44%, depending on the headgear model) head accelerations compared to men when wearing headgear (potentially due to women heading the ball harder when wearing headgear due to feeling protected). The overall difference in impact metrics between headers completed with or without headgear was not significant. Broglio et al. (2003) however, found that headgear reduced peak impact force compared to a no headgear condition although, the methodology was not representative of real-world heading (i.e., heading that occurs during training and in game scenarios) since the headgear was not tested using human participants.

Only one study has assessed the effectiveness of headgear by assessing functional brain response to headers completed with and without headgear. Elbin et al. (2015) measured neurocognitive performance and concussion symptoms before and after 15 headers, finding impaired cognitive function in the headgear group following heading. Although the authors concluded that the tested headgear model may have exacerbated the effects of heading it is noteworthy that the study had some methodological limitations. Firstly, the sample size was small (≤ 13 participants per group) and secondly, the number of 'successful' headers differed between groups, albeit the difference was not significant. All the other studies (described in the previous paragraph) evaluated the use of headgear for heading by measuring differences in impact metrics. Acceleration data alone, however, does not provide information about the impact effects on the brain. Furthermore, research to date has examined only a small selection of headgear models. The small number of available research, the methodological limitations and inconclusive findings emphasise the need for further research. Especially, using new, and so far, untested models and products of headgear. Continuous research investigating the protective properties of headgear is important as technology and product design develop. This study is

the first to investigate the effectiveness of a headgear prototype supplied by Storelli Sports (USA), examining whether it can prevent or lessen heading induced acute brain changes.

The present research is based on the initial findings of electrophysiological changes, impaired cognitive function and decreased vestibular control observed following RSHI. We utilised those variables with the aims of (1) examining the acute dose response relationship between impact quantity (10 vs 20) and functional brain changes and (2) assessing whether headgear can mitigate the functional brain response to subconcussive head impacts. We postulated that if acute brain changes are impact dose dependent, then those previously reported acute brain changes should scale in response to the dose, and if headgear is protective, then wearing it should limit or eliminate brain changes following subconcussive impacts.

4.2 Methodology

4.2.1 Participant Characteristics and Ethical Approval

Thirty-one soccer players were recruited to participate in experiment one (dose response study) and experiment two (headgear study); 30 participants (26 M; 4 F) completed experiment one and 28 volunteers (24 M, 4 F) completed experiment two. One participant was excluded due to reporting experiencing a prolonged headache following a testing session where they performed headers. Participants were considered eligible to participate if they were aged 18 or above and self-reported to regularly heading the ball (approximately 15 times per week) as part of recreational or organised soccer participation. Participants with (1) head trauma in the last six months (i.e., a concussion), (2) history of a neurological or psychiatric conditions, (3) family history of epilepsy, (4) lower extremity injury affecting gait, or (5) participants reporting consumption of psychoactive drugs were excluded from the study. The study received ethical approval from the NHS, Invasive and Clinical Research Ethics Committee, University of Stirling (project reference: NICR 16/17 - 005) and conformed to the Declaration of Helsinki 2013 (World Medical Association, 2013) guidelines except for registering the trial due to the uncertainties caused by the Coronavirus pandemic (potential for sudden changes in design and trial stoppage). All participants provided written informed consent prior to participation.

4.2.2 Study Design

This study employed a within-subject repeated measures design with both experiments (dose response study and headgear study) having three conditions. The dose response study had two heading sessions, one with 10 and other with 20 headers, and a control condition where

participants kicked the ball 20 times. The headgear study also incorporated the control condition and had two heading sessions: one where participants headed the ball 20 times without headgear and another session participants completed 20 headers while wearing headgear. The headgear tested was a prototype provided by Storelli Sports LLC (Brooklyn New York, USA; Figure 4.1). The two experiments were run in parallel with a nested design, making use of the same control condition and the same condition in which participants performed 20 headers. The order of the conditions was counterbalanced and heading sessions were carried out in different weeks to limit head impact exposure to the participants.

Prior to the testing sessions participants attended the laboratory for a familiarisation session where they were screened against the inclusion and exclusion criteria and were acquainted with the testing procedures to minimise later learning effects. In the familiarisation session, height and mass were measured using a stadiometer (Marsden HM-250P, Rotherham, UK) and electronic scales (Seca 804, Hamburg, Germany), respectively. Self-reported information including football participation and concussion history were also gathered.

Figure 4.1. Headgear prototype.

In each of the testing sessions, measures of silent period duration (SP), ratio of silent period duration to motor evoked potential amplitude (SP:MEP), motor control (temporal

parameters of gait and motor unit firing rate) and cognitive functioning were collected at baseline and immediately following heading and kicking activity. Silent period duration (including SP:MEP) and motor unit firing rate were also measured at one hour post. A schematic diagram of the session design is displayed in Figure 4.2.

Participants were instructed to refrain from high intensity exercise, head impacts, alcohol and smoking for 24 hours prior to the testing sessions. Participants were also asked to avoid consuming caffeine on the day of the testing sessions. Moreover, participants were instructed to record their food and liquid intake on the day of the first testing session so that they could follow the same diet, including timing of the consumption, prior the subsequent sessions.

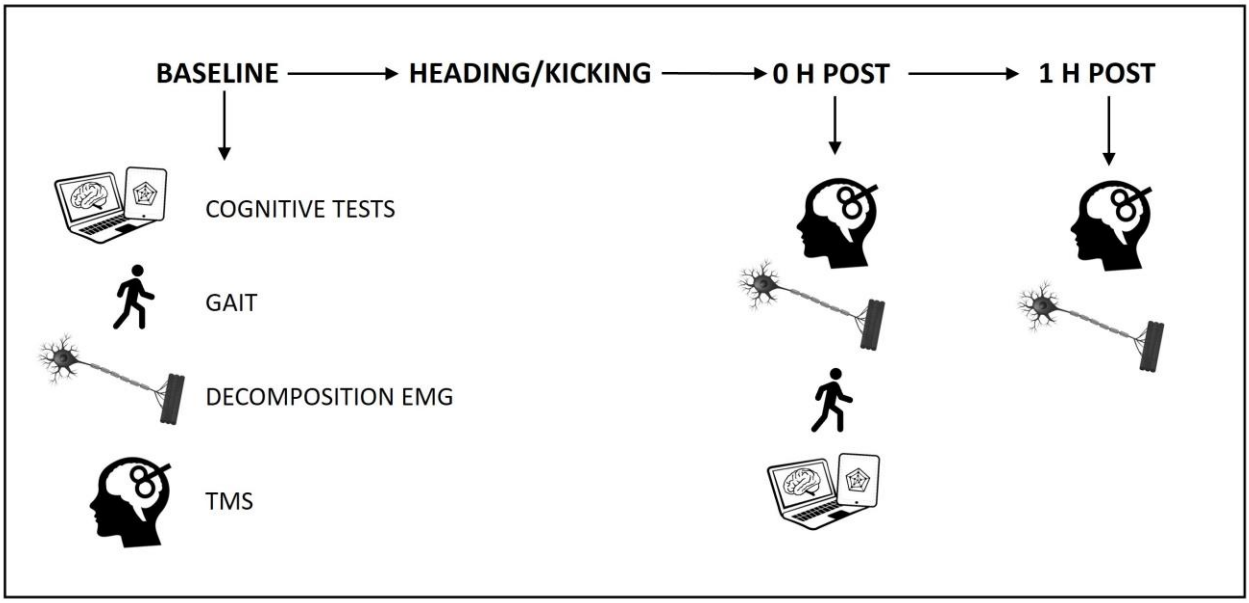


Figure 4.2. Schematic diagram of the study session design. TMS – transcranial magnetic stimulation (used to measure silent period and silent period to motor evoked potential ratio); EMG – electromyography (decomposition EMG was used to measure motor unit firing rate).

4.2.3 Procedures and Apparatus

4.2.3.1 Heading Procedure

A standard size 5 (diameter 22 cm, circumference 70 cm) and pressure (8 psi) football was projected using a JUGS soccer dispensing machine (JUGS Sports, Tualatin, Oregon, USA) from 12 meters at a speed between 32 to 48 km per hour. Participants were instructed to head the ball rotationally mimicking a header from a corner kick (i.e., redirect the ball's trajectory perpendicularly). The balls were projected at the rate of one header per minute. The ball projection speed was determined for each participant by their perceived ability to head the ball at

that speed. Ball speed was kept constant for each participant for the subsequent testing sessions (maximum deviation between sessions was 1.6 km/h). The ball release angle (angle between the ground and the midline of the rotating wheels; displayed in Figure 4.3) was adjusted depending on the participants' height and the ball projection speed so that the participant could perform standing headers. Any headers that failed to make proper contact with the head (e.g., skimmed the head), as determined by the experimenter observation and participant feedback, were repeated.

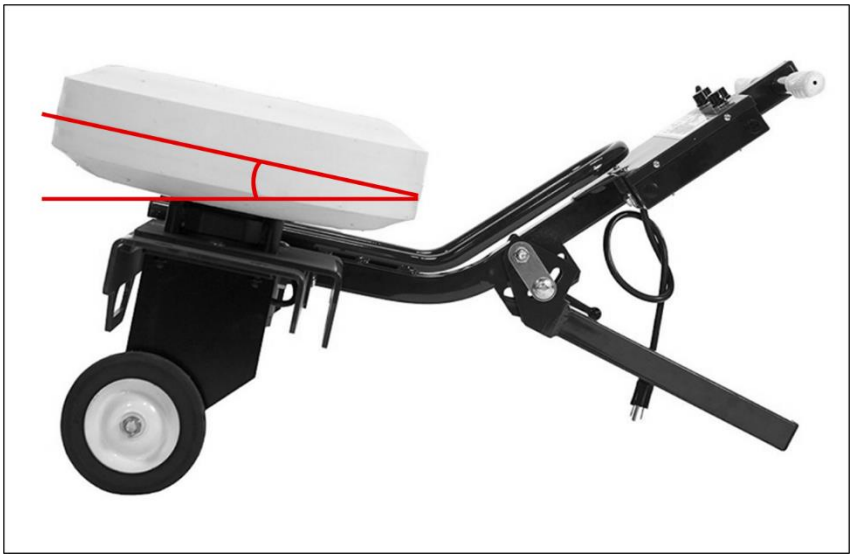


Figure 4.3. Soccer ball dispensing machine and the release angle (marked in red).

To compare physical exertion between heading and kicking mean heart rate (HR; beats per minute) was recorded using a HR sensor (Polar H1, Kempele, Finland) strapped around the participants chest. HR activity was analysed as a percentage of age predicted maximum HR, where age-predicted maximum HR equates to 220 minus the age. The physical activity level was then classified as follows: < 35% very light, 35–59% light, 60–79% moderate, 80–89% heavy and ≥ 90% very heavy (Kenney et al., 2012).

4.2.3.2 Transcranial Magnetic Stimulation

Corticomotor inhibition was assessed by measuring silence in the EMG activity following TMS using the same methodology as Di Virgilio et al. (2016, 2019).

Surface EMG activity was recorded using a MP100 system, a wireless BioNomadix transmitter and AcqKnowledge software (BIOPAC Systems, Inc., Goleta, CA, USA), sampling at the rate of 2000 Hz and filtered using 500 Hz low and 1 Hz high band filters. For recording surface EMG activity participants were seated on a resistance exercise chair, connected to the

data collection hardware described, with their dominant leg secured to a load cell using an ankle cuff. Two Ag/AgCl surface electrodes (Ambu Ltd., Cambridgeshire, UK) were placed over the rectus femoris (RF) muscle with an inter electrode distance of two cm, as described by the Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines (in the middle of the distance between anterior superior iliac spine and the superior part of the patella) (*Recommendations for Sensor Locations in Hip or Upper Leg Muscles*, n.d.). Prior to electrode placement over the muscle, the area was shaved, slightly abraded and cleaned with an alcohol swab (70% isopropyl). A ground electrode was placed over the patella. The transmitter was strapped around the participants leg (between electrodes placed on RF and patella). Participants performed isometric contractions with their leg at a 60-degree knee angle (0 degrees being a fully extended limb). Prior to the TMS participants were asked to perform three five second maximum voluntary isometric contractions (pre-MVC) with a minute rest in between each trial. Participants were verbally encouraged while performing maximum contractions. Participants were then instructed to sustain an isometric contraction equal to 20% of their maximum pre-MVC for determining (1) the primary motor cortex (M1) hotspot and (2) active motor threshold (aMT) (described below). Participants were seated approximately 1.5 meters from the monitor (distance between eyes and the screen) where they obtained visual feedback to maintain the appropriate contraction force.

Motor evoked potentials (MEPs) were evoked over M1 using a magnetic stimulator (Magstim 200^2 unit, The Magstim Company Ltd., Whitland, UK) and a 110 mm double cone coil placed contralateral to the vertex. To determine the location of M1 the distance from (1) nasion to inion and (2) from the left to the right tragi were measured and a marking was made at the halfway point using a surgical skin marker pen. The location was cross-referenced with participants' verbal confirmation: slight pressure was placed on the marking using the tip of the pen and participants were asked whether the location felt central in frontal and lateral plane. Optimal stimulation location was then determined by delivering suprathreshold stimulations while participants maintained an isometric contraction equal to 20% of their maximum pre-MVC until finding the location where highest MEP amplitude was elicited. This location was marked on the scalp to ensure correct positioning throughout the testing session.

To determine aMT, stimulations were delivered during an isometric contraction at 20% of pre-MVC starting at 20% stimulator output, increasing the intensity by 1–5% until discernible MEPs were visible (stimulation intensity was determined to the closest 1%).

Silent period duration was then measured three times by delivering a stimulation at 130% of aMT intensity during a five second isometric maximal leg contraction with approximately one-minute rest between trials. Participants received energetic verbal encouragement from the researcher during the contraction trials to push.

4.2.3.2.1 Data Processing

Data was processed on completion of the study. A detailed data extraction protocol was agreed and finalised prior to the start of data processing due to the manual analysis element which had a level of subjectivity. Manual analysis was opted for more accurate evaluation of the data, allowing careful examination of individual EMG traces that may have been missed by automated methods. Manual processing ensured that any artifacts and signal abnormalities were identified, improving the overall accuracy and validity of the results. The protocol was derived at consensus meetings with Professor Alan Pearce, Dr Thomas Di Virgilio and Dr Magdalena Ietswaart. The detailed protocol can be found in Appendix C.

Signals were analysed offline using AcqKnowledge software (version 3.9.1.6, BIOPAC Systems, Inc., Goleta, CA, USA). Silent period duration was defined as the duration between the offset of the TMS trigger and return of the visually observed voluntary EMG activity (Figure 1.1, page 25). Following initial training and piloting, the timepoint of EMG resumption was extracted by two reviewers (L-ML and AM[2]) independently. Reviewers were blinded to the participant, condition and sampling time information when extracting the data. Where a discrepancy of ≤ 4 ms occurred, data extracted by L-ML were used for analysis. Any signals where the EMG resumption timepoint was ≥ 5 ms different between reviewers, were reviewed by a third reviewer (TDV[3]). If the third reviewer (TDV) did not agree with either of the reviewers (difference of ≥ 5 ms), then the signal was discussed between L-ML, AM and TDV to reach consensus. Intraclass correlation coefficient (ICC) was calculated to assess interrater reliability for SP duration data between L-ML and AM. The peak-to-peak amplitude (mV) of motor evoked potentials was also extracted as a measure of excitability. The data extraction results are provided in Appendix C. For data analysis the median of the three SP durations was used. The ratio of inhibition to excitability was also computed as SP:MEP. The median of the three SP:MEP ratios was used in the analysis.

[2] Ali Muqtadir (AM) – a PhD candidate at University of Stirling, Psychology Division
[3] Dr Thomas Di Virgilio (TDV) – a researcher and lecturer at University of Stirling, Physiology, Exercise and Nutrition Research Group

Muscular fatigue/participant effort throughout each testing session was assessed by comparing the mean force of the three maximum voluntary isometric contractions (during the measurement of corticomotor inhibition) at baseline, immediately and one hour post.

4.2.3.3 Decomposition Electromyography

Motor unit firing rates were measured from the rectus femoris muscle belly during an isometric leg contraction at 60% pre-MVC. Participants were seated on the same resistance exercise chair as during the TMS trials, with their dominant leg secured to a load cell via an ankle cuff (leg was positioned at a 60-degree knee angle during the contraction with zero degrees being a fully extended limb). Motor unit activity was recorded using a four-pin surface EMG sensor (Trigno Galileo, Delsys Inc., Boston, USA; Figure 4.4) and EMGworks Acquisition software (version 4.7.3, Delsys Inc., Boston, USA) sampling at 2222 Hz (per channel) and bandwidth filtered at 20 to 450 Hz. The area of the sensor placement was shaved, dry skin cells were removed by dabbing the skin with medical tape and the skin was cleaned using an alcohol swab (70% isopropyl). The sensor location was marked with a pen to ensure the same placement throughout the testing session and the sensor was attached using Transpore medical tape. The dual on-board stabilizing reference for the electrode was placed on another muscle parallel to the recording sensor (lateral side of the upper leg).

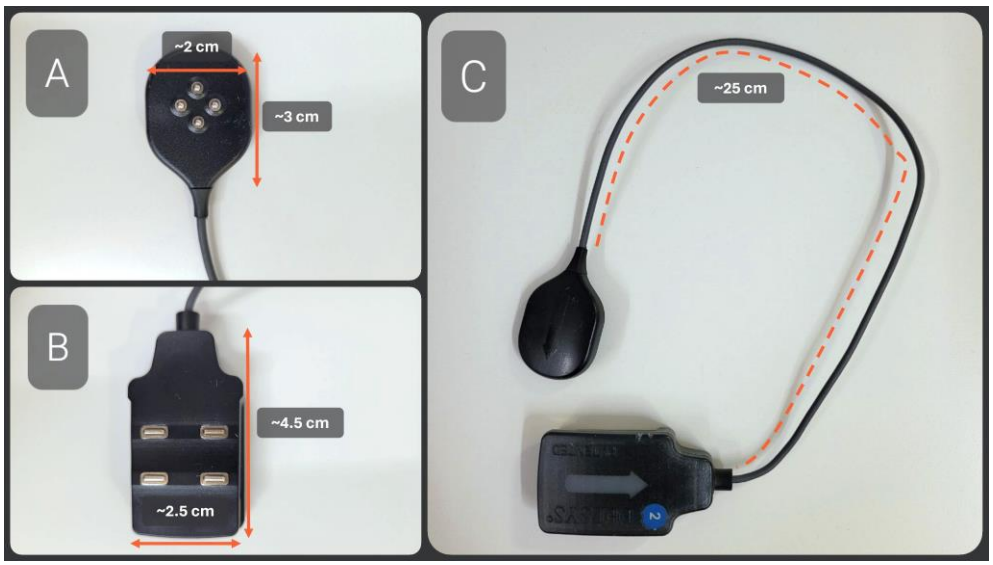


Figure 4.4. Trigno Galileo sensor from Delsys Inc. used for decomposition EMG. A – four-pin surface EMG sensor; B – reference contacts; C – sensor arrows were positioned in the same direction as the muscle fibres. Reference (B) for the EMG sensor (A) was placed on another muscle parallel to the recording sensor (lateral side of the upper leg).

Participants were instructed to perform a knee extension trapezoid-shaped contraction with a total duration of 22 seconds, where participants started out by increasing their force at the rate of 10% MVC per second until reaching 60% MVC, maintained a steady-state contraction at 60% MVC for 10 seconds and then decreased the force at 10% MVC per second (Figure 4.5). Participants obtained visual feedback from a monitor placed in front of them in order to maintain the appropriate contraction force.

Raw EMG signals were decomposed into constituent motor unit action potential trains using NeuroMap (version 1.1.0.0, Delsys Inc., Boston, USA). Motor unit firing rate data were extracted for a three-second section in the latter stages of the 10-second steady-state contraction, as the last three seconds of the steady rate contraction have previously displayed greatest reliability (Balshaw et al., 2017) using Neuromap Explorer software (version 1.2.2.0, Delsys Inc., Boston, USA). Only motor units with accuracy ≥ 80% were included in the data and mean firing rate curves were smoothed using 0.6 second Hanning window. Mean motor unit firing rate and percentage variability in mean motor unit firing rate were put forward for analysis, where the variability was assessed using coefficient of variation [CV%; calculated using the following formula: (*SD*/mean) × 100].

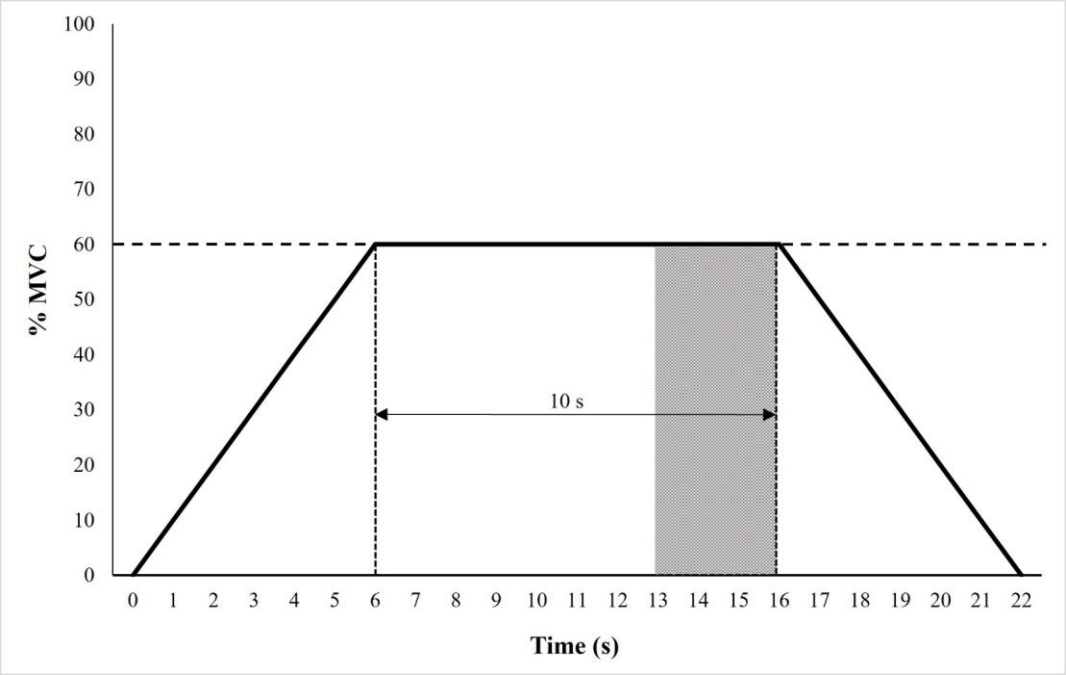


Figure 4.5. Decomposition EMG contraction protocol. Last three seconds (greyed area) of the 10 second isometric contraction at 60% MVC force was used in the analysis.

4.2.3.4 Gait

Temporal parameters of gait were measured during a continuous three-minute walk under two conditions: divided (no distractions) and undivided attention (performing a cognitive task while walking). Participants were instructed to walk at their habitual pace for three minutes in a circle (Figure 4.6). During divided attention participants were instructed to perform subtractions out loud using the number seven, the starting number was given to the participant immediately prior to the commencement of the walk. If participants reached zero (or value closest to zero) before the three minutes were up, they were instructed to continue subtracting with number six instead starting from the same number that they had been given before the start of the trial. The number of errors were recorded as a measure of accuracy and the total number of answers given was recorded as performance. Ratio of accuracy to performance was calculated (total number of answers divided by number of correct answers) and used in the analysis, where lower ratio indicates better performance (less mistakes for the number of answers given).

Walking speed was measured over a four-meter walkway (a section of the circle) with a custom-built infrared light beam system using Arduino hard- and software (version 1.8.9). The average and CV% of walking speed (m/s) were used in the analysis, calculated across the laps completed within the three-minute walk. Data from the first lap were excluded from the analysis for all trials to account for potential acceleration.

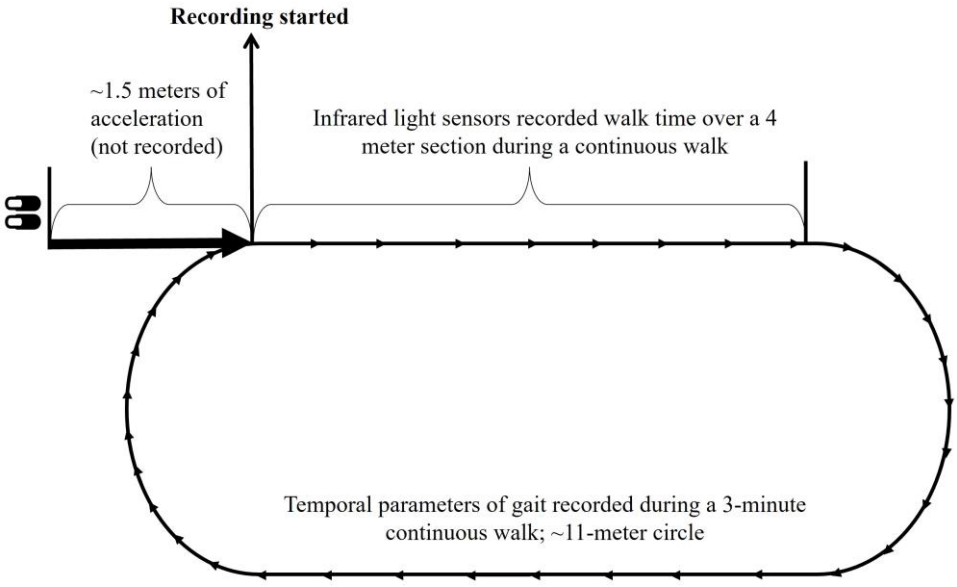


Figure 4.6. Schematic illustration of the testing protocol for measuring temporal parameters of gait.

Insoles (Pedar-X, Novel GmbH, Munich, Germany), sampling at 50 Hz, were fitted to participants' shoes to record the following eight temporal parameters of gait: stride time (s), step time (s), stance time (s), swing time (s), stance (as % of gait cycle [GC]), swing (% GC), single support (% GC) and double support (% GC) (Figure 4.7). The mean and CV% were calculated for each three-minute trial and used in the analysis; the first and last two steps of each trial were excluded to account for acceleration and deceleration.

Participants were asked to wear the same shoes for all testing sessions.

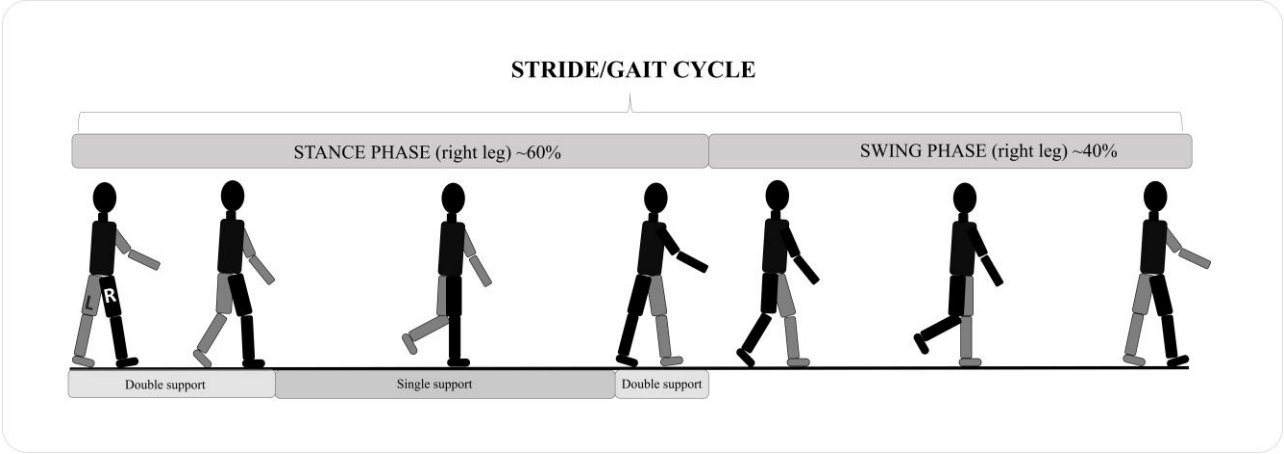


Figure 4.7. Illustration of the phases of the gait cycle.

4.2.3.5 Cognitive Tests

Cognitive function was measured using the Cambridge Neuropsychological Test Automated Battery (CANTAB) high-functioning (i.e., extended) version, consisting of two tests: (1) paired associates learning test (PAL) assessing episodic memory function and (2) working memory task (SWM) assessing working memory and executive function. Participants completed practice tests prior to the start of each testing session to minimise learning effects.

In the PAL test, participants were shown blank boxes on an iPad screen, each box revealing an abstract stimulus one at the time in a random order. Participants were required to remember which stimulus belonged to which box (link to the test demonstration). The test started with four boxes with two boxes added at each level, the final level contained 12 boxes. Participants were able to move to the next level only if they answered everything correctly. If participants made a mistake, they were shown the stimuli again. Participants had four attempts to complete a level, if they were unsuccessful the test was considered complete. The software provided an estimated number of errors the participants would have made in the levels that they

did not complete based on the number of errors they made in the test. Total number of errors (adjusted) was used in the analysis.

During the SWM test participants were required to find tokens hidden in the boxes, participants were instructed to not choose a box where they had already found a token (link to the demonstration). The test started with four boxes and four tokens to be found. Two boxes and tokens were added at each level with 12 boxes and tokens in the final level. Number of errors (participants revisiting a box where a token was already found) was used in the analysis as a measure of working memory performance. Strategy was used in the analysis as a measure executive function, where a low score indicated high strategy use.

4.2.4 Statistical Analysis

The required sample size for this study was determined *a priori* by performing a power analysis (G*Power, version 3.1.9.7, Heinrich Heine University Düsseldorf, Düsseldorf, Germany) for the primary outcome measure (silent period duration); parameters for the analysis (ES = 0.48, $\alpha = 0.05$, power $(1-\beta) = 0.8$; see Appendix B for the power analysis details) were based on previous research from our laboratory that assessed the acute effects of soccer heading (Di Virgilio et al., 2016). The power analysis revealed that 28 participants were required to detect heading induced increase in SP duration.

Data were assessed for outliers using boxplots. Extreme outliers (≥ three times IQR) were transformed using winsorization method whereby extreme outlier values were replaced with mean + 3 × *SD* (Field, 2012). A combination of visual inspection (histograms and normal Q-Q plots), normality testing (Shapiro-Wilk test) and assessment of skewness and kurtosis (skewness < 2 and kurtosis [excess] < 4 indicating normal distribution) was used to determine if the data were normally distributed (Kim, 2013). Square root (sqrt) and logarithmic (log10) transformations were applied to positively skewed data to achieve normal distribution (sqrt transformation was used for SWM errors data and log10 for PAL errors and walking speed CV% data). A constant of 1 was added when logarithmically transforming variables where values of 0 occurred. Where normal distribution could not be achieved, equivalent non-parametric test was used (for cognitive test during walking mean difference between baseline and post scores was compared across the conditions using Friedman's test). Data analysis was performed only on complete set of data, listwise deletion was used to deal with missing data. Data are reported as mean ± *SD* unless stated otherwise.

To assess the effect of conditions (kicking and heading) and time (baseline, immediately and one hour post) on outcome variables two-way repeated measures ANOVAs were computed where the condition and time were the independent variables and outcome data were the dependent variables (i.e., SP duration, SP:MEP, MVC force, mean motor unit firing rate, mean motor unit firing rate CV%, PAL errors, SWM errors, executive function score). Walking speed and walking speed CV% data were analysed using three-way repeated measures ANOVAs where the independent variables were condition, time and attention type (divided and undivided). The mean and CV% data for the eight temporal parameters of gait (recorded using the insoles), were analysed using three-way repeated measures MANOVAs. When assumption of sphericity was violated Greenhouse-Geisser correction was used. Bonferroni correction was applied to post-hoc pairwise comparisons to reduce false-positive findings (type I errors). One-way ANOVAs were used to compare aMT and mean HR across the sessions. Partial eta squared (η_p^2: 0.0099 = small; 0.0588 = medium; 0.1379 = large; Richardson, 2011) and Kendall's *W* (*W*: ranges 0 to 1, with 0 = no agreement and 1 = complete agreement between the ranked data; Field, 2013) were used to quantify effect sizes.

Statistical tests were carried out using IBM SPSS (version 28.0.0.0, SPSS Inc., Chicago, USA). Statistical significance was set at $p \leq 0.05$.

4.3 Results

4.3.1 Characteristics

Participant characteristics for experiment one (dose response study) and two (headgear study) are displayed in Table 4.1. The average soccer playing experience was 12 ± 4 years.

Table 4.1. Participant characteristics, displayed as mean ± *SD* (range).

	Experiment one (*n* = 30)	**Experiment two (*n* = 28)**
Age (yr)	24 ± 4 (18–34)	24 ± 4 (18–34)
Height (m)	1.75 ± 0.07 (1.65–1.92)	1.75 ± 0.07 (1.65–1.92)
Mass (kg)	71.3 ± 11.7 (47.3–98.7)	70.8 ± 12.0 (47.3–98.7)
Years playing football	12 ± 4 (4–25)	12 ± 4 (4–25)
Heading experience (yr)	12 ± 4 (2–25)	12 ± 4 (2–25)
Level of education (*n*)		
Undergraduate degree or equivalent	12	12
Master's degree or equivalent	16	14
PhD or equivalent	2	2
Race (*n*)		
White	16	14
Black	1	1
Asian	13	13

4.3.2 Physical Exertion Between Conditions

4.3.2.1 Experiment One

Heart rate data were not analysed for seven participants (23.3%) due to equipment failure in detecting HR ($n = 4$) or due to measurement error (sensor movement). The highest activity level during heading and kicking for the remaining participants ($n = 23$) was light (≤ 59% HR_{max}). There was no significant difference in the mean HR (Table 4.2) between conditions, $F(2,44) = 1.059$, $p = 0.355$, $\eta^2 = 0.046$. Therefore, there is no evidence to suggest that physical exertion differed between the control (kicking) and the heading conditions in the dose response experiment.

4.3.2.2 Experiment Two

HR data were not analysed for five participants (17.9%) due to equipment failure to detect HR ($n = 3$) or due to measurement error (sensor movement). The highest activity level during heading and kicking for the remaining participants ($n = 23$) was light. There was no significant difference in the mean HR (Table 4.2) between conditions, $F(2,44) = 0.537$, $p =$

0.588, $\eta^2 = 0.024$. Therefore, there is no evidence to suggest that physical exertion differed between the control (kicking) and the heading conditions in the headgear experiment.

Table 4.2. Mean heart rate (beats per minute) with standard deviation.

	Kicking	10 headers	20 headers	20 headers with HG
Experiment one	73 ± 8	75 ± 9	75 ± 10	N/A
Experiment two	72 ± 8	N/A	74 ± 9	73 ± 10

HG – headgear

4.3.3 Changes in Maximum Voluntary Contraction Force

Changes in maximum voluntary contraction force were first analysed to ensure that silent period duration and corticomotor excitability (motor evoked potential amplitude) data were not affected by any possible changes in contraction force (i.e., submaximal) levels due to fatigue or effort.

4.3.3.1 Experiment One

There was no significant effect of time [$F(2,58) = 1.420$, $p = 0.250$, $\eta^2 = 0.047$], condition [$F(2,58) = 0.774$, $p = 0.466$, $\eta^2 = 0.026$] or condition*time interaction [$F(3.082, 89.364) = 1.418$, $p = 0.242$, $\eta^2 = 0.047$] on mean MVC force (Table D.1). These results confirm that there were no significant changes in the contraction force between baseline and post measures or between sessions in the dose response study.

4.3.3.2 Experiment Two

There was no significant effect of time [$F(2, 54) = 0.815$, $p = 0.448$, $\eta^2 = 0.029$], condition [$F(1.623,43.823) = 0.448$, $p = 0.601$, $\eta^2 = 0.016$] or condition*time interaction [$F(4, 108) = 0.821$, $p = 0.515$, $\eta^2 = 0.029$] on mean MVC force (Table D.1). These results confirm that there were no significant changes in the contraction force between baseline and post measures or between sessions in the headgear study.

4.3.4 Active Motor Threshold, Silent Period Duration and Silent Period to Motor Evoked Potential Ratio

Silent period data for one participant were excluded from the analysis from both experiments due their data not being analysable in approximately half of the trials (e.g., resumption of EMG was not distinguishable).

4.3.4.1.1 Experiment One

The average aMT in this cohort (n = 30) was 36 ± 6% of the maximum stimulator output. Within participant difference in the aMT between sessions was a maximum of 3% and fluctuated 1% on average. There was no significant difference in the aMT between sessions, $F(2,58) = 0.555$, $p = 0.577$, $\eta^2 = 0.019$.

There was no significant effect of condition [$F(2,56) = 0.157$, $p = 0.855$, $\eta^2 = 0.006$], time [$F(2,56) = 2.989$, $p = 0.058$, $\eta^2 = 0.096$] or condition*time interaction [$F(4,112) = 0.627$, $p = 0.645$, $\eta^2 = 0.022$] on median silent period duration (Figure 4.8 [a], Table D.1). These results confirm that silent period duration was not affected by heading (10 or 20 times) or kicking activity.

Furthermore, there was no significant effect of condition, $F(1.561,43.710) = 0.950$, $p = 0.374$, $\eta^2 = 0.033$, or condition*time interaction, $F(2.930,82.045) = 0.330$, $p = 0.799$, $\eta^2 = 0.012$ on median SP to MEP ratio, whereas the effect of time was significant, $F(2,56) = 14.856$, $p < 0.001$, $\eta^2 = 0.347$, with the ratio increasing significantly from baseline (Figure 4.9 [a], Table D.1). Since silent period duration did not change significantly across timepoints, the findings suggest that motor evoked potential amplitude decreased following heading and kicking activity.

4.3.4.1.2 Experiment Two

The average aMT in this cohort (n = 28) was 35 ± 6% of the maximum stimulator output. Within participant difference in the aMT between sessions was a maximum of 3% and fluctuated 1% on average. There was no significant difference in the aMT between testing sessions, $F(2,54) = 1.169$, $p = 0.318$, $\eta^2 = 0.041$.

There was no significant effect of condition [$F(2,52) = 0.080$, $p = 0.923$, $\eta^2 = 0.003$], time [$F(2,52) = 2.813$, $p = 0.069$, $\eta^2 = 0.098$] or condition*time interaction [$F(4,104) = 0.258$, $p = 0.904$, $\eta^2 = 0.010$] on median silent period duration (Figure 4.8 [b], Table D.1). These results confirm that silent period duration was not affected by heading (with or without headgear) or kicking activity.

Furthermore, there was no significant effect of condition, $F(2,52) = 1.104$, $p = 0.339$, $\eta^2 = 0.041$, or condition*time interaction, $F(4,104) = 0.277$, $p = 0.892$, $\eta^2 = 0.011$ on median SP to MEP ratio, whereas the effect of time was significant, $F(2,52) = 9.848$, $p < 0.001$, $\eta^2 = 0.275$, with the ratio increasing significantly from baseline (Figure 4.9 [b], Table D.1). Since

silent period duration did not change significantly across timepoints, the findings suggest that motor evoked potential amplitude decreased following heading and kicking activity.

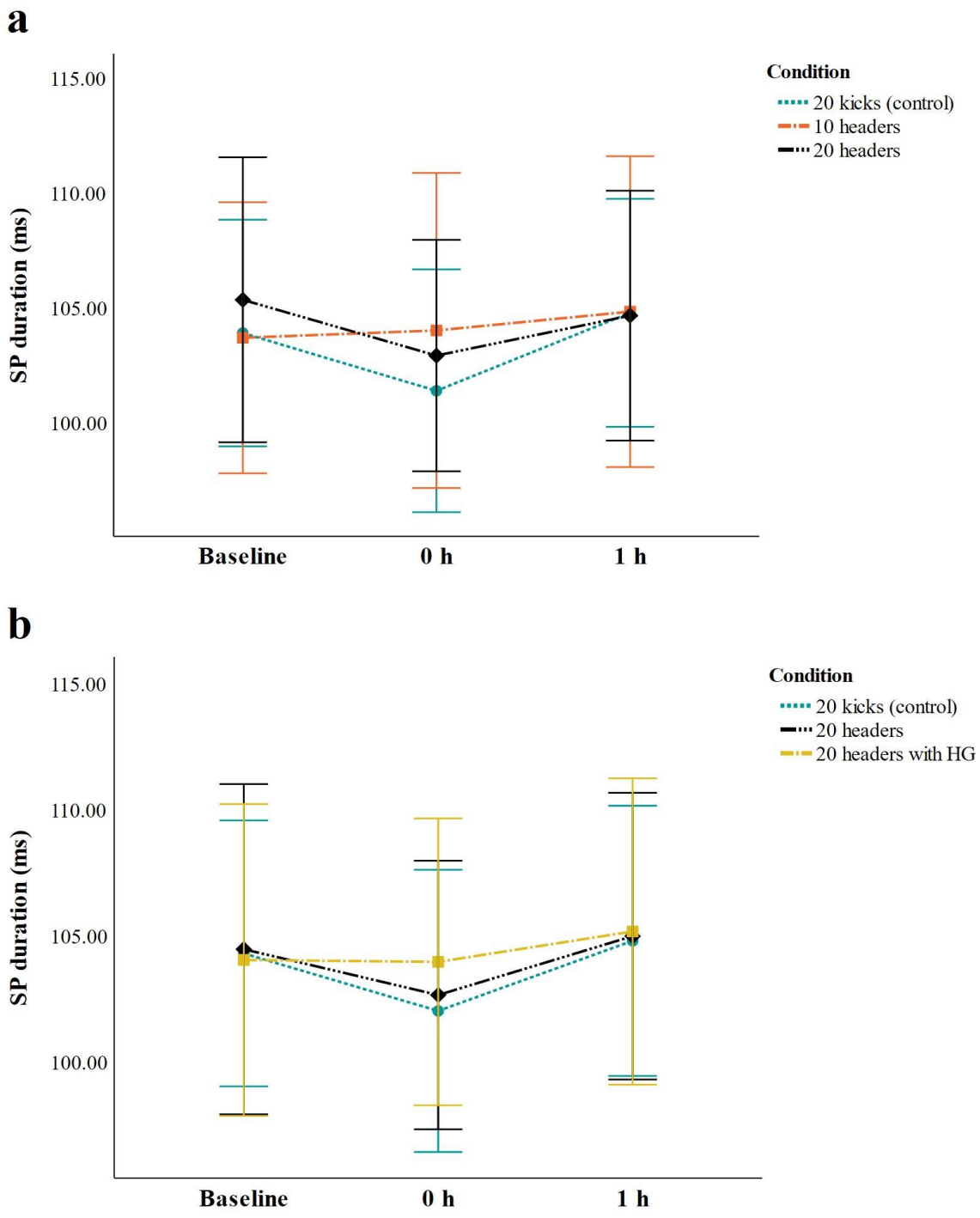


Figure 4.8. Silent period duration (SP) in milliseconds measured at baseline and immediately (0 h) and 1 hour after kicking (control condition) and heading conditions. Experiment one (a) – dose response study included 10 and 20 header conditions; experiment two (b) – headgear study included 20 headers completed with and without headgear (HG) conditions. Error bars denote 95% confidence intervals.

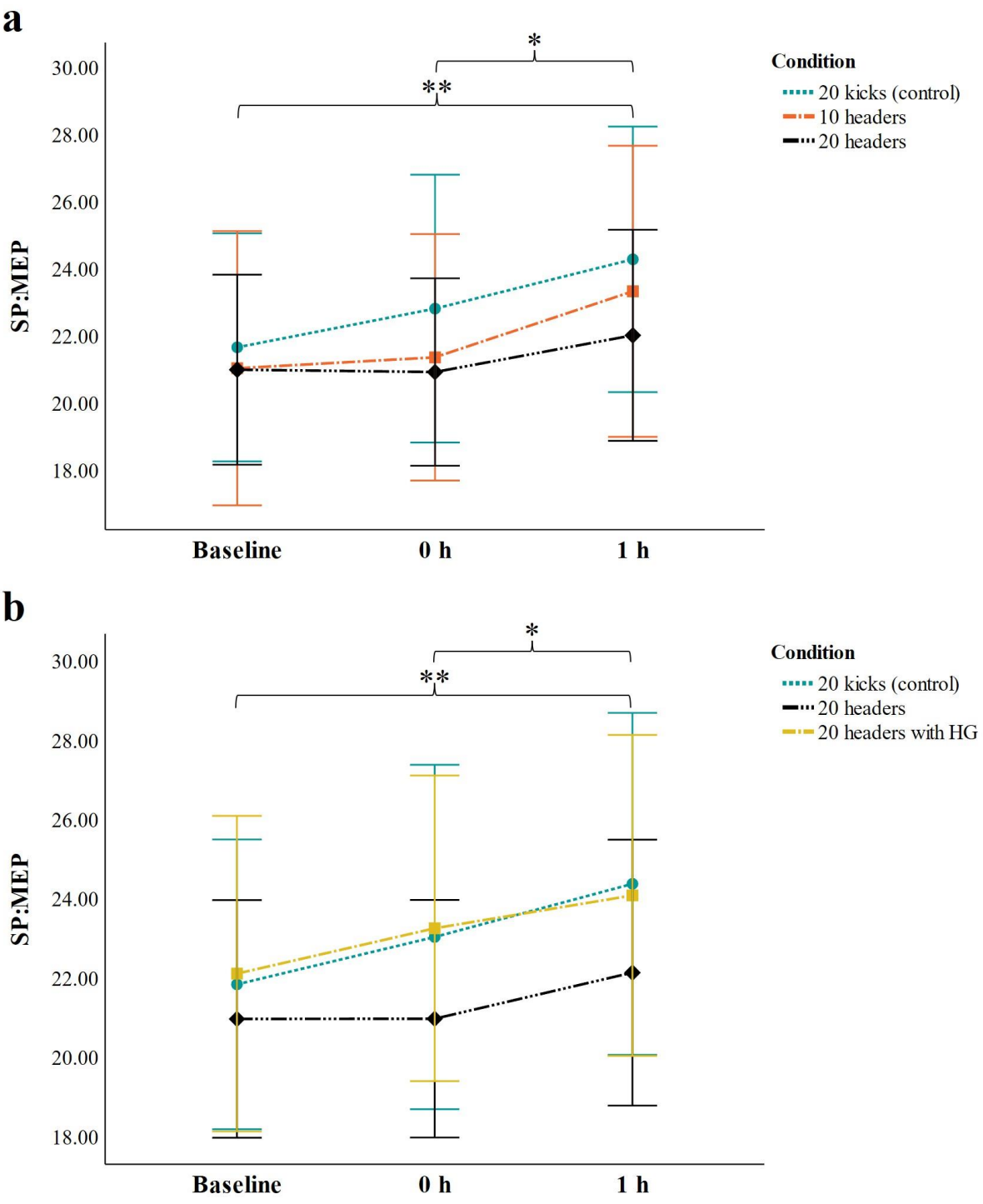


Figure 4.9. Silent period (SP) duration to motor evoked potential (MEP) amplitude ratio at baseline and immediately (0 h) and 1 hour after kicking (control condition) and heading conditions. Experiment one (a) – dose response study included 10 and 20 header conditions; experiment two (b) – headgear study included 20 headers completed with and without headgear (HG) conditions. Error bars denote 95% confidence intervals. **Baseline vs 1 h post ($p < 0.01$); *0 h vs 1 h post ($p < 0.05$).

4.3.5 Mean Motor Unit Firing Rate

4.3.5.1 Experiment One

Data were available for 20 participants, 33.3% of data were missing due to poor signal quality during data collection (i.e., low motor unit accuracy). There was no significant effect of condition [$F(1.477,28.070) = 0.554$, $p = 0.579$, $\eta^2 = 0.028$], time [$F(2,38) = 0.081$, $p = 0.922$, $\eta^2 = 0.004$] or condition*time interaction [$F(4,76) = 0.900$, $p = 0.468$, $\eta^2 = 0.045$] for mean firing rate (Figure 4.10 [a], Table D.2). Moreover, there was no significant effect of condition [$F(2,38) = 0.023$, $p = 0.977$, $\eta^2 = 0.001$], time [$F(2,38) = 0.114$, $p = 0.892$, $\eta^2 = 0.006$] or time*condition interaction [$F(2.617,49.723) = 0.665$, $p = 0.618$, $\eta^2 = 0.034$] on motor unit firing rate CV% (Figure 4.11 [a], Table D.2). Therefore, mean motor unit firing rate or variation in motor unit firing rate were not affected by heading or kicking activity in the dose response study.

4.3.5.2 Experiment Two

Data were available for 20 participants, 28.6% of data were missing due to poor signal quality during data collection (i.e., low motor unit accuracy). There was no significant effect of condition [$F(2,38) = 0.044$, $p = 0.957$, $\eta^2 = 0.002$], time [$F(1.551,29.474) = 1.051$, $p = 0.360$, $\eta^2 = 0.052$] or condition*time interaction [$F(4,76) = 1.136$, $p = 0.346$, $\eta^2 = 0.056$] for mean firing rate (Figure 4.10 [b], Table D.2). Furthermore, there was no significant effect of condition [$F(2,38) = 0.102$, $p = 0.903$, $\eta_p^2 = 0.005$], time [$F(2,38) = 1.882$, $p = 0.166$, $\eta_p^2 = 0.090$] or condition*time interaction [$F(4,76) = 0.557$, $p = 0.695$, $\eta_p^2 = 0.028$] on motor unit firing rate CV% (Figure 4.11 [b], Table D.2). Therefore, motor unit firing rate or variation in motor unit firing rate were not affected by heading or kicking activity in the headgear study.

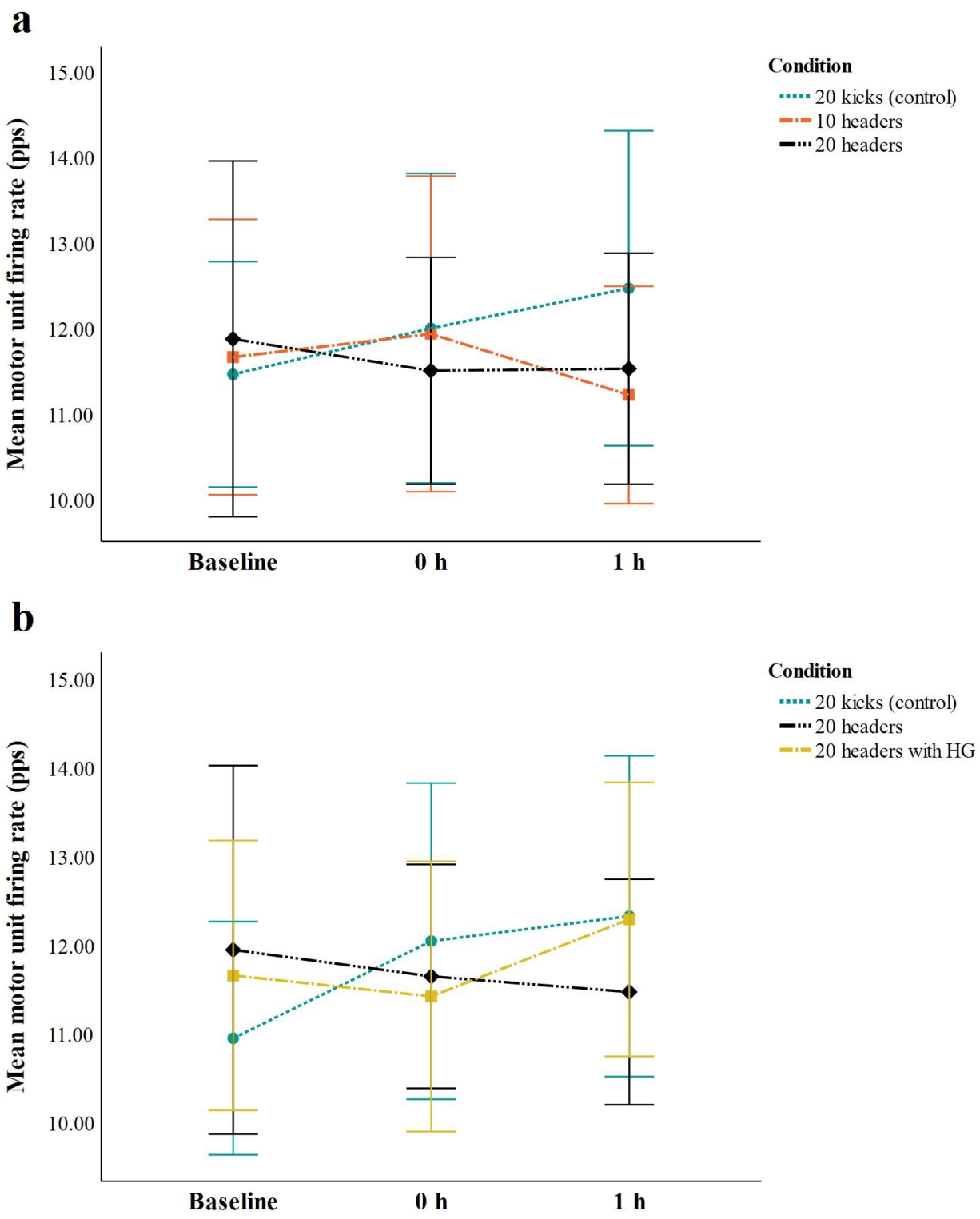


Figure 4.10. Mean motor unit firing rate at baseline and immediately (0 h) and 1 hour after kicking (control condition) and heading conditions. Experiment one (a) – dose response study included 10 and 20 header conditions; experiment two (b) – headgear study included 20 headers completed with and without headgear (HG) conditions. Error bars denote 95% confidence intervals; pps – pulses per second.

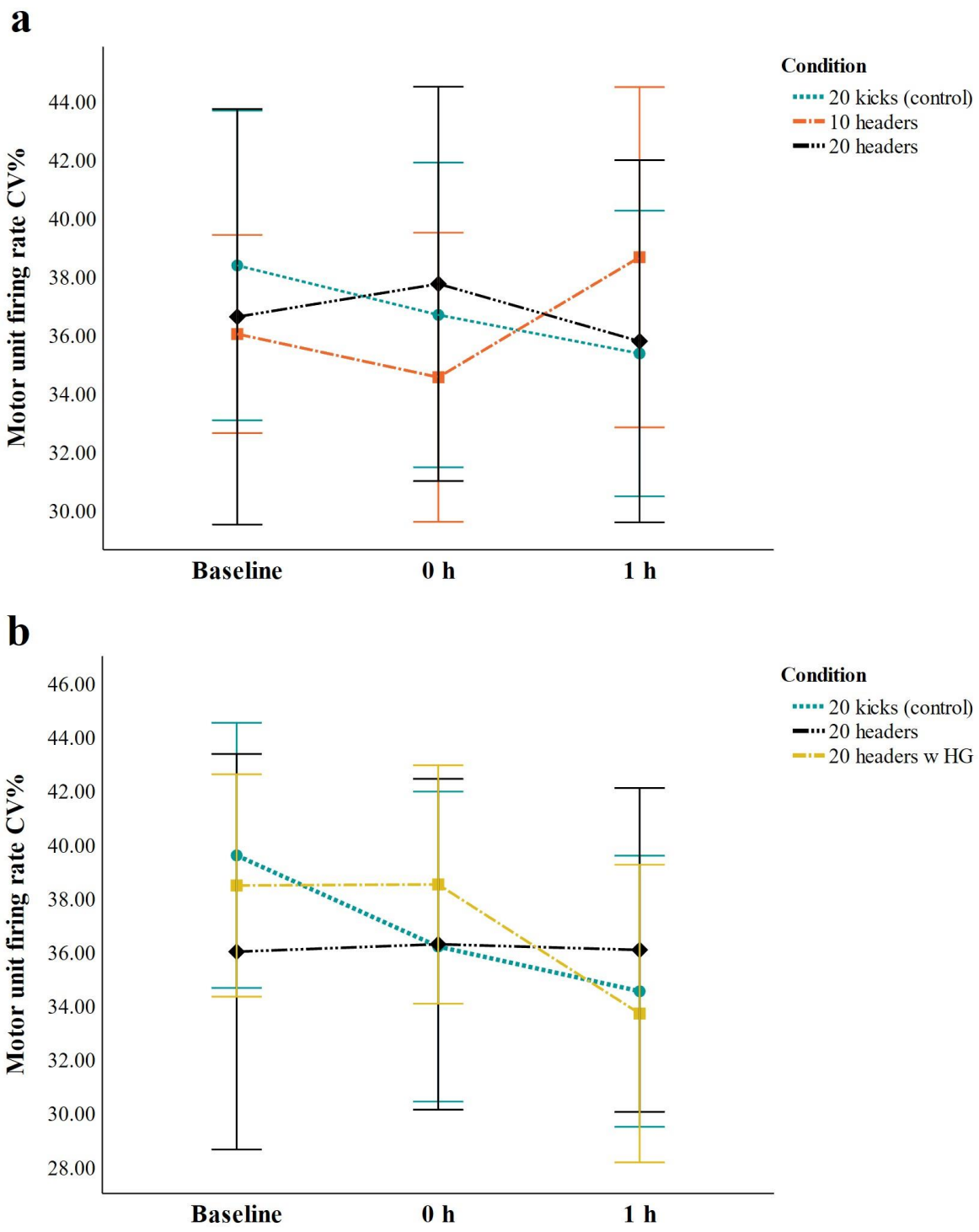


Figure 4.11. Motor unit firing rate CV% at baseline and immediately (0 h) and 1 hour after kicking (control condition) and heading conditions. Experiment one (a) – dose response study included 10 and 20 header conditions; experiment two (b) – headgear study included 20 headers completed with and without headgear (HG) conditions. Error bars denote 95% confidence intervals.

4.3.6 Gait

4.3.6.1 Cognitive Function During Walking

Cognitive function during walking was first analysed to ensure that gait was not maintained at the expense of impaired cognitive performance.

4.3.6.1.1 Experiment One

There was no significant change in cognitive performance between baseline and post measurements across the conditions, $\chi^2(2) = 0.054$, $p = 0.974$, $W = 0.001$ (Table D.3).

4.3.6.1.2 Experiment Two

There was no significant change in cognitive performance across the conditions, $\chi^2(2) = 0.059$, $p = 0.971$, $W = 0.001$ (Table D.3).

4.3.6.2 Temporal Parameters of Gait

4.3.6.2.1 Experiment One

Walking speed data are missing for one participant due to equipment failure. There was no significant effect of condition [$F(1.494,41.839) = 0.157$, $p = 0.855$, $\eta^2 = 0.006$], time [$F(1,28) = 0.007$, $p = 0.932$, $\eta^2 = 0.000$] or an overall interaction effect [condition*time*attention type, $F(2,56) = 0.943$, $p = 0.396$, $\eta^2 = 0.033$] on the average walking speed (Table D.4). However, there was a significant effect of attention (divided versus undivided attention) on walking speed, $F(1,28) = 42.060$, $p < 0.001$, $\eta^2 = 0.600$, where participants were walking slower when their attention was divided. Further, there was no effect of condition [$F(1.668,46.697) = 0.612$, $p = 0.518$, $\eta^2 = 0.021$], time [$F(1,28) = 0.002$, $p = 0.966$, $\eta^2 = 0.000$], attention [$F(1,28) = 3.674$, $p = 0.066$, $\eta^2 = 0.116$] or an interaction effect [condition*time*attention type, $F(1.652,46.268) = 0.666$, $p = 0.491$, $\eta^2 = 0.023$] on walking speed CV% (Table D.5).

Temporal data recorded using the insoles were affected by system error and six participants' data were excluded from the analysis due to significant data loss in the affected trials (≤ 8% data available in some trials, i.e., ≤ 16 steps available). The average number of steps completed during a continuous three-minute walk was 259 (range: 30–354; calculated across all participants and trials that were included in the analysis).

There was no significant interaction effect between condition (kicking, 10 and 20 headers), time (baseline and post) and trial type (undivided and divided attention) on temporal

variables of gait mean data, $F(16, 78) = 0.913$, $p = 0.558$, Wilks'$\Lambda = 0.709$, $\eta_p^2 = 0.158$ (Table D.4) or CV% data, $F(16, 78) = 0.868$, $p = 0.607$, Wilks'$\Lambda = 0.721$, $\eta_p^2 = 0.151$ (Table D.5). The temporal variables of gait mean and CV% data had a significant effect for the type of trial (undivided vs divided attention) only [$F(8,16) = 2.675$, $p = 0.045$, Wilks'$\Lambda = 0.428$, $\eta_p^2 = 0.572$ and $F(8,16) = 3.578$, $p = 0.014$, Wilks'$\Lambda = 0.359$, $\eta_p^2 = 0.641$, respectively]. Follow-up univariate test results for temporal gait variables that were significantly different between trials of divided and undivided attention are in Appendix E. None of the other factors or interactions (condition*time, time*trial, condition*trial) were significant. Therefore, there is no evidence to suggest that heading 10 or 20 times (or kicking activity) negatively affected any of the temporal parameters of gait.

4.3.6.2.2 Experiment Two

Walking speed data for two participants are missing due to equipment malfunction. There was no significant effect of condition [$F(1.376,34.390) = 2.074$, $p = 0.136$, $\eta_p^2 = 0.077$], time [$F(1,25) = 0.109$, $p = 0.744$, $\eta_p^2 = 0.004$] or an overall interaction effect [condition*time*attention type, $F(2,50) = 0.444$, $p = 0.644$, $\eta_p^2 = 0.017$] on the average walking speed (Table D.4). However, there was a significant effect of attention type (divided versus undivided attention) on walking speed, $F(1,25) = 32.966$, $p < 0.001$, $\eta_p^2 = 0.569$, with participants walking slower during divided attention. Furthermore, there was no effect of condition [$F(2,50) = 0.125$, $p = 0.883$, $\eta_p^2 = 0.005$], time [$F(1,25) = 0.066$, $p = 0.800$, $\eta_p^2 = 0.003$], attention type [$F(1,25) = 2.504$, $p = 0.126$, $\eta_p^2 = 0.091$] or interaction effect [condition*time*attention type, $F(2,50) = 0.668$, $p = 0.571$, $\eta_p^2 = 0.026$] on walking speed CV% (Table D.5).

Due to system error seven participants' temporal data collected with the insoles were excluded (≥ 95% data loss in some of the affected trials, i.e., ≤ 18 steps available). The average number of steps completed during a continuous three-minute walk was 265 (range: 47–371 steps; calculated across all participants and trials that were included in the analysis).

There was no significant interaction effect between condition (kicking, 20 headers with and without headgear), time (baseline and post) and attention type (undivided and divided attention) on the temporal variables of gait mean data, $F(16, 66) = 1.327$, $p = 0.208$, Wilks'$\Lambda = 0.572$, $\eta_p^2 = 0.243$ (Table D.4) or CV% data, $F(16, 66) = 1.160$, $p = 0.323$, Wilks'$\Lambda = 0.609$, $\eta_p^2 = 0.219$ (Table D.5). Furthermore, there were no significant effects of condition, time, trial or other interaction effects (condition*time, time*trial, condition*trial) for temporal variables of gait mean data. The temporal variables of gait CV% data had a significant effect for the type

of attention (undivided or divided attention), $F(8,13) = 3.865$, $p = 0.015$, Wilks'$\Lambda = 0.296$, $\eta_p^2 = 0.704$ only. Follow-up univariate test results for temporal gait variables that were significantly affected by performing a cognitive task while walking are in Appendix E. None of the other factors or interactions were significant. Therefore, there is no evidence to suggest that heading with or without headgear (or kicking activity) negatively affected any of the temporal parameters of gait.

4.3.7 Cognitive Function

4.3.7.1 Paired Associates Learning Test

4.3.7.1.1 Experiment One

There was no significant effect of condition, $F(2, 58) = 1.027$, $p = 0.364$, $\eta^2 = 0.034$ or condition*time interaction, $F(2,58) = 0.191$, $p = 0.826$, $\eta^2 = 0.007$, however, the effect of time was significant, $F(1,29) = 8.412$, $p = 0.007$, $\eta^2 = 0.225$ with the number of errors increasing after heading and kicking (Table 4.3). Therefore, episodic memory function was negatively affected by both heading (10 and 20 times) and kicking activity in the dose response study.

4.3.7.1.2 Experiment Two

There was no significant effect of condition [$F(2, 54) = 1.529$, $p = 0.226$, $\eta_p^2 = 0.054$], time [$F(1, 27) = 4.026$, $p = 0.055$, $\eta_p^2 = 0.130$] or condition*time interaction [$F(2,54) = 0.570$, $p = 0.569$, $\eta_p^2 = 0.021$] (Table 4.3). Therefore, there is no evidence to suggest that episodic memory function was affected by heading (with or without headgear) or kicking in the headgear study.

4.3.7.2 Spatial Working Memory Task

4.3.7.2.1 Experiment One

There was no significant effect of condition [$F(2, 58) = 0.879$, $p = 0.421$, $\eta^2 = 0.029$], time [$F(1,29) = 0.620$, $p = 0.437$, $\eta^2 = 0.021$] or condition*time interaction [$F(2,58) = 1.317$, $p = 0.276$, $\eta^2 = 0.043$] on spatial working memory task error score (Table 4.3). Further, there was no significant effect of condition [$F(2, 58) = 0.040$, $p = 0.961$, $\eta^2 = 0.001$], time [$F(1,29) = 3.156$, $p = 0.086$, $\eta^2 = 0.098$] or condition*time interaction [$F(2,58) = 0.318$, $p = 0.729$, $\eta^2 = 0.011$] on strategy score (Table 4.3). These results show that working memory nor executive function were affected by heading (10 or 20 times) or kicking activity.

4.3.7.2.2 Experiment Two

There was no significant effect of condition [$F(2, 54) = 1.739$, $p = 0.361$, $\eta_p^2 = 0.037$], time [$F(1,27) = 0.674$, $p = 0.419$, $\eta_p^2 = 0.024$] or condition*time interaction [$F(2,54) = 0.326$, $p = 0.723$, $\eta_p^2 = 0.012$] on spatial working memory task error score (Table 4.3). There was no significant effect of condition [$F(2, 54) = 0.192$, $p = 0.826$, $\eta_p^2 = 0.007$], time [$F(1,27) = 2.653$, $p = 0.115$, $\eta_p^2 = 0.089$] or condition*time interaction [$F(2,54) = 0.501$, $p = 0.609$, $\eta_p^2 = 0.018$] on strategy score (Table 4.3). These results show that working memory nor executive function were affected by heading (with or without headgear) or kicking activity.

Table 4.3. Cambridge Neuropsychological Test Automated Battery (CANTAB) high-functioning (i.e., extended) version test results for PAL and SWM; higher error and strategy scores denote worse performance.

			Baseline	0 h
PAL (log errors)	**Experiment one**	**20 kicks (control)**	0.83 ± 0.43	0.92 ± 0.42
		10 headers	0.83 ± 0.51	0.97 ± 0.42
		20 headers	0.88 ± 0.40	1.01 ± 0.51
	Experiment two	**20 kicks (control)**	0.78 ± 0.40	0.90 ± 0.42
		20 headers	0.88 ± 0.41	0.97 ± 0.51
		20 headers with HG	0.86 ± 0.44	0.88 ± 0.46
SWM:				
Working memory (sqrt errors)	**Experiment one**	**20 kicks (control)**	3.90 ± 2.14	3.43 ± 2.38
		10 headers	3.59 ± 2.18	3.25 ± 2.21
		20 headers	3.67 ± 2.38	4.01 ± 2.46
	Experiment two	**20 kicks (control)**	3.78 ± 2.16	3.44 ± 2.46
		20 headers	3.80 ± 2.36	3.90 ± 2.48
		20 headers with HG	3.62 ± 2.61	3.38 ± 2.02
Strategy score	**Experiment one**	**20 kicks (control)**	10.10 ± 5.12	10.33 ± 5.18
		10 headers	9.97 ± 5.04	10.23 ± 5.14
		20 headers	9.80 ± 5.67	10.53 ± 5.26
	Experiment two	**20 kicks (control)**	9.64 ± 4.99	10.25 ± 5.27
		20 headers	9.68 ± 5.85	10.25 ± 5.30
		20 headers with HG	9.68 ± 5.75	9.75 ± 4.91

HG – headgear; PAL – paired associates learning test; SWM – spatial working memory task

4.4 Discussion

The aims of the experiments described in this chapter were to (1) examine whether acute brain changes from subconcussive head impacts are dose dependent and (2) compare the functional brain response to headers completed with and without headgear. The study was not able to determine whether acute brain changes are subconcussive impact dose dependent or whether headgear can prevent heading induced acute brain changes since no acute brain changes following RSHI exposure were detected. These findings either suggest that the soccer headers did not cause acute brain changes, or that the changes were too subtle for the measures that were used to detect them.

The results of the current study do not support the findings of the previously published research from University of Stirling looking at the acute effects of RSHI on corticomotor inhibition, cognitive function and motor unit recruitment strategies. The differences in findings could be in some parts perhaps be explained by differences between the current and prior research and methodological limitations.

Previously, Di Virgilio et al. (2016, 2019) reported significant elongation of the silent period immediately following a bout of soccer headers and one hour following sparring in boxing. It is noteworthy that the observed increases in the silent period means were approximately five and less than eight milliseconds for the soccer headers and sparring, respectively. These effects are small compared to the ~33 millisecond increase in the silent period duration observed by McNabb et al (2020) immediately following a drill of rugby tackling (n = 9; p = 0.001). Additionally, during the extraction of data reported in this chapter we observed fluctuations in the silent period duration between the three trials that exceeded the effects reported by Di Virgilio et al. (2016, 2019). Interestingly, McNabb et al. (2020) reports non-significant changes from baseline to post measurements in the silent period duration of approximately six milliseconds in the control group which exceeds the effects reported by Di Virgilio et al. (2016) that were associated with increased inhibition due to soccer heading. Since the soccer heading study in question did not employ a control condition or group, it is not possible to determine whether the increased silent period was truly an effect of RSHI exposure. Therefore, in light of the contrast between the findings reported by McNabb et al. (2020) and Di Virgilio (2016, 2019) and the observations we made during the silent period data extraction, the effects previously observed in the laboratory at University of Stirling are questionable due to the methodological limitations.

It is likely that the issue with the previous and current findings is due to the low number of silent period trials used to test the effects of RSHI. Research comparing the effect of number of trials averaged (10, 20, 30, 40 and 50) on silent period duration, reported that the duration elongated with the number of trials added, with the difference in the mean duration being almost seven milliseconds between 10 and 50 trials (Garvey et al., 2001). Although the latter findings were not statistically significant, this fluctuation is bigger than the effects reported in the research done at our laboratory. Around 20 to 30 trials are recommended for an accurate estimation of silent period duration and motor evoked potential amplitude (Groppa et al., 2012; Hupfeld et al., 2020). While lower number of trials have also been suggested suitable for measuring silent period duration, i.e., an average of five to six stimulations (Hupfeld et al., 2020) then that is still nearly double the number of trials used in our laboratory. Moreover, it may be more appropriate to use the average of 20 or more trials to get an accurate estimation of the silent period duration when the aim is to detect subtle changes in corticomotor inhibition.

The rationale for performing only three stimulations for the assessment of silent period was to prevent muscular fatigue while recruiting a large pool of motor neurons to detect subtle changes in inhibition in the motor cortex. However, this reasoning relies on the assumption that the number of lower motor neurons recruited, responsible for maintaining the contraction, is equivalent to the number of upper motor neurons being activated, which may not necessarily be the case (Nozaki et al., 2003). At present, there is no consensus on whether the intensity of the muscle contraction affects silent period duration due to mixed findings (Hupfeld et al. 2020). Since McNabb et al. (2020) were able to detect significant changes in silent period duration during a 10% voluntary contraction it can be presumed that performing contractions at maximum intensity is not necessary to detect subtle changes in corticomotor inhibition. Furthermore, to ensure the detection of subtle changes in motor cortex inhibition it may be advantageous to test finger or hand instead of (leg) muscles that are not involved in movements that require precision. Muscles performing precise movements, such as finger muscles, have proportionally larger representation in the cortical area than muscles that perform unrefined movements such as rectus femoris, responsible for flexion and extending the knee and flexing the hip (Gleitman et al., 2011; Zewdie & Kirton, 2016). The substantially larger effect in elongation of the silent period observed following the drill of rugby tackles by McNabb et al. (2020) compared to changes observed in University of Stirling laboratory could therefore be a result of their TMS protocol where they tested a first dorsal interosseous muscle which abducts the index finger and has therefore greater corticomotoneuronal connections than rectus femoris muscle

tested in Stirling. Furthermore, due to the deep motor cortical location of the lower limbs, compared to the hands, higher TMS intensities and a coil that can target deeper cortical areas are required (Hupfeld et al., 2020); this in turn may make TMS protocols targeting lower limbs more uncomfortable for the participant, potentially limiting the number of stimulations that the participant may consider tolerable. Taken together, performing a sufficient number of trials to get an accurate estimate of the silent period duration is more important than collecting the silent period data during a maximum contraction which limits the number of trials that can be performed since fatigue is considered to affect silent period duration (Hupfeld et al. 2020). Moreover, to ensure the detection of subtle effects it is potentially beneficial to use a muscle with large neuronal representation in the motor cortex.

We found also no changes in the motor control in the lower limbs through assessing gait and motor unit firing rates. Static balance testing has not been considered a sensitive measure for detecting the effects of RSHI (Stephen et al. 2022). The current evidence suggests that dynamic gait measures are also not sensitive to the effects of RSHI. Especially considering that the temporal parameters of gait were sensitive to the trial type, where participants' gait was affected by performing a cognitive task while walking. There has been only one previous study examining the same gait parameters in relation to RSHI exposure as reported here however, that study assessed changes in gait following a season of American football participation (n = 34) rather than in an acute setting (Buckley et al., 2019). Nonetheless, the study also concluded that dynamic postural control may not be affected by a season of RSHI exposure. We also observed no changes in motor unit firing rate while Di Virgilio et al. (2019) observed changes in motor unit recruitment strategies following sparring in boxing. The different findings are potentially caused by technological limitations where the system used in the present research could not record the force of the contractions during motor unit firing measurements and as such, we were not able to assess the threshold at which early and late recruited motor units were activated, which is where Di Virgilio et al. (2019) previously found significant changes. Considering that the present study found no changes in motor control measured through assessing gait parameters and motor unit firing rates, it is possible that measures of motor control are not particularly sensitive to the effects of RSHI.

Furthermore, unlike Di Virgilio et al. (2016, 2019) the present studies found no statistically significant changes in cognitive function related to the RSHI exposure despite using the same cognitive tests. Moreover, the current studies used the high-performance version of the tests containing 12 levels instead of eight, making the tasks even more sensitive to detecting

changes in cognitive function. Participants performed the cognitive tests in the experiments described in this chapter numerous times (during the familiarisation and three times in every session). Also, completing a practice trial always before the baseline test. This could have affected the participants' motivation, leading to sub-optimal performance during baseline testing potentially masking any decrease in the performance following heading. It is, however, more likely that participants became that skilled at performing the cognitive tasks that any small detriments that would have otherwise been sensitive to the effects or RSHI were removed with the amount of practice. Moreover, considering that the CANTAB assessments are recommended for evaluating cognitive function in TBI, neurodegenerative diseases and neurological disorders where impairments may be easier to detect it is perhaps unsurprising that present findings were not able to demonstrate changes in cognitive function in relation to subconcussive impacts. Furthermore, Stephen et al. (2022) reported in a scoping review that studies finding decreased cognitive function following RSHI exposure had considerably larger sample sizes than studies that found no changes (median, n = 126 vs 72) in cognitive performance. The authors concluded that the effect size of RSHI on cognitive function may be small and thus, large sample sizes are needed to be able to detect an effect. It is therefore possible that one of the reasons the experiments described in the present chapter failed to detect statistically significant changes in the cognitive function are due to the sample size being too small. Nonetheless, it is perhaps unsurprising that we did not find changes in cognitive function since we also did not observe electrophysiological changes or decreased motor control.

It is possible that the effects of RSHI previously reported by Di Virgilio et al. (2016) were caused by differences in the soccer player characteristics, and/or differences in the heading procedure. The present cohort consisted of soccer players who had been heading on average for 12 years and alongside recreational players included athletes playing at junior, semi- and professional level (for details see Chapter 5) and as such were potentially more experienced than the sample studied by Di Virgilio et al. (2016) consisting of amateur players only. It is possible that athletes who are used to sustaining routine impacts to the head are less susceptible to the acute effects of subconcussive impacts through acquired brain adaptations. There were also differences in the heading procedure between the studies which may have affected the response to the impacts. Firstly, balls were delivered from approximately 12 meters in the present study, as recommended in the *subconcussive soccer heading model* (Bevilacqua et al. 2019), whereas the distance before was only six meters (due to the availability of testing facilities at the time) potentially not giving the ball enough flight distance to descend. It is

noteworthy that heading ascending balls is considered more dangerous (Babbs, 2001). Interestingly however, the linear acceleration reported by Di Virgilio et al. (2016) was not higher than in the present study (see Chapter 5 for impact details). The lack of considerable differences in the impact accelerations between the studies could be caused by using different equipment and the anatomical accelerometer placement. Another difference in the impact exposure between the studies was the frequency at which the headers were performed. Di Virgilio et al. (2016) had participants perform two headers per minute whereas, the current study had a minute interval between headers, using the same protocol as developed by Bevilacqua et al (2019). Even though the minute interval between headers was mostly chosen due to practical considerations, such as giving the headgear time to deform to its original shape following the compression from the impact, the effects of subconcussive impact frequency on the brain are not known and may also affect the brains' susceptibility to injury. In a game scenario the interval between headers would likely be even longer than a minute which is also an important consideration for future research. Nonetheless, the interval between headers during a heading drill practice may be more similar to the time between headers performed in this study.

4.5 Conclusion

The cognitive, vestibular and electrophysiological measures and techniques used in the experiments described in this chapter were not able to detect the effects of RSHI on the brain. As such, we could not determine whether limiting impact dose or using headgear could be utilised as preventative strategies for mitigating the acute effects of RSHI. The present findings highlight the need for sensitive measures that can reliably detect the acute effects of RSHI on the brain in order for the field of subconcussive research to advance. However, finding such measures may take time and until such methods are found research in the field of subconcussive impacts should utilise what is available even if those measures are vague.

Chapter 5: Methods for Reducing Subconcussive Impacts

5.1 Introduction

Experiments described in the previous chapter were not able to determine strategies for limiting the acute effects of subconcussive impacts on the brain. This may have been the result of the measures not being sensitive enough to detect the effects of subconcussive impacts however, it is also possible that the RSHI exposure did not affect the brain. Nonetheless, lack of sensitive measures (that are also accessible) is an issue in the field of RSHI research. As such, caution should still be warranted regards to subconcussive impacts, despite inconclusive or mixed findings in the field of RSHI research. Especially considering that there is considerable amount of evidence demonstrating acute and chronic brain health implications following RSHI exposure. It is therefore paramount that research on ways to limit the burden of subconcussive impacts on athletes' brain is continued. Without reliable and sensitive measures of brain function, alternative methods should be sought and used for conducting such research. One way of testing the impact burden is through impact monitoring by using accelerometers. This chapter was set out to test ways of limiting subconcussive impacts on athletes' that could be implemented in the real-world setting. Of particular interest was the use of headgear for limiting impacts from soccer heading since in soccer, unlike in many other contact sports, headgear is not routinely used.

Soccer players can experience a vast number of subconcussive impacts (see Chapter 1 for details). Last known report estimated that there were 265 million soccer players worldwide in 2006 (FIFA, 2007), making it the most popular sport worldwide. Moreover, with over 128 thousand professional players in the world (not taking into account female players; Ruiz-Ocaña et al., 2023), even a low percentage of players being negatively affected by the effects of heading may have a substantial effect. Despite this, the use of headgear is not encouraged or even legislated in soccer, unlike it other sports where head impacts are routine such as American football, boxing and rugby. In soccer, athletes sustain direct, intentional head impacts (as opposed to head impacts caused by whiplash often occurring in rugby and American football through tackling) when redirecting the ball's trajectory with their unprotected head. Furthermore, players may head balls travelling at 85 km per hour (Erkmen, 2009) and while most balls that can be headed travel at lower velocities (< 65 km per hour; Levendusky et al., 1988, as cited in Erkmen, 2009), impacts at such speeds can still incur in forces of 850 to 912 N to the head (30 to 55 g; Armstrong et al., 1988 and Delaney & Drummond 1999, as cited in Erkmen,

2009) which are comparable to impact accelerations occurring in contact sports like American football and ice hockey where headgear is worn (Naunheim et al., 2000). Of course, soccer players are also not protected from other types of head impacts, for example resulting from player-to-player contact (i.e., elbow to face etc.) which may occur from attempts to head the ball. Although the occurrence of unintentional head impacts is much lower in soccer than intentional heading (Lamond et al., 2018; Langdon et al., 2022), unexpected impacts also pose a greater risk of injury (Babbs, 2001). Therefore, the use of headgear in soccer should be considered to attenuate the impacts from heading and from other, unintentional head impacts.

To date, it is thought that headgear is ineffective at attenuating impacts from heading (Naunheim et al., 2003; Withnall et al., 2005) due to the relative stiffness of the objects (Naunheim et al., 2003; Niedfeldt, 2011; Withnall et al., 2005). Usually, headgear dissipates energy through deforming, subsequently reducing the impact force; however, Withnall et al. (2005) demonstrated that during heading, the ball deformed almost ten times the thickness of the headband meaning that the headgear was unable to dissipate the required energy to reduce the force of heading induced impact. Although unable to reduce impact on the head from heading a ball, the study demonstrated that soccer headgear could reduce impact force between stiff objects (e.g., head-to-head impacts). As such, the authors suggested that whilst the headgear models were not able to effectively dissipate energy during heading, they could protect players from stiff impacts (e.g., impacts between players and objects such as goal post). Nonetheless, research by Broglio et al. (2003) suggested that headgear can attenuate impacts that are representative of heading. While the aforementioned study had methodological limitations (using a force platform instead of a head form or participants), their findings suggest a further need to examine the effectiveness of headgear. Especially, new, and so far, untested models and products of headgear. As such, the study described in this chapter will investigate the effectiveness of a headgear prototype produced by Storelli Sports, USA for attenuating heading induced impacts.

Moreover, there have been concerns surrounding behavioural changes, such as heading a ball with more force or playing more aggressively, potentially caused by the perceived feelings of safety from wearing headgear (Niedfeldt, 2011). There is limited evidence about the behavioural aspect of wearing soccer headgear. In light of the potential dangers associated with behavioural change caused by wearing headgear, it is important to also consider other methods for reducing impact severity such as anthropometric and strength variables. Previous research by Caccese et al. (2018) has demonstrated that head size and neck girth as well as

sternocleidomastoid and upper trapezius strength are associated with linear and rotational accelerations, whereas that heading technique is not. A systematic review of five research papers (190 participants), examining the relationship between neck strength and head acceleration also concluded that stronger neck strength can significantly reduce head acceleration from soccer heading (Peek et al., 2020). Unlike headgear, neck strength may also help against the whiplash injury as a strong neck may help prevent excessive head movement. Moreover, researching the relationship between strength and impact attenuation is also beneficial for other contact sports where impacts occur.

The overall aims of this study were to (1) compare the impact metrics of headers completed with and without headgear, (2) investigate whether neck girth (as a proxy for neck strength), grip strength (as a proxy for upper body strength), and years of heading experience affect impact metrics and (3) assess the behavioural aspect of wearing headgear.

5.2 Methodology

5.2.1 Participant Characteristics and Ethical Approval

This chapter describes data from the same cohort as in Chapter 4 experiment two: the headgear study. The recruitment method, participant inclusion and exclusion criteria and ethical approval are the same as described in Chapter 4 (see page 105).

5.2.2 Study Design

The study implement a within-subject repeated measures design. Participants attended the laboratory for a familiarisation and two heading sessions: (1) 20 headers performed without headgear and (2) 20 headers done while wearing headgear. The headgear prototype was provided by Storelli Sports LLC (Brooklyn New York, USA; see Figure 4.1, page 106). The order of the conditions was counterbalanced and heading sessions were carried out in different weeks to limit head impact exposure to the participants.

Prior to the testing sessions participants attended the laboratory for a familiarisation session where they were screened against the inclusion and exclusion criteria and were acquainted with the testing procedures described in Chapter 4. In the familiarisation session anthropometric measures of height, mass and neck circumference were taken in accordance with the International Society for the Advancement of Kinanthropometry (ISAK) standards (Stewart et al., 2011). Further, maximum grip strength (kg) was measured in the familiarization session using Takei T.K.K.5001 Hand Grip A Dynamometer (Takei Scientific Instruments Co., Ltd.,

Tokyo, Japan). Participants were instructed to stand in an upright position, feet hip width apart, arms by the side of their body and squeeze the dynamometer as hard as they can for five seconds. Three contractions were performed with each hand, alternating hands, with a minute rest between each trial. Participants received verbal encouragement during the contractions. Maximum force from the six trials was put forward for the analysis. Self-reported information about football participation and concussion history were also gathered in the familiarization session. For participants who reported more than one playing position or level of play (e.g., identified as recreational and amateur player since they played in two different teams), the player position where more headers have been reported to occur (Langdon et al., 2022) and the highest level of level of play are reported in the results.

5.2.3 Impact Assessment Procedure

Participants performed 20 headers during both heading conditions (with and without headgear) using the same methodology and equipment as described in Chapter 4 (see pages 107–108). For the duration of heading, an impact sensor containing a triaxial accelerometer and a gyroscope (Protxx Inc., California, USA) was secured behind the participants' ear using double sided adhesive tape (Figure 5.1). The threshold for recording impacts was set at 8 g by the manufacturer. The mean of the impacts was used in the analysis. If data upload was partial due to technical error (e.g., data for 15 out of 20 impacts uploaded) then the average of the available impacts was used in the analysis. In case there were additional impact data available (i.e., a header was repeated that had resulted in an impact ≥ 8 g) then the extra impact data were included in the average. Finally, if there were missing data due to impacts being below the recording threshold of 8 g then the mean was calculated for the available data, ignoring the impacts below the sensor threshold.

After participants had completed both heading sessions, they were asked to complete a short questionnaire assessing (1) whether they felt safer heading the ball when wearing the headgear, (2) whether they think they behaved differently when wearing the headgear, (3) whether they wear headgear outside of this study, and (4) whether they would consider starting to wear football headgear if it proved to be protective.

Figure 5.1. PROTXX impact sensor.

5.2.4 Statistical Analysis

Data were assessed for outliers using boxplots and extreme outliers (≥ three times IQR) were winsorized using the method described in Chapter 4 (see page 115). The distribution of data was investigated through normality testing, visual assessment and by examining the skewness and kurtosis scores (more details in Chapter 4, page 115). Data analysis was performed only on complete set of data, listwise deletion was used to deal with missing data.

Two-tailed paired *t*-tests were carried out to compare mean peak linear acceleration (PLA) and peak rotational acceleration (PRA) between the heading conditions, where Cohen's *d* (*d*: 0.2 = small; 0.5 = medium; 0.8 = large; Cohen, 1977) was used to quantify effect sizes. Two multiple regression analyses were conducted to assess the combined effect of (1) neck circumference, (2) maximum grip strength and (3) self-reported years of heading experience on PLA and PRA.

Statistical tests were carried out using IBM SPSS (version 28.0.0.0, SPSS Inc., Chicago, USA). Statistical significance was set at $p \leq 0.05$.

5.3 Results

The results first detail participant characteristics, followed by (1) a comparison of impact metrics of headers completed with and without the headgear and (2) an analysis examining whether neck girth (as a proxy for neck strength), grip strength (as a proxy for upper body strength), and years of heading experience affect impact metrics. Finally, the results document the behavioural questionnaire data about wearing headgear.

5.3.1 Characteristics

Overview of participant characteristics are in Table 5.1 and summary of participants' level of play and playing position are displayed in Figure 5.2. Overall, the player level ranged from recreational to professional, with majority of this cohort consisting of amateur players (46%).

Table 5.1. Participant characteristics.

	Mean ± *SD* (range)
Age (yr)	24 ± 5 (18–34)
Years playing football	12 ± 4 (4–25)
Heading experience (yr)	12 ± 4 (2–25)
Height (m)	1.75 ± 0.07 (1.65–1.92)
Mass (kg)	70.8 ± 12.0 (47.3–98.7)
BMI*	23.1 ± 3.1 (16.6–30.1)
Neck circumference (cm)	36.1 ± 2.8 (30.0–40.3)
Max grip (kg)	39.8 ± 9.0 (24.0–56.5)
Handedness (*n*)	
Left	2
Right	26

* BMI = mass (kg)/height (m^2)

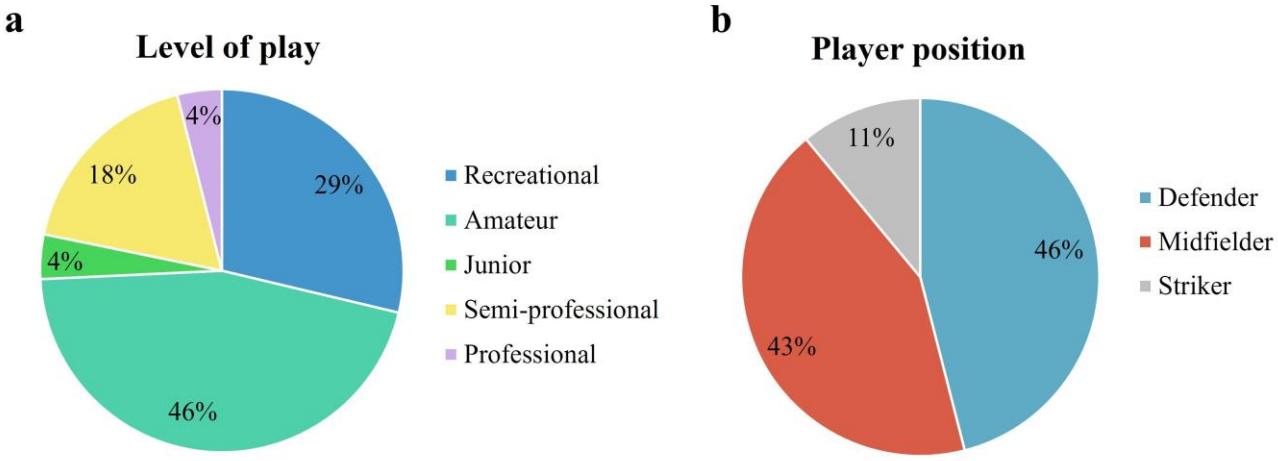


Figure 5.2. Percentage of participants based on their (a) level of play and (b) playing position.

5.3.2 Head Impact Metrics and Headgear Questionnaire

5.3.2.1 Impact Metrics

All headers, in all of the sessions landed on the headgear. One participant's data were excluded from the analysis due to impact data upload failure in one of the testing sessions. Eight (29%) participants' data were affected by partial impact data upload failure in one of the heading sessions (on average 58% of the data uploaded, range: 25–80%). Sixty-five percent of the impact data used in the analysis contained impacts < 8 g in PLA, meaning that those impacts were not detected by the sensor (67% of data in 20 headers without headgear and 63% in headgear condition contained impacts < 8 g). In eight sessions, extra impacts were recorded (four in each condition; i.e., a header was repeated that was above the accelerometer threshold of 8 g). In seven of the eight sessions, there was one additional impact recorded (21 headers instead of 20) and on one occasion there were four extra impacts in the data (20 headers without headgear condition).

There was no significant difference in the mean peak linear, $t(26) = -0.710$, $p = 0.484$, $d = -0.137$ or rotational, $t(26) = -0.493$, $p = 0.626$, $d = -0.095$ acceleration of the head between 20 headers done with and without headgear (Table 5.2).

Table 5.2. Impact data for headers completed with and without headgear (mean ± *SD*).

	20 headers with HG	**20 headers**
PLA (g)	15.5 ± 2.8	14.9 ± 2.3
PRA (krad/s^2)	1.22 ± 0.46	1.17 ± 0.41

HG – headgear; PLA – peak linear acceleration; PRA – peak rotational acceleration

Multiple regressions were performed to assess whether years of heading, neck girth and maximum grip strength predict PLA and PRA. These independent variables did not significantly predict PLA [$F(3,23) = 1.040$, $p = 0.393$, $R^2 = 0.119$, $R^2_{adjusted} = 0.005$] or PRA [$F(3,23) = 1.619$, $p = 0.212$, $R^2 = 0.174$, $R^2_{adjusted} = 0.067$]. None of the variables added significantly to the PLA prediction ($p > 0.05$); whereas self-reported years of heading experience was the only variable that added significantly to the PRA prediction ($p = 0.044$), indicating that participants with a greater number of years of heading experience sustained higher PRA.

5.3.2.2 Headgear Questionnaire

None of the participants reported wearing soccer headgear before this study. Only 25% of the participants believed that wearing headgear affected their behaviour, while heading as part of the study. Notably, the majority (71%) of participants answered that they felt safer heading the ball when they were wearing headgear (Figure 5.3 [a]). Almost a third (29%) of the participants responded that they would consider starting to wear headgear if it proved to be protective and a further 14% replied that they would consider wearing headgear but only during training. Lastly, 29% of the participants responded that they were not sure if they would wear headgear and another 29% answered that they wouldn't consider it (Figure 5.3 [b]).

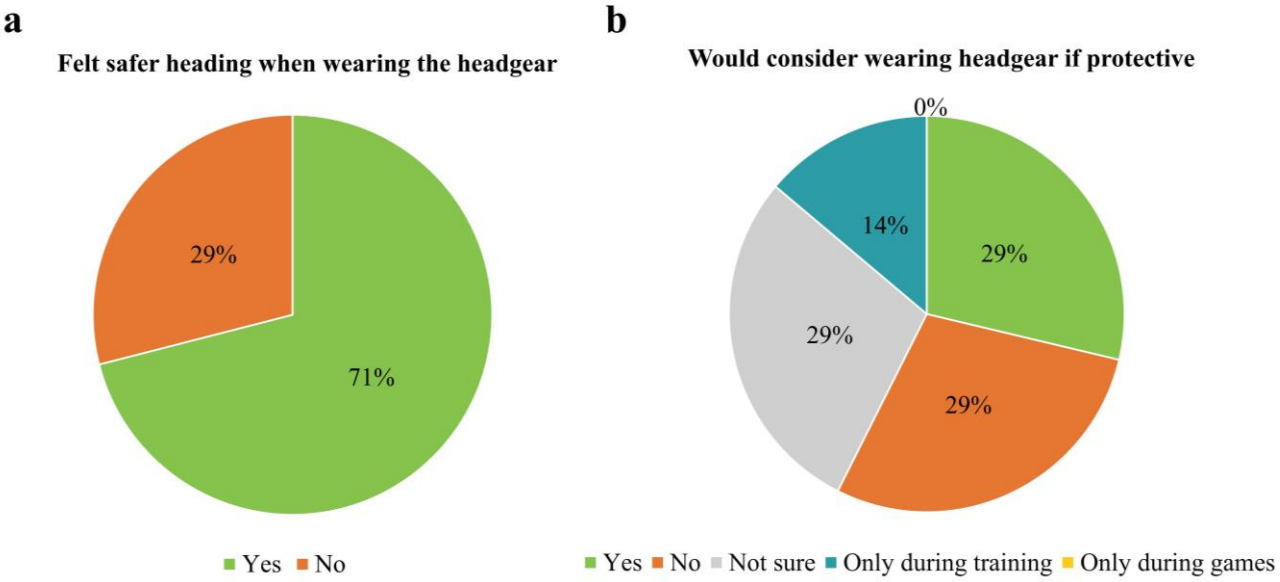


Figure 5.3. Headgear questionnaire results.

5.4 Discussion

The aims of this study were to assess the effectiveness of headgear by comparing impact metrics between headers performed with and without headgear and to assess whether strength

and heading experience affect impact accelerations. We also assessed the behavioural aspect of heading the ball while wearing headgear by employing a questionnaire to assess (1) whether participants felt safer heading the ball when wearing the headgear and (2) whether participants think they headed the ball differently while wearing the headgear.

Previous research examining various other soccer headgear models has reported that headgear does not attenuate impact metrics from soccer heading (Naunheim et al. 2003; Withnall et al. 2005). Despite no statistically significant difference in the impact metrics between headers done with and without headgear, around 70% of the participants reported feeling safer heading the ball when wearing headgear in the present study. Therefore, it is possible that participants put more force into headers performed while wearing headgear due to feeling safer, resulting in no differences in the impact metrics. Future studies should examine whether more force is put into headers when wearing headgear, for example, by comparing muscle activation during the heading movement with and without headgear and measuring the speed of the ball following the header.

If footballers put more force into headers when wearing headgear, then the potential detriments of headgear should be considered. Especially, if the 'feeling safe' state when wearing headgear is not limited to heading behaviour alone and causes athletes to play more aggressively in general, potentially increasing the risk of injuries. Nonetheless, a large field study examining contact-related injury incidence and severity during a soccer season reported no differences between soccer players who wore headgear (n = 1498) and who did not (n = 1539) (McGuine et al. 2019) suggesting that headgear does not affect playing style. Although the field study by McGuine et al. (2019) reported no differences in concussion incidence between players wearing soccer headgear or not, the use of headgear in general has demonstrated to reduce the force of strong impacts in laboratory setting (Frizzell et al. 2018; Withnall et al., 2005). Despite not attenuating impact accelerations, wearing headgear did not lead to increased impact metrics compared to the no headgear condition in this study. As such the use of headgear may potentially still be beneficial for injury prevention from player-to-player and player-to-object (e.g., ground and goalpost) impacts, even if it does not reduce the impacts of heading.

In contrast to previous findings (Gutierres et al. 2014; Caccese et al. 2018), grip strength, a proxy for strength, nor neck girth statistically predicted impact metrics. Men have bigger (Esopenko et al. 2020; Tierney et al. 2008) and stronger necks (Tierney et al. 2008) and experience lower head accelerations than women (Tierney et al. 2008) suggesting that strength

and size are aspects that affect impact metrics. Moreover, the biomechanics of heading suggests that training may enhance the tension of neck muscles leading to more effective mass behind the header subsequently decreasing the head acceleration from impact (Babbs, 2001). When the neck muscles are strong and engaged, then the neck connects the head and torso, placing the entire body mass behind bracing the force from a head impact. However, when the neck musculature is not engaged then the impact force is applied to the mass of the head alone, leading to substantially increased head acceleration. Therefore, despite the present findings, utilising strengthening exercises as a preventative strategy may be considered beneficial. Especially, since the current findings are limited by the methodology of using a proxy instead of direct measures of strength. Perhaps the only disadvantage of stronger musculature to consider is the ability to put more force into the headers which may lead to higher impacts, but this is a consideration for future research.

Interestingly, we found that years of heading experience significantly contributed to the rotational, but not linear, acceleration of the head. It is possible that this finding is caused by the heading paradigm where participants performed rotational as opposed to linear headers. Participants who were less skilled were observed to experience more difficulty in re-directing the ball perpendicular to the ball's initial trajectory, sometimes resulting in the ball being directed back towards the researcher or upwards. Previous research has shown that headers from corner kicks (i.e., rotational headers) induce higher rotational acceleration than headers from scenarios where the ball is directed in a linear fashion (Harriss et al., 2019). Therefore, it is possible that participants with less heading experience sustained lower rotational forces compared to the more skilled participants in this study as they failed to perform rotational headers using the correct technique. However, this finding may also be the result of multiple testing, and it is noteworthy that the result is based on a small sample size.

While carrying out the present research interesting observations were made during the heading procedure that future research could examine. Although the observations are anecdotal it appeared that players who were skilled at heading were able to put considerable force into headers and the opposite, perform headers that made good contact with the head and were directed perpendicularly with great accuracy, yet being less than 8 g in linear acceleration, falling below the detection limit of the sensor. The present study did not have a representative sample from different levels of play or player positions to assess differences in impact metrics between the groups. We also did not document the quality of the headers (we did not assess the accuracy of the headers or the technique). Nonetheless, future research could assess how

heading experience affects impact metrics since it is currently unclear whether experienced players are better able to control the force they put into headers and therefore can control the accelerations that they sustain.

5.5 Conclusion

In conclusion, the present study did not find support for the use of headgear or strength of the player for reducing impact metrics. The current evidence base for the protective properties of headgear in soccer is scarce and there has been a long gap in time between prior and current research in the area. Despite, the non-significant findings presented here, product development and further research have the potential to aid the development of headgear that may be able to attenuate heading and non-header impacts. Implementing the use of headgear in soccer may be easier than changing the rules of the game to remove heading, however, we also need to understand how headgear may affect the athletes' playing style. Strengthening exercises for reducing impact accelerations also requires more research. The rationale for strength being advantageous for reducing impacts is strong, yet the present study could not confirm this, potentially due to the methods that were used in our lab (i.e., using a proxy as opposed to a direct measure of strength). Strength and heading technique are both variables that can be manipulated with training and are therefore a good avenue for protecting the athletes. However, research should first determine whether better technique would encourage players to perform more headers in general (since better technique may lead to better accuracy, performance and reward) and whether increased strength would lead to athletes performing more forceful headers leading to higher accumulative impact burden. Lastly, it is important to acknowledge the limitation of research examining how to prevent or mitigate brain injury using acceleration data, considering that there is limited information about acceleration threshold that may lead to impact induced brain changes.

Chapter 6: General Discussion and Conclusions

6.1 Discussion

This thesis had the overarching aims of finding sensitive measures for detecting, and methods for ameliorating the acute effects of sport-related repetitive subconcussive head impacts on the brain. Chapters 2 and 3 explored whether brain injury markers sampled from biofluids are elevated following exposure to RSHI. First by comprehensively scoping and reviewing all evidence in the field, and then by analysing data collected in our laboratory in earlier years that had remained dormant until now. The aim was to determine whether and which biofluid markers could be utilised as effective measures for detecting the effects of subconcussive impacts, as well as gain an insight about when to sample biofluid markers in relation to subconcussive impact exposure. The results from Chapters 2 and 3 demonstrated that biofluid markers of brain injury do elevate following RSHI however, not consistently. These findings suggest that the use of biofluid markers for assessing the effect of RSHI is currently premature. As such, Chapter 4 investigated whether reducing the number of subconcussive impacts and using headgear could mitigate acute brain changes using cognitive, motor control and electrophysiological measures that had previously shown sensitivity to the effects of RSHI. However, we were unable to find answers to either of the questions since the measures were not affected by the soccer heading paradigm that we used. It is likely that the measures were not sensitive to the effects of RSHI. Nonetheless, it is also possible that the impacts did not cause acute brain changes or that the effects took longer to develop than the sampling time window. As a result, Chapter 5 assessed ways that could be used to reduce the impact metrics, as a potential avenue for reducing the cumulative impact burden on athletes. We found that headgear did not attenuate the impact metrics. Furthermore, we did not find support for the idea that (neck) strength can reduce the impact force. The findings from this thesis highlight the complexity of conducting research in the field of sport-related subconcussive impacts due to the subtle effects of RSHI and lack of reliable and sensitive measures. The following discussion covers some methodological considerations that arose from the current research and aspects that future research could consider and potentially advance from.

Firstly, we observed significant changes in biofluid markers that were not related to the baseline (in Chapter 3). Considering the subtle effects of subconcussive impacts, it is essential to consider intrapersonal semidiurnal and day-to-day variability of biological outcome measures in future research. Moreover, to have confidence in the findings, it is essential to

implement a within-subject design with a control condition. A between-subject design would only be appropriate if a considerable sample size is acquired, considering the interpersonal variability of the variable and the small effects of subconcussive impacts. NfL for example, has large between subject variability, where values in healthy individuals can range from 2.1 to 19.1 pg/mL (Hviid et al., 2022). Furthermore, biofluid marker evidence base would benefit from studies that implement serial sampling. This would provide an understanding of normal fluctuations in the biofluid marker levels and give an insight into the temporal trend of subconcussive effects.

The nonsignificant changes in the outcome variables described in the experiments in this thesis were potentially caused by the methods used. The methodological limitations of the protocol that we used for measuring corticomotor inhibition using TMS were covered in detail in Chapter 4's discussion. The primary downfall of our procedure was the limited number of trials performed to establish the duration of silent period. Nonetheless, TMS is a useful technique considering that it is cheaper and faster than most imaging methods currently available. Moreover, findings from McNabb et al. (2020) demonstrating elongated silent period duration measured from the first dorsal interosseous muscle following a drill of rugby tackles suggest that TMS may still prove useful in future RSHI research. Nonsignificant results from this thesis coupled with the significant findings from McNabb et al. (2020) suggest that future research may potentially benefit from testing silent period duration using hand or finger muscles that have large cortical representation (Gleitman et al., 2011; Zewdie & Kirton, 2016). Interestingly however, a recently published study also found no acute effects of 20 soccer headers on silent period duration despite measuring SP from first dorsal interosseous muscle (Hamel et al., 2024). Specifically, the authors reported a similar significant increase in SP duration from baseline in both, the heading and the kicking (control) group (n = 30 in both groups; p = 0.025, η^2_p = 0.084). Nonetheless, the study did report significant increase in symptoms following heading, potentially since the sample included participants who were not soccer players and therefore, were not used to heading. It is possible that one of the reasons the recently published study did not find changes in SP is because they used hand-thrown balls, resulting in lower impacts than reported by Di Virgilio et al. (2016). Hamel et al. (2024) reported 12.5 g as the average head acceleration from heading, compared to 13.1 g reported by Di Virgilio et al. (2016). Although the difference in the accelerations between the studies does not appear substantial it is noteworthy that the impacts were recorded only in a subset of participants (10 out of 30) by Hamel et al. (2024) and therefore, the true average acceleration of impacts in their sample is unknown.

Future research using lower limb muscles for TMS measures would likely benefit from recording EMG from muscles such as tibialis anterior which has similar cortical projection magnitude to finger muscles due to the muscle being involved in movements that require precision such as clearing the toes during swing motion in gait (Zewdie & Kirton, 2016). The rationale for using a leg muscle in our laboratory research was based on the observation that athletes who have suffered from a concussion may be at increased risk of lower extremity injury due to impaired motor control (Jildeh et al., 2022; McPherson et al., 2019). Therefore, if we had observed increased corticomotor inhibition measured from the lower limb muscle together with impaired vestibular function, following subconcussive head impacts then it would have suggested that routine head impacts in contact sports also place athletes at increased risk of lower limb injuries. Instead, our results suggest that vestibular control, similarly to cognitive function, is not a particularly sensitive measure when it comes to subconcussive impacts. It is possible that athletes become accustomed to the sensation of RSHI symptoms, where they are able to mask its effects on behavioural and cognitive function.

A scoping review looking at the effects of RSHI on vestibular, cognitive and oculomotor function concluded that oculomotor function was the most effective method of assessing the acute effects of RSHI with 43% of the studies included in the review reporting impaired oculomotor function compared to 21% of the studies reporting reduction in vestibular function and 33% cognitive impairment (Stephen et al. 2022). Noteworthily, reductions in oculomotor function were detected primarily by measuring near point of convergence (NPC), while using King-Devick test (KDT) and Vestibular Oculo-Motor Screening (VOMS) assessment were either less sensitive or not sensitive at all, respectively, to the effects of RSHI (Stephen et al. 2022). Therefore, future studies would potentially benefit from implementing oculomotor assessment by measuring NPC instead of assessing cognitive and vestibular function. Moreover, taking a multimodal approach by combining oculomotor and electrophysiological assessments may increase the possibility of encapsulating the effects of RSHI on the brain.

Besides the subtleness of the effects of RSHI, the difficulty in finding sensitive measures to detect brain responses to subconcussive impacts are potentially confounded by (1) the unknowns surrounding the number and magnitude of impacts that trigger brain changes, and (2) individual differences, where not everyone may be affected the same by the impacts. These confounders potentially give rise to the mixed and conflicting findings observed in the RSHI research. The following two paragraphs will address potential reasons why some people have

adverse brain response to sub-concussive impacts and others do not (i.e., factors affecting the degree of responsiveness).

We know from retrospective cohort studies that the head impacts from contact sport participation do not affect all contact sport athletes the same. While contact sport athletes have been found generally at increased risk of neurodegenerative disease compared to the general population, not all contact sport athletes go on to develop adverse brain health (Lehman et al., 2012; Mackay et al., 2019; Russell et al., 2022; Ueda et al., 2023). It is tenable that the distinction between athletes who develop neurodegenerative disorders versus athletes who do not is the cumulative impact burden. Montenigro et al. (2017) reported a dose-response relationship between cumulative head impact exposure in former football players and later-life cognitive, mood and behavioural impairment. However, the issue is possibly more complex. Data from Di Virgilio et al. (2019) and McNabb et al. (2020) demonstrated that silent period duration did not prolong for all participants acutely following exposure to the subconcussive impacts. This suggests that not everybody is affected by subconcussive impacts the same. It is possible that accumulative impact burden makes athletes more vulnerable to subsequent impacts and their effects on the brain, similarly to concussion. Evidence shows that prior concussions is a risk factor for sustaining subsequent concussions (Abrahams et al., 2014) and number of previous concussions prolongs the symptom duration in the following concussions (Hänni et al., 2020). However, a study, looking at whether cumulative (subconcussive) and singular impact magnitude (linear and rotational) predict the likelihood of sustaining a concussion, reported that the linear and rotational acceleration of a single (concussive) impact were associated with the probability of sustaining a concussion, while prior (same day to a season of) cumulative impact magnitudes were not (O'Connor et al., 2017). This suggests that cumulative impact burden (i.e., sum of accelerations) does not make the brain more susceptible for sustaining an injury, while the magnitude of the impact acceleration does; meaning that an accumulation of low acceleration events may be less dangerous than a single high acceleration impact. Similar findings have also been reported by Eckner et al. (2011) where the authors reported that the number of prior (non-concussive) head impacts, cumulative linear or rotational acceleration during the practice or game and one week prior did not differ between impacts that were concussive versus impacts that did not result in concussion (Eckner et al., 2011). Another, perhaps even a more telling finding that the authors report is that all but one of the athletes who sustained a concussion were able to tolerate impacts that were higher in magnitude at some point during the study without sustaining a concussion. This suggests that brain injury threshold is variable even

within person. In addition, a study monitoring impacts in American football, where impacts up to 200 g occurred, found that an impact of 81 g resulted in a concussion, while many impact at higher magnitudes did not (Duma et al., 2005). Therefore, any proposed injury threshold is likely elusive, as many factors such as prior concussions (Abrahams et al., 2014) and impact location (Guskiewicz & Mihalik, 2011) are likely to contribute to the risk of brain injury. Similar observations were also made by the authors of the systematic review with meta-analysis (Brennan et al., 2017) who also observed that many head impacts above the mean values associated with sustaining a concussion did not result in one.

Severity of TBI, genetics (possession of APOE ε4 allele), smoking and alcohol consumption affect and modulate the risk of developing neurodegenerative disease (Maas et al. 2017). Therefore, it is likely that a combination of impact related (history of head impacts, time since last injury), biological (genetics, sex) and lifestyle factors (e.g., smoking, alcohol consumption and diet) also affect the athlete's susceptibility to the acute and long-term effects of subconcussive impacts. For example, soccer players with APOE ε4 allele of the APOE gene (n = 81), which is a known risk factor for developing Alzheimer's disease, demonstrated worse memory function that was associated with heading in the prior year than APOE ε4-negative players (n = 271) (Hunter et al., 2020). Therefore, even with sensitive measures without knowing (1) how individual differences (including previous impact history and its' effects on the individual) and (2) the number and magnitude of impacts that increase the likelihood of brain response, the research in this field is likely to continue to yield mixed results.

Bailes and Parel (2014) suggested that the most definitive way to protect the acute and long-term brain health is by preventing or lessening any cellular and structural damage, that may add to the cumulative burden. While it may be difficult to assess the damage to the brain from subconcussive impacts due to the subtleness of its effects, we aimed to find potential ways in which to limit the cumulative impact burden. We examined limiting the number of head impacts, the use of headgear and neck strength as strategies for mitigating the effects of subconcussive forces to the head without finding any positive outcomes.

It is noteworthy that when we initially started data collection, we used a headgear prototype that only covered the forehead however, after a couple of testing sessions it became clear that headers do not always land on the forehead. Therefore, we realised that for headgear to provide effective protection from heading, it should also cover the top of the skull. As such, we restarted the testing using a prototype, not yet in production, that also covered the top of the

head. It has been found that headers landing on top of the head result in significantly higher rotational acceleration than headers performed with forehead (1215 vs 952 rad/s^2; $p = 0.0001$) (Harriss et al., 2019). Further highlighting the importance of soccer headgear also covering the top of the head. From all the headgear models that have previously been tested for heading only one (*Kangaroo Soccer Headgear*; Withnall et al., 2005) has covered the top of the head (Broglio et al. 2003; Elbin et al., 2015; Naunheim et al. 2003; Tierney et al., 2008; Withnall et al., 2005). The importance of headgear also covering the top of the head has gone either unnoticed or unaddressed potentially due to previous research utilising a force platform (Broglio et al. 2003) or a head form (Naunheim et al. 2003) instead of human participants and by not assessing headgear in the real-world scenarios. We tested the headgear while participants performed rotational headers, mimicking the corner kick in a soccer game scenario. Whereas most studies using a soccer heading paradigm ask participants to complete linear headers which are more likely to land on the forehead as opposed to headers from the corner kick. Noteworthily, there are various soccer headgear models available for purchase, including models that cover the top and the front of the head however, the efficacy of these headgears for preventing the effects of soccer heading has not been studied. Moreover, there are still several aspects of soccer headgear that need addressing. For example, how the use of headgear affects the playability of the ball and what the stance of athletes towards the introduction of routine headgear wear in soccer is. Taken together this highlights the importance of testing headgear with human participants in both controlled environments and in real-world settings to also observe their utility.

The criticism towards headgear is that it may have limited ability to protect the brain inside the skull from sloshing against the cranium as it can only externally cushion the head (Myer et al., 2016; Yuan et al., 2021). An alternative protective method that addresses this issue is jugular vein compression collar. It works by increasing the blood volume in the brain's venous structures, subsequently increasing the brain's stiffness and reducing the space between the brain and the cranium which mitigates excessive sloshing of the brain against the skull (Myer et al., 2016). Routine wearing of jugular vein compression collar has been investigated by a group of researchers in America for preventing concussive (Yuan et al., 2021) and subconcussive (Myer et al., 2016, 2019) impact related brain changes in contact sport athletes with some promising initial results. Reduced microstructural brain changes in white matter integrity, quantified using DTI, were observed following a season of American football in players who wore the collar ($n = 32$) in comparison to athletes who did not ($n = 30$) (Myer et al., 2016). Similarly, a study assessing post-season DTI changes in female soccer players found that

significant white matter alterations only occurred in the athletes who did not wear the collar (*n* = 22 and 24, collar and non-collar, respectively) (Myer et al., 2019). Brain alterations (white matter microstructural integrity and network organisation) have also been observed significantly reduced in athletes who wore the collar (*n* = 20) , compared to the athletes who did not (*n* = 20), when sustaining a concussion (Yuan et al., 2021). Therefore, jugular vein compression collar has demonstrated promising effects for preventing concussive and subconcussive impact related brain alterations and may be a more protective strategy than traditional helmets and headgear. Future research could examine whether a combination of headgear and jugular vein compression collar provides better overall performance compared to either of the wearables in isolation.

There are also other lines of enquiry in research looking at ways to prevent or attenuate the negative effects of RSHI exposure. There has been research looking at the use of nutritional supplements for preventing injury and aiding recovery from concussion (Ashbaugh & McGrew, 2016; Bailes & Patel, 2014). Research has looked at supplements such as omega-3 fatty acids (particularly docosahexaenoic acid [DHA]), curcumin, resveratrol, melatonin and creatine (Ashbaugh & McGrew, 2016; Bailes & Patel, 2014). Although there is some evidence to suggest that supplements may help prevent damage from concussive impacts and aid recovery, research in this field is preliminary and majority of the findings are based on the rodent model (Ashbaugh & McGrew, 2016; Bailes & Patel, 2014). Nonetheless, research investigating the potential benefits of supplements for preventing acute brain changes from head impacts is promising and merits attention. Moreover, supplementing with omega 3 fatty acids have also been examined in the field of subconcussive head impacts with promising first results. A study comparing increases in serum NfL throughout an American football season, demonstrated that athletes (*n* = 31) receiving omega-3 polyunsaturated fatty acids supplementation (mixture of ecosapentaneoic acid, docosapentaenoic acid and DHA) generally experienced significantly lower increases in NfL levels compared to the athletes (*n* = 35) who did not supplement (Heileson et al., 2021). Therefore, nutritional supplementation may protect the brain from the effects of RSHI exposure and possibly accelerate recovery. Nonetheless, potential benefits of prophylactic supplementation should be considered among potential negative side effects such as digestive issues.

Moreover, with any preventative interventions and protective measures, there is a concern about athletes potentially gaining a (false) sense of security towards exposure to head impacts which could lead to more aggressive play and increased injury risk. Such behaviour

would counteract any possible benefits of the intervetion and measures and as such the risk-benefit ratio of any measure should be assessed via behavioural research. Moreover, any preventative measures should be accompanied by raised awareness, especially considering that athletes are known to sacrifice their health in pursuit of athletic goals (Chen et al., 2019).

Lastly, it is important to highlight that future research should study the effects of sub-concussive impacts in females and young athletes. The results from systematic (Mainwaring et al., 2018; Walter et al., 2022) and scoping (Lember et al., 2024; Stephen et al., 2022) reviews in the field of subconcussive research have brought attention to female and youth-aged athletes being understudied. It is currently unclear what effects subconcussive impacts have on their brain health. Interestingly, Stephen et al. (2022) reported that studies including women participants were more likely to observe cognitive decline as a result of RSHI. Females tend to have smaller necks and lower muscle mass (Tierney et al., 2008) as well as smaller axons with fewer microtubules (Dollé et al., 2018), which may make them more susceptible to injury, similarly to youth athletes. Moreover, developing brains may be more vulnerable to injury and therefore have long-lasting implications. This highlights the importance of future research considering these understudied populations and incorporating them into future studies.

6.2 Conclusions

Participation in sports has many psychological and physical health benefits however, it also carries the risk of acute and chronic injury, where contact sport athletes are of particular concern due to the vast number of head impacts that they sustain. Moreover, evidence suggests that there is a dose-response relationship between impact exposure and adverse brain health in former contact sport athletes which highlights the importance of removing or mitigating the exposure to head impacts in contact sports. Currently it is unclear what the threshold for triggering acute brain changes, that likely contribute to the long-term effects, from subconcussive impacts is. Moreover, based on concussion research this threshold is likely to be elusive and may be affected by both the number and magnitude (i.e., force) of the impacts as well as the frequency and other biological and lifestyle factors. Future research is needed to find feasible methods for reducing the forces of routine sport-related head impacts on the brain that would be accepted by the athletes and the governing bodies. Lastly, athletes' awareness should also be raised regarding the potential negative consequences of routine exposure to head impacts.

References

Abrahams, S., McFie, S., Patricios, J., Posthumus, M., & September, A. V. (2014). Risk factors for sports concussion: An evidence-based systematic review. *British Journal of Sports Medicine*, *48*(2), 91–97. https://doi.org/10.1136/bjsports-2013-092734

Akkurt, S., Tanriverdi, F., Kalay, N., Karaca, Z. C. O., Unluhizarci, K., Sucan, S., Karakus, M., & Kelestimur, H. F. (2020). Investigation of pituitary dysfunction in retired professional soccer players. *Revista Brasileira de Medicina Do Esporte*, *26*(6), 503–507. https://doi.org/10.1590/1517-869220202606215617

Alosco, M. L., & Stern, R. A. (2019). The long-term consequences of repetitive head impacts: Chronic traumatic encephalopathy. *Handbook of Clinical Neurology*, *167*, 337–355. https://doi.org/10.1016/B978-0-12-804766-8.00018-2

Alosco, M. L., Tripodis, Y., Fritts, N. G., Heslegrave, A., Baugh, C. M., Conneely, S., Mariani, M., Martin, B. M., Frank, S., Mez, J., Stein, T. D., Cantu, R. C., McKee, A. C., Shaw, L. M., Trojanowski, J. Q., Blennow, K., Zetterberg, H., & Stern, R. A. (2018). Cerebrospinal fluid tau, Aβ and sTREM2 in Former National Football League Players: Modeling the relationship between repetitive head impacts, microglial activation, and neurodegeneration. *Alzheimer's and Dementia*, *14*(9), 1159–1170. https://doi.org/10.1016/j.jalz.2018.05.004

Alosco, M. L., Tripodis, Y., Jarnagin, J., Baugh, C. M., Martin, B., Chaisson, C. E., Estochen, N., Song, L., Cantu, R. C., Jeromin, A., & Stern, R. A. (2017). Repetitive head impact exposure and later-life plasma total tau in former National Football League players. *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring*, *7*, 33–40. https://doi.org/10.1016/j.dadm.2016.11.003

Antonio, J., Cabrera, D., Knafo, S., Thomas, J., Peacock, C., & Tartar, J. (2021). Neurofilament Light (NFL) in Division II Female Soccer Players: A Potential Biomarker for Brain Trauma. *Journal of Exercise Physiology Online*, *24*(1), 1–6.

Arslan, F., Büyükyazi, G., Ulman, C., Taneli, F., Gözlükaya, F., & Çalkan, M. (2010). Examining acute changes in some serum biochemical markers of brain tissue damage after free and Greco-Roman style wrestling. *Turkish Journal of Biochemistry*, *35*(4), 307–312.

Ashbaugh, A., & McGrew, C. (2016). The role of nutritional supplements in sports concussion

treatment. *Current Sports Medicine Reports*, *15*(1), 16–19. https://doi.org/10.1249/JSR.0000000000000219

Asken, B. M., Bauer, R. M., Dekosky, S. T., Houck, Z. M., Moreno, C. C., Jaffee, M. S., Dubose, D. N., Boone, J. K., Weber, A. G., & Clugston, J. R. (2018a). Concussion basics II Baseline serum biomarkers, head impact exposure, and clinical measures. *Neurology*, *91*(23), E2123–E2132. https://doi.org/10.1212/WNL.0000000000006616

Asken, B. M., Bauer, R. M., DeKosky, S. T., Houck, Z. M., Moreno, C. C., Jaffee, M. S., Weber, A. G., & Clugston, J. R. (2018). Concussion Biomarkers Assessed in Collegiate Student-Athletes (BASICS) I. *Neurology*, *91*(23), E2109–E2122. https://doi.org/10.1212/WNL.0000000000006613

Asken, B. M., Tanner, J. A., Vandevrede, L., Mantyh, W. G., Casaletto, K. B., Staffaroni, A. M., La Joie, R., Iaccarino, L., Soleimani-Meigooni, D., Rojas, J. C., Gardner, R. C., Miller, B. L., Grinberg, L. T., Boxer, A. L., Kramer, J. H., & Rabinovici, G. D. (2022). Plasma P-tau181 and P-tau217 in Patients with Traumatic Encephalopathy Syndrome with and Without Evidence of Alzheimer Disease Pathology. *Neurology*, *99*(6). https://doi.org/10.1212/WNL.0000000000200678

Austin, K., Lee, B. J., Flood, T. R., Toombs, J., Borisova, M., Lauder, M., Heslegrave, A., Zetterberg, H., & Smith, N. a. (2021). Serum neurofilament light concentration does not increase following exposure to low velocity football heading. *Science & Medicine in Football*, *5*(3), 188–194. https://doi.org/10.1080/24733938.2020.1853210

Azar, S., Hasan, A., Younes, R., Najdi, F., Baki, L., Ghazale, H., Kobeissy, F. H., Zibara, K., & Mondello, S. (2017). Biofluid proteomics and biomarkers in traumatic brain injury. In *Methods in Molecular Biology, 1598,* 45–63. https://doi.org/10.1007/978-1-4939-6952-4_3

Babbs, C. F. (2001). Biomechanics of heading a soccer ball: implications for player safety. *TheScientificWorldJournal*, *1*. https://doi.org/10.1100/tsw.2001.56

Bailes, J. E., & Patel, V. (2014). The potential for DHA to mitigate mild traumatic brain injury. *Military Medicine*, *179*(11), 112–116. https://doi.org/10.7205/MILMED-D-14-00139

Balshaw, T. G., Pahar, M., Chesham, R., Macgregor, L. J., & Hunter, A. M. (2017). Reduced firing rates of high threshold motor units in response to eccentric overload. *Physiological*

Reports, *5*(2), 1–12. https://doi.org/10.14814/phy2.13111

Bamaç, B., Tamer, G. S., Colak, T., Colak, E., Seyrek, E., Duman, C., Colak, S., & Özbek, A. (2011). Effects of repeatedly heading a soccer ball on serum levels of two neurotrophic factors of brain tissue, BDNF and NGF, in professional soccer players. *Biology of Sport*, *28*(3), 177–181. https://doi.org/10.5604/959284

Battista, A. P. D., Rhind, S. G., Richards, D., Churchill, N., Baker, A. J., & Hutchison, M. G. (2016). Altered blood biomarker profiles in athletes with a history of repetitive head impacts. *PLoS ONE*, *11*(7). https://doi.org/10.1371/journal.pone.0159929

Bazarian, J. J., Abar, B., Merchant-Borna, K., Pham, D. L., Rozen, E., Mannix, R., Kawata, K., Chou, Y., Stephen, S., & Gill, J. M. (2022). Effects of Physical Exertion on Early Changes in Blood-Based Brain Biomarkers: Implications for the Acute Point of Care Diagnosis of Concussion. *Journal of Neurotrauma*. https://doi.org/10.1089/neu.2022.0267

Benussi, A., Karikari, T. K., Ashton, N., Gazzina, S., Premi, E., Benussi, L., Ghidoni, R., Rodriguez, J. L., Emeršič, A., Simrén, J., Binetti, G., Fostinelli, S., Giunta, M., Gasparotti, R., Zetterberg, H., Blennow, K., & Borroni, B. (2020). Diagnostic and prognostic value of serum NfL and p-Tau 181 in frontotemporal lobar degeneration. *Journal of Neurology, Neurosurgery and Psychiatry*, *91*(9). https://doi.org/10.1136/jnnp-2020-323487

Bernick, C., Zetterberg, H., Shan, G., Banks, S., & Blennow, K. (2018). Longitudinal Performance of Plasma Neurofilament Light and Tau in Professional Fighters: The Professional Fighters Brain Health Study. *Journal of Neurotrauma*, *35*(20), 2351–2356. https://doi.org/10.1089/neu.2017.5553

Bouvier, D., Duret, T., Abbot, M., Stiernon, T., Pereira, B., Coste, A., Chazal, J., & Sapin, V. (2017). Utility of S100B Serum Level for the Determination of Concussion in Male Rugby Players. *Sports Medicine*, *47*(4), 781–789. https://doi.org/10.1007/s40279-016-0579-9

Brayne, C. E. G., Dow, L., Calloway, S. P., & Thompson, R. J. (1982). Bood Creatine Kinase Isoenzyme BB In Boxers. *Lancet*, 1308–1309.

Brennan, J. H., Mitra, B., Synnot, A., McKenzie, J., Willmott, C., McIntosh, A. S., Maller, J. J., & Rosenfeld, J. V. (2017). Accelerometers for the Assessment of Concussion in Male Athletes: A Systematic Review and Meta-Analysis. *Sports Medicine*, *47*(3), 469–478.

https://doi.org/10.1007/s40279-016-0582-1

Broglio, S. P., Guskiewicz, K. M., Sell, T. C., & Lephart, S. M. (2004). No acute charges in postural control after soccer heading. *British Journal of Sports Medicine*, *38*(5), 561–567. https://doi.org/10.1136/bjsm.2003.004887

Buckley, T. A., Oldham, J. R., Watson, D. J., Murray, N. G., Munkasy, B. A., & Evans, K. M. (2019). Repetitive Head Impacts in Football Do Not Impair Dynamic Postural Control. *Medicine and Science in Sports and Exercise*, *51*(1). https://doi.org/10.1249/MSS.0000000000001761

Caccese, J. B., Buckley, T. A., Tierney, R. T., Arbogast, K. B., Rose, W. C., Glutting, J. J., & Kaminski, T. W. (2018). Head and neck size and neck strength predict linear and rotational acceleration during purposeful soccer heading. *Sports Biomechanics*, *17*(4), 462–476. https://doi.org/10.1080/14763141.2017.1360385

Cente, M., Perackova, J., Peracek, P., Majdan, M., Toth, I., Mikulic, M., Hanes, J., Porubska, S., Spajdel, M., Kazickova, B., Jurisica, I., & Filipcik, P. (2023). Association of Nonconcussive Repetitive Head Impacts and Intense Physical Activity With Levels of Phosphorylated Tau181and Total Tau in Plasma of Young Elite Soccer Players. *JAMA Network Open*, *6*(3). https://doi.org/10.1001/jamanetworkopen.2023.6101

Champagne, A. A., Coverdale, N. S., Germuska, M., Bhogal, A. A., & Cook, D. J. (2020). Changes in volumetric and metabolic parameters relate to differences in exposure to sub-concussive head impacts. *Journal of Cerebral Blood Flow and Metabolism*, *40*(7). https://doi.org/10.1177/0271678X19862861

Chen, Y., Buggy, C., & Kelly, S. (2019). Winning at all costs: a review of risk-taking behaviour and sporting injury from an occupational safety and health perspective. *Sports Medicine - Open*, *5*(1). https://doi.org/10.1186/s40798-019-0189-9

Chiò, A., Benzi, G., Dossena, M., Mutani, R., & Mora, G. (2005). Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. *Brain*, *128*(3), 472–476. https://doi.org/10.1093/brain/awh373

Chrisman, S. P. D., Donald, C. L. M., Friedman, S., Andre, J., Rowhani-Rahbar, A., Drescher, S., Stein, E., Holm, M., Evans, N., Poliakov, A. V., Ching, R. P., Schwien, C. C., Vavilala, M. S., & Rivara, F. P. (2016). Head Impact Exposure during a Weekend Youth Soccer

Tournament. *Journal of Child Neurology*, *31*(8), 971–978. https://doi.org/10.1177/0883073816634857

Christensen, S. H., Hviid, C. V. B., Madsen, A. T., Parkner, T., & Winther-Larsen, A. (2022). Short-term biological variation of serum glial fibrillary acidic protein. *Clinical Chemistry and Laboratory Medicine*, *60*(11). https://doi.org/10.1515/cclm-2022-0480

Cicchetti, D. V. (1994). Guidelines, Criteria, and Rules of Thumb for Evaluating Normed and Standardized Assessment Instruments in Psychology. *Psychological Assessment*, *6*(4). https://doi.org/10.1037/1040-3590.6.4.284

Cohen, J. (1977). *Statistical power analysis for the behavioral sciences* (Revised ed). Academic Press.

Collie, A., Maruff, P., Darby, D. G., & McStephen, M. (2003). The effects of practice on the cognitive test performance of neurologically normal individuals assessed at brief test-retest intervals. *Journal of the International Neuropsychological Society, 9*(3), 419–428. https://doi.org/10.1017/S1355617703930074

Comper, P., Hutchison, M., Magrys, S., Mainwaring, L., & Richards, D. (2010). Evaluating the methodological quality of sports neuropsychology concussion research: A systematic review. *Brain Injury*, *24*(11), 1257–1271. https://doi.org/10.3109/02699052.2010.506854

Czeiter, E., Amrein, K., Gravesteijn, B. Y., Lecky, F., Menon, D. K., Mondello, S., Newcombe, V. F. J., Richter, S., Steyerberg, E. W., Vyvere, T. Vande, Verheyden, J., Xu, H., Yang, Z., Maas, A. I. R., Wang, K. K. W., & Büki, A. (2020). Blood biomarkers on admission in acute traumatic brain injury: Relations to severity, CT findings and care path in the CENTER-TBI study. *EBioMedicine*, *56*, 1–11. https://doi.org/10.1016/j.ebiom.2020.102785

Dementia: applying All Our Health - GOV.UK. (n.d.). Retrieved March 10, 2025, from https://www.gov.uk/government/publications/dementia-applying-all-our-health/dementia-applying-all-our-health

Di Virgilio, T. G., Hunter, A., Wilson, L., Stewart, W., Goodall, S., Howatson, G., Donaldson, D. I., & Ietswaart, M. (2016). Evidence for Acute Electrophysiological and Cognitive Changes Following Routine Soccer Heading. *EBioMedicine*, *13*, 66–71. https://doi.org/10.1016/j.ebiom.2016.10.029

Di Virgilio, T. G., Ietswaart, M., Wilson, L., Donaldson, D. I., & Hunter, A. M. (2019). Understanding the Consequences of Repetitive Subconcussive Head Impacts in Sport: Brain Changes and Dampened Motor Control Are Seen After Boxing Practice. *Frontiers in Human Neuroscience*, *13*. https://doi.org/10.3389/fnhum.2019.00294

Dollé, J. P., Jaye, A., Anderson, S. A., Ahmadzadeh, H., Shenoy, V. B., & Smith, D. H. (2018). Newfound sex differences in axonal structure underlie differential outcomes from in vitro traumatic axonal injury. *Experimental Neurology*, *300*. https://doi.org/10.1016/j.expneurol.2017.11.001

Dorminy, M., Hoogeveen, A., Tierney, R. T., Higgins, M., McDevitt, J. K., & Kretzschmar, J. (2015). Effect of soccer heading ball speed on S100B, sideline concussion assessments and head impact kinematics. *Brain Injury*, *29*(10), 1158–1164. https://doi.org/10.3109/02699052.2015.1035324

Duma, S. M., Manoogian, S. J., Bussone, W. R., Brolinson, P. G., Goforth, M. W., Donnenwerth, J. J., Greenwald, R. M., Chu, J. J., & Crisco, J. J. (2005). Analysis of real-time head accelerations in collegiate football players. *Clinical Journal of Sport Medicine*, *15*(1), 3–8. https://doi.org/10.1097/00042752-200501000-00002

Echemendia, R. J., Brett, B. L., Broglio, S., Davis, G. A., Giza, C. C., Guskiewicz, K. M., Harmon, K. G., Herring, S., Howell, D. R., Master, C. L., Valovich McLeod, T. C., McCrea, M., Naidu, D., Patricios, J., Putukian, M., Walton, S. R., Schneider, K. J., Burma, J. S., & Bruce, J. M. (2023). Introducing the Sport Concussion Assessment Tool 6 (SCAT6). *British Journal of Sports Medicine, 57*(11). https://doi.org/10.1136/bjsports-2023-106849

Eckner, J. T., Sabin, M., Kutcher, J. S., & Broglio, S. P. (2011). No evidence for a cumulative impact effect on concussion injury threshold. *Journal of Neurotrauma*, *28*(10), 2079–2090. https://doi.org/10.1089/neu.2011.1910

Elbin, R. J., Beatty, A., Covassin, T., Schatz, P., Hydeman, A., & Kontos, A. P. (2015). A preliminary examination of neurocognitive performance and symptoms following a bout of soccer heading in athletes wearing protective soccer headbands. *Research in Sports Medicine*, *23*(2). https://doi.org/10.1080/15438627.2015.1005293

Erkmen, N. (2009). Evaluating the heading in professional soccer players by playing positions. *Journal of Strength and Conditioning Research*, *23*(6).

https://doi.org/10.1519/JSC.0b013e3181b42633

FDA authorizes marketing of first blood test to aid in the evaluation of concussion in adults | FDA. (n.d.). Retrieved September 13, 2024, from https://www.fda.gov/news-events/press-announcements/fda-authorizes-marketing-first-blood-test-aid-evaluation-concussion-adults

Field, A. P. (2013). *Discovering statistics using IBM SPSS statistics and sex and drugs and rock "n" roll* (4th ed.). Sage.

FIFA. (2007). Big Count 2006. *FIFA Communications Division, Information Services*, *31*, 1–12.

Fino, P. C., Parrington, L., Pitt, W., Martini, D. N., Chesnutt, J. C., Chou, L. S., & King, L. A. (2018). Detecting gait abnormalities after concussion or mild traumatic brain injury: A systematic review of single-task, dual-task, and complex gait. *Gait and Posture*, *62*, 157–166. https://doi.org/10.1016/j.gaitpost.2018.03.021

Förstl, H., Haass, C., Hemmer, B., Meyer, B., & Halle, M. (2010). Boxing-acute complications and late sequelae: from concussion to dementia. *Deutsches Arzteblatt International*, *107*(47), 835–839. https://doi.org/10.3238/arztebl.2010.0835

Garvey, M. A., Ziemann, U., Becker, D. A., Barker, C. A., & Bartko, J. J. (2001). New graphical method to measure silent periods evoked by transcranial magnetic stimulation. *Clinical Neurophysiology*, *112*(8). https://doi.org/10.1016/S1388-2457(01)00581-8

Gleitman, H., Gross, J. J., & Reisberg, D. (2011). *Psychology* (8th ed.). W. W. Norton & Company.

Graham, M. R., Myers, T., Evans, P., Davies, B., Cooper, S. M., Bhattacharya, K., Grace, F. M., & Baker, J. S. (2011). Direct hits to the head during amateur boxing is associated with a rise in serum biomarkers for brain injury. *International Journal of Immunopathology and Pharmacology*, *24*(1), 119–125. https://doi.org/10.1177/039463201102400114

Graham, M. R., Pates, J., Davies, B., Cooper, S. M., Bhattacharya, K., Evans, P. J., & Baker, J. S. (2015). Should an increase in cerebral neurochemicals following head kicks in full contact karate influence return to play? *International Journal of Immunopathology and Pharmacology*, *28*(4), 539–546. https://doi.org/10.1177/0394632015577045

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., Kaelin-Lang, A.,

Mima, T., Rossi, S., Thickbroom, G. W., Rossini, P. M., Ziemann, U., Valls-Solé, J., & Siebner, H. R. (2012). A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clinical Neurophysiology, 123*(5). https://doi.org/10.1016/j.clinph.2012.01.010

Guerriero, R. M., Giza, C. C., & Rotenberg, A. (2015). Glutamate and GABA Imbalance Following Traumatic Brain Injury. *Current Neurology and Neuroscience Reports, 15*(5). https://doi.org/10.1007/s11910-015-0545-1

Guskiewicz, K. M., & Mihalik, J. P. (2011). Biomechanics of sport concussion: Quest for the elusive injury threshold. *Exercise and Sport Sciences Reviews*, *39*(1), 4–11. https://doi.org/10.1097/JES.0b013e318201f53e

Gutierrez, G. M., Conte, C., & Lightbourne, K. (2014). The relationship between impact force, neck strength, and neurocognitive performance in soccer heading in adolescent females. *Pediatric Exercise Science*, *26*(1), 33–40. https://doi.org/10.1123/pes.2013-0102

Gysland, S. M., Mihalik, J. P., Register-Mihalik, J. K., Trulock, S. C., Shields, E. W., & Guskiewicz, K. M. (2012). The relationship between subconcussive impacts and concussion history on clinical measures of neurologic function in collegiate football players. *Annals of Biomedical Engineering*, *40*(1), 14–22. https://doi.org/10.1007/s10439-011-0421-3

Halford, J., Shen, S., Itamura, K., Levine, J., Chong, A. C., Czerwieniec, G., Glenn, T. C., Hovda, D. A., Vespa, P., Bullock, R., Dietrich, W. D., Mondello, S., Loo, J. A., & Wanner, I. B. (2017). New astroglial injury-defined biomarkers for neurotrauma assessment. *Journal of Cerebral Blood Flow and Metabolism*, *37*(10). https://doi.org/10.1177/0271678X17724681

Hamel, R., Waltzing, B. M., Massey, T., Blenkinsop, J., McConnell, L., Osborne, K., Sesay, K., Stoneman, F., Carter, A., Maaroufi, H., & Jenkinson, N. (2024). Sub-concussive head impacts from heading footballs do not acutely alter brain excitability as compared to a control group. *PLoS ONE*, *19*, 1–18. https://doi.org/10.1371/journal.pone.0306560

Hänni, S., Vedung, F., Tegner, Y., & Marklund, N. (2020). *Soccer-Related Concussions Among Swedish Elite Soccer Players: A Descriptive Study of 1,030 Players Study Design and Participants*. *11*(September), 1–8. https://doi.org/10.3389/fneur.2020.510800

Haran, F. J., Tierney, R. T., Wright, W. G., Keshner, E., & Silter, M. (2013). Acute changes in postural control after soccer heading. *International Journal of Sports Medicine*, *34*(4), 350–354. https://doi.org/10.1055/s-0032-1304647

Harriss, A., Johnson, A. M., Walton, D. M., & Dickey, J. P. (2019). Head impact magnitudes that occur from purposeful soccer heading depend on the game scenario and head impact location. *Musculoskeletal Science and Practice*, *40*(January), 53–57. https://doi.org/10.1016/j.msksp.2019.01.009

Hasselblatt, M., Mooren, F. C., Von Ahsen, N., Keyvani, K., Fromme, A., Schwarze-Eicker, K., Senner, V., & Paulus, W. (2004). Serum S100β increases in marathon runners reflect extracranial release rather than glial damage. *Neurology*, *62*(9). https://doi.org/10.1212/01.WNL.0000123092.97047.B1

Heileson, J. L., Anzalone, A. J., Carbuhn, A. F., Askow, A. T., Stone, J. D., Turner, S. M., Hillyer, L. M., Ma, D. W. L., Luedke, J. A., Jagim, A. R., & Oliver, J. M. (2021). The effect of omega-3 fatty acids on a biomarker of head trauma in NCAA football athletes: a multi-site, non-randomized study. *Journal of the International Society of Sports Nutrition*, *18*(1), 1–13. https://doi.org/10.1186/s12970-021-00461-1

Helmrich, I. R. A. R., Czeiter, E., Amrein, K., Büki, A., Lingsma, H. F., Menon, D. K., Mondello, S., Steyerberg, E. W., von Steinbüchel, N., Wang, K. K. W., Wilson, L., Xu, H., Yang, Z., van Klaveren, D., & Maas, A. I. R. (2022). Incremental prognostic value of acute serum biomarkers for functional outcome after traumatic brain injury (CENTER-TBI): an observational cohort study. *The Lancet Neurology*, *21*(9), 792–802. https://doi.org/10.1016/S1474-4422(22)00218-6

Henry, L. C., Tremblay, S., Boulanger, Y., Ellemberg, D., & Lassonde, M. (2010). Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. *Journal of Neurotrauma*, *27*(1). https://doi.org/10.1089/neu.2009.0962

Hicks, S. D., Onks, C., Kim, R. Y., Zhen, K. J., Loeffert, J., Loeffert, A. C., Olympia, R. P., Fedorchak, G., DeVita, S., Gagnon, Z., McLoughlin, C., Madeira, M. M., Zuckerman, S. L., Lee, T., Heller, M., Monteith, C., Campbell, T. R., Neville, C., Fengler, E., & Dretsch, M. N. (2021). Refinement of saliva microRNA biomarkers for sports-related concussion. *Journal of Sport and Health Science, 12*(3), 369–378. https://doi.org/10.1016/j.jshs.2021.08.003

Hirsch, G. V, Bauer, C. M., & Merabet, L. B. (2015). Using structural and functional brain imaging to uncover how the brain adapts to blindness. *Journal of Psychiatry and Brain Functions*, *2*(1). https://doi.org/10.7243/2055-3447-2-7

Hoffman, J. R., Ostfeld, I., Zamir, A., Amedi, R., Fonville, T. R., Horstemeyer, M. F., & Gepner, Y. (2022). Examination of Cognitive Function, Neurotrophin Concentrations, and both Brain and Systemic Inflammatory Markers Following a Simulated Game of American Football. *Journal of Strength & Conditioning Research (Lippincott Williams & Wilkins)*, *36*(3), 686–694. https://doi.org/10.1519/JSC.0000000000004218

Horner, E. B., Lee, T. C., Tipton, K. F., O'Brien, M., & Phillips, J. P. (1993). Creatine kinase and neuron-specific enolase: Serum markers of cell damage in the central nervous system in boxers. *Clinical Journal of Sport Medicine*, *3*(3), 144–148. https://doi.org/10.1097/00042752-199307000-00002

Huibregtse, M. E., Ejima, K., Chen, Z., Kalbfell, R. M., Koppineni, A., & Kawata, K. (2020a). Acute Time-Course Changes in CCL11, CCL2, and IL-10 Levels after Controlled Subconcussive Head Impacts: A Pilot Randomized Clinical Trial. *Journal of Head Trauma Rehabilitation*, *35*(5), 308–316. https://doi.org/10.1097/HTR.0000000000000597

Huibregtse, M. E., Nowak, M. K., Kim, J. E., Kalbfell, R. M., Koppineni, A., Ejima, K., & Kawata, K. (2020b). Does acute soccer heading cause an increase in plasma S100B? A randomized controlled trial. *PLOS ONE*, *15*(10). https://doi.org/10.1371/journal.pone.0239507

Hunter, L. E., Freudenberg-Hua, Y., Davies, P., Kim, M., Lipton, R. B., Stewart, W. F., Srinivasan, P., Hu, S., & Lipton, M. L. (2020). Associations of Apolipoprotein e ϵ4 Genotype and Ball Heading with Verbal Memory in Amateur Soccer Players. *JAMA Neurology*, *77*(4), 419–426. https://doi.org/10.1001/jamaneurol.2019.4828

Hupfeld, K. E., Swanson, C. W., Fling, B. W., & Seidler, R. D. (2020). TMS-induced silent periods: A review of methods and call for consistency. *Journal of Neuroscience Methods*, *346*. https://doi.org/10.1016/j.jneumeth.2020.108950

Hviid, C. V. B., Madsen, A. T., & Winther-Larsen, A. (2022). Biological variation of serum neurofilament light chain. *Clinical Chemistry and Laboratory Medicine*, *60*(4), 569–575. https://doi.org/10.1515/cclm-2020-1276

Hwang, S., Ma, L., Kawata, K., Tierney, R. T., & Jeka, J. J. (2017). Vestibular dysfunction after subconcussive head impact. *Journal of Neurotrauma*, *34*(1), 8–15. https://doi.org/10.1089/neu.2015.4238

Iverson, G. L., Gardner, A. J., Shultz, S. R., Solomon, G. S., McCrory, P., Zafonte, R., Perry, G., Hazrati, L. N., Dirk Keene, C., & Castellani, R. J. (2019). Chronic traumatic encephalopathy neuropathology might not be inexorably progressive or unique to repetitive neurotrauma. *Brain*, *142*(12), 3672–3693. https://doi.org/10.1093/brain/awz286

Jansen, A. E., McGrath, M., Samorezov, S., Johnston, J., Bartsch, A., & Alberts, J. (2021). Characterizing Head Impact Exposure in Men and Women During Boxing and Mixed Martial Arts. *Orthopaedic Journal of Sports Medicine*, *9*(12), 1–9. https://doi.org/10.1177/23259671211059815

Jildeh, T. R., Castle, J. P., Buckley, P. J., Abbas, M. J., Hegde, Y., & Okoroha, K. R. (2022). Lower Extremity Injury After Return to Sports From Concussion: A Systematic Review. *Orthopaedic Journal of Sports Medicine*, *10*(1), 1–7. https://doi.org/10.1177/23259671211068438

Jordan, B. D. (2000). Chronic traumatic brain injury associated with boxing. *Seminars in Neurology*, *20*(2), 179–185. https://doi.org/10.1055/s-2000-9826

Jordan, B. D., Jahre, C., Hauser, W. A., Zimmerman, R. D., Zarrelli, M., Lipsitz, E. C., Johnson, V., Warren, R. F., Tsairis, P., & Folk, F. S. (1992). CT of 338 active professional boxers. *Radiology*, *185*(2), 509–512. https://doi.org/10.1148/radiology.185.2.1410364

Joseph, J. R., Swallow, J. S., Willsey, K., Lapointe, A. P., Khalatbari, S., Korley, F. K., Oppenlander, M. E., Park, P., Szerlip, N. J., & Broglio, S. P. (2019). Elevated markers of brain injury as a result of clinically asymptomatic high-acceleration head impacts in high-school football athletes. *Journal of Neurosurgery*, *130*(5), 1642–1648. https://doi.org/10.3171/2017.12.JNS172386

Kawata, K. (2016). *Subconcussive head impact effect on plasma expression of S100-beta and PINCH proteins in collegiate football players*. Temple University.

Kawata, K., Liu, C. Y., Merkel, S. F., Ramirez, S. H., Tierney, R. T., & Langford, D. (2016). Blood biomarkers for brain injury: What are we measuring? *Neuroscience and Biobehavioral Reviews, 68*. https://doi.org/10.1016/j.neubiorev.2016.05.009

Kawata, K., Mitsuhashi, M., & Aldret, R. (2018a). A preliminary report on brain-derived extracellular vesicle as novel blood biomarkers for sport-related concussions. *Frontiers in Neurology*, *9*, 1–11. https://doi.org/10.3389/fneur.2018.00239

Kawata, K., Rubin, L. H., Takahagi, M., Lee, J. H., Sim, T., Szwanki, V., Bellamy, A., Tierney, R. T., & Langford, D. (2017). Subconcussive impact-dependent increase in plasma S100β levels in collegiate football players. *Journal of Neurotrauma*, *34*(14), 2254–2260. https://doi.org/10.1089/neu.2016.4786

Kawata, K., Rubin, L. H., Wesley, L., Lee, J. H., Sim, T., Takahagi, M., Bellamy, A., Tierney, R. T., & Langford, D. (2018b). Acute Changes in Plasma Total Tau Levels Are Independent of Subconcussive Head Impacts in College Football Players. *Journal of Neurotrauma*, *35*(2), 260–266. https://doi.org/10.1089/neu.2017.5376

Kelestimur, F., Tanriverdi, F., Atmaca, H., KUnluhizarci, Selcuklu, A., & Casanueva, F. F. (2004). Boxing as a sport activity associated with isolated GH deficiency. *Journal of Endocrinological Investigation*, *27*(11). https://doi.org/10.1007/BF03345299

Kelly, D. F., Chaloner, C., Evans, D., Mathews, A., Cohan, P., Wang, C., Swerdloff, R., Sim, M. S., Lee, J., Wright, M. J., Kernan, C., Barkhoudarian, G., Yuen, K. C. J., & Guskiewicz, K. (2014). Prevalence of pituitary hormone dysfunction, metabolic syndrome, and impaired quality of life in retired professional football players: A prospective study. *Journal of Neurotrauma*, *31*(13), 1161–1171. https://doi.org/10.1089/neu.2013.3212

Kenney, W. L., Wilmore, J. H., & Costill, D. L. (2012). Monitoring Exercise Intensity. In *Physiology of Sport and Exercise* (5th ed., pp. 510–515). Human Kinetics.

Kenny, R., Elez, M., Clansey, A., Virji-Babul, N., & Wu, L. C. (2024). Individualized monitoring of longitudinal heading exposure in soccer. *Scientific Reports*, *14*(1), 1–10. https://doi.org/10.1038/s41598-024-52163-8

Kim, H.-Y. (2013). Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restorative Dentistry & Endodontics*, *38*(1). https://doi.org/10.5395/rde.2013.38.1.52

King, D., Hume, P. A., Brughelli, M., & Gissane, C. (2015). Instrumented mouthguard acceleration analyses for head impacts in amateur rugby union players over a season of

matches. *American Journal of Sports Medicine*, *43*(3), 614–624. https://doi.org/10.1177/0363546514560876

Klomjai, W., Katz, R., & Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Annals of Physical and Rehabilitation Medicine, 58*(4). https://doi.org/10.1016/j.rehab.2015.05.005

Koerte, I. K., Bahr, R., Filipcik, P., Gooijers, J., Leemans, A., Lin, A. P., Tripodis, Y., Shenton, M. E., Sochen, N., Swinnen, S. P., & Pasternak, O. (2022). REPIMPACT - a prospective longitudinal multisite study on the effects of repetitive head impacts in youth soccer. *Brain Imaging & Behavior*, *16*(1), 492–502. https://doi.org/10.1007/s11682-021-00484-x

Koerte, I. K., Ertl-Wagner, B., Reiser, M., Zafonte, R., & Shenton, M. E. (2012). White matter integrity in the brains of professional soccer players without a symptomatic concussion. *JAMA, 308*(18). https://doi.org/10.1001/jama.2012.13735

Koerte, I. K., Lin, A. P., Muehlmann, M., Merugumala, S., Liao, H., Starr, T., Kaufmann, D., Mayinger, M., Steffinger, D., Fisch, B., Karch, S., Heinen, F., Ertl-Wagner, B., Reiser, M., Stern, R. A., Zafonte, R., & Shenton, M. E. (2015). Altered Neurochemistry in Former Professional Soccer Players without a History of Concussion. *Journal of Neurotrauma*, *32*(17), 1287–1293. https://doi.org/10.1089/neu.2014.3715

Koerte, I. K., Schultz, V., Sydnor, V. J., Howell, D. R., Guenette, J. P., Dennis, E., Kochsiek, J., Kaufmann, D., Sollmann, N., Mondello, S., Shenton, M. E., & Lin, A. P. (2020). Sex-Related Differences in the Effects of Sports-Related Concussion: A Review. *Journal of Neuroimaging, 30*(4). https://doi.org/10.1111/jon.12726

Kontos, A. P., Braithwaite, R., Chrisman, S. P. D., McAllister-Deitrick, J., Symington, L., Reeves, V. L., & Collins, M. W. (2017). Systematic review and meta-analysis of the effects of football heading. *British Journal of Sports Medicine*, *51*(15), 1118–1124. https://doi.org/10.1136/bjsports-2016-096276

Korley, F. K., Jain, S., Sun, X., Puccio, A. M., Yue, J. K., Gardner, R. C., Wang, K. K. W., Okonkwo, D. O., Yuh, E. L., Mukherjee, P., Nelson, L. D., Taylor, S. R., Markowitz, A. J., Diaz-Arrastia, R., Manley, G. T., Adeoye, O., Badatjia, N., Duhaime, A. C., Ferguson, A., … Zafonte, R. (2022). Prognostic value of day-of-injury plasma GFAP and UCH-L1 concentrations for predicting functional recovery after traumatic brain injury in patients from the US TRACK-TBI cohort: an observational cohort study. *The Lancet Neurology*,

21(9), 803–813. https://doi.org/10.1016/S1474-4422(22)00256-3

Lamond, L. C., Caccese, J. B., Buckley, T. A., Glutting, J., & Kaminski, T. W. (2018). *Linear Acceleration in Direct Head Contact Across Impact Type, Player Position, and Playing Scenario in Collegiate Women's Soccer Players*. *53*(2), 115–121. https://doi.org/10.4085/1062-6050-90-17

Langdon, S., Goedhart, E., Oosterlaan, J., & Königs, M. (2022). Heading Exposure in Elite Football (Soccer): A Study in Adolescent, Young Adult, and Adult Male and Female Players. *Medicine and Science in Sports and Exercise*, *54*(9), 1459–1465. https://doi.org/10.1249/MSS.0000000000002945

Lee, E. B., Kinch, K., Johnson, V. E., Trojanowski, J. Q., Smith, D. H., & Stewart, W. (2019). Chronic traumatic encephalopathy is a common co-morbidity, but less frequent primary dementia in former soccer and rugby players. *Acta Neuropathologica*, *138*(3), 389–399. https://doi.org/10.1007/s00401-019-02030-y

Lee, H., Sullivan, S. J., & Schneiders, A. G. (2013). The use of the dual-task paradigm in detecting gait performance deficits following a sports-related concussion: A systematic review and meta-analysis. *Journal of Science and Medicine in Sport, 16*(1). https://doi.org/10.1016/j.jsams.2012.03.013

Lehman, E. J., Hein, M. J., Baron, S. L., & Gersic, C. M. (2012). Neurodegenerative causes of death among retired National Football League players. *Neurology*, *79*(19), 1970–1974. https://doi.org/10.1212/WNL.0b013e31826daf50

Lember, L.-M., Ntikas, M., Mondello, S., Wilson, L., Di Virgilio, T. G., Hunter, A. M., Kobeissy, F., Mechref, Y., Donaldson, D. I., & Ietswaart, M. (2024). The Use of Biofluid Markers to Evaluate the Consequences of Sport-Related Subconcussive Head Impact Exposure: A Scoping Review. *Sports Medicine - Open, 10*(1). https://doi.org/10.1186/s40798-023-00665-6

Lember, L.-M., Ntikas, M., Mondello, S., Wilson, L., Hunter, A., Di Virgilio, T., Santoro, E., & Ietswaart, M. (2021). Effects of sport-related repetitive subconcussive head impacts on biofluid markers: a scoping review protocol. *BMJ Open*, *11*(6). https://doi.org/10.1136/bmjopen-2020-046452

Ling, H., Morris, H. R., Neal, J. W., Lees, A. J., Hardy, J., Holton, J. L., Revesz, T., &

Williams, D. D. R. (2017). Mixed pathologies including chronic traumatic encephalopathy account for dementia in retired association football (soccer) players. *Acta Neuropathologica*, *133*(3), 337–352. https://doi.org/10.1007/s00401-017-1680-3

Lipton, M. L., Kim, N., Zimmerman, M. E., Kim, M., Stewart, W. F., Branch, C. A., & Lipton, R. B. (2013). Soccer heading is associated with white matter microstructural and cognitive abnormalities. *Radiology*, *268*(3), 850–857. https://doi.org/10.1148/radiol.13130545

Livingston, G., Huntley, J., Liu, K. Y., Costafreda, S. G., Selbæk, G., Alladi, S., Ames, D., Banerjee, S., Burns, A., Brayne, C., Fox, N. C., Ferri, C. P., Gitlin, L. N., Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Nakasujja, N., Rockwood, K., … Mukadam, N. (2024). Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. *The Lancet*, *404*(10452), 572–628. https://doi.org/10.1016/S0140-6736(24)01296-0

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns, A., Cohen-Mansfield, J., Cooper, C., Costafreda, S. G., Dias, A., Fox, N., Gitlin, L. N., Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Ogunniyi, A., … Mukadam, N. (2020). Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *The Lancet, 396*(10248). https://doi.org/10.1016/S0140-6736(20)30367-6

Maas, A. I. R., Menon, D. K., Adelson, P. D., Andelic, N., Bell, M. J., Belli, A., Bragge, P., Brazinova, A., Büki, A., Chesnut, R. M., Citerio, G., Coburn, M., Cooper, D. J., Crowder, A. T., Czeiter, E., Czosnyka, M., Diaz-Arrastia, R., Dreier, J. P., Duhaime, A. C., … Zumbo, F. (2017). Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *The Lancet Neurology*, *16*(12), 987–1048. https://doi.org/10.1016/S1474-4422(17)30371-X

Mackay, D. F., Russell, E. R., Stewart, K., MacLean, J. A., Pell, J. P., & Stewart, W. (2019). Neurodegenerative Disease Mortality among Former Professional Soccer Players. *New England Journal of Medicine*, *381*(19), 1801–1808. https://doi.org/10.1056/nejmoa1908483

Mainwaring, L., Ferdinand Pennock, K. M., Mylabathula, S., & Alavie, B. Z. (2018). Subconcussive head impacts in sport: A systematic review of the evidence. *International Journal of Psychophysiology*, *132*, 39–54. https://doi.org/10.1016/j.ijpsycho.2018.01.007

Major, B. P., McDonald, S. J., O'Brien, W. T., Symons, G. F., Clough, M., Costello, D., Sun,

M., Brady, R. D., Mccullough, J., Aniceto, R., Lin, I.-H., Law, M., Mychasiuk, R., O'Brien, T. J., Agoston, D. V, & Shultz, S. R. (2020). Serum Protein Biomarker Findings Reflective of Oxidative Stress and Vascular Abnormalities in Male, but Not Female, Collision Sport Athletes. *Frontiers in Neurology*, *11*. https://doi.org/10.3389/fneur.2020.549624

Manor, B., Zhou, J., Lo, O. Y., Zhu, H., Gouskova, N. A., Yu, W., Zafonte, R., Lipsitz, L. A., Travison, T. G., & Pascual-Leone, A. (2020). Self-Reported Head Trauma Predicts Poor Dual Task Gait in Retired National Football League Players. *Annals of Neurology*, *87*(1). https://doi.org/10.1002/ana.25638

Marchi, N., Bazarian, J. J., Puvenna, V., Janigro, M., Ghosh, C., Zhong, J., Zhu, T., Blackman, E., Stewart, D., Ellis, J., Butler, R., & Janigro, D. (2013). Consequences of Repeated Blood-Brain Barrier Disruption in Football Players. *PLoS ONE*, *8*(3). https://doi.org/10.1371/journal.pone.0056805

Martland, H. S. (1928). Punch drunk. *Journal of the American Medical Association*, *91*(15), 1103–1107. https://doi.org/10.1001/jama.1928.02700150029009

Matser, E. J. T., Kessels, A. G. H., Lezak, M. D., & Troost, J. (2001). A dose-response relation of headers and concussions with cognitive impairment in professional soccer players. *Journal of Clinical and Experimental Neuropsychology*, *23*(6), 770–774. https://doi.org/10.1076/jcen.23.6.770.1029

Matser, E. J. T., Kessels, A. G., Lezak, M. D., Jordan, B. D., & Troost, J. (1999). Neuropsychological impairment in amateur soccer players. *Journal of the American Medical Association*, *282*(10), 971–973. https://doi.org/10.1001/jama.282.10.971

Matuk, R., Pereira, M., Baird, J., Dooner, M., Cheng, Y., Wen, S., Rao, S., Quesenberry, P., & Raukar, N. P. (2021). The role of salivary vesicles as a potential inflammatory biomarker to detect traumatic brain injury in mixed martial artists. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-87180-4

McAllister, T. W., Ford, J. C., Flashman, L. A., Maerlender, A., Greenwald, R. M., Beckwith, J. G., Bolander, R. P., Tosteson, T. D., Turco, J. H., Raman, R., & Jain, S. (2014). Effect of head impacts on diffusivity measures in a cohort of collegiate contact sport athletes. *Neurology*, *82*(1), 63–69. https://doi.org/10.1212/01.wnl.0000438220.16190.42

McCann, H., Bahar, A. Y., Burkhardt, K., Gardner, A. J., Halliday, G. M., Iverson, G. L., & Shepherd, C. E. (2022). Prevalence of chronic traumatic encephalopathy in the Sydney Brain Bank. *Brain Communications*, *4*(4). https://doi.org/10.1093/braincomms/fcac189

Mcdonald, S. J., Shultz, S. R., & Agoston, D. V. (2021). The Known Unknowns: An Overview of the State of Blood-Based Protein Biomarkers of Mild Traumatic Brain Injury. *Journal of Neurotrauma*, *38*(19), 2652–2666. https://doi.org/10.1089/neu.2021.0011

McGuinness, L. A., & Higgins, J. P. T. (n.d.). Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. *Research Synthesis Methods*, *n/a*(n/a). https://doi.org/10.1002/jrsm.1411

McKee, A. C., Cairns, N. J., Dickson, D. W., Folkerth, R. D., Dirk Keene, C., Litvan, I., Perl, D. P., Stein, T. D., Vonsattel, J. P., Stewart, W., Tripodis, Y., Crary, J. F., Bieniek, K. F., Dams-O'Connor, K., Alvarez, V. E., & Gordon, W. A. (2016). The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. *Acta Neuropathologica*, *131*(1), 75–86. https://doi.org/10.1007/s00401-015-1515-z

McKee, A. C., Daneshvar, D. H., Alvarez, V. E., & Stein, T. D. (2014). The neuropathology of sport. *Acta Neuropathologica*, *127*(1), 29–51. https://doi.org/10.1007/s00401-013-1230-6

McNabb, C., Reha, T., Georgieva, J., Jacques, A., Netto, K., & Lavender, A. P. (2020). The effect of sub-concussive impacts during a rugby tackling drill on brain function. *Brain Sciences*, *10*(12), 1–12. https://doi.org/10.3390/brainsci10120960

McPherson, A. L., Nagai, T., Webster, K. E., & Hewett, T. E. (2019). Musculoskeletal Injury Risk After Sport-Related Concussion: A Systematic Review and Meta-analysis. *American Journal of Sports Medicine*, *47*(7), 1754–1762. https://doi.org/10.1177/0363546518785901

Mez, J., Daneshvar, D. H., Kiernan, P. T., Abdolmohammadi, B., Alvarez, V. E., Huber, B. R., Alosco, M. L., Solomon, T. M., Nowinski, C. J., McHale, L., Cormier, K. A., Kubilus, C. A., Martin, B. M., Murphy, L., Baugh, C. M., Montenigro, P. H., Chaisson, C. E., Tripodis, Y., Kowall, N. W., … McKee, A. C. (2017). Clinicopathological evaluation of chronic traumatic encephalopathy in players of American football. *JAMA - Journal of the American Medical Association*, *318*(4). https://doi.org/10.1001/jama.2017.8334

Mihalik, J. P., Bell, D. R., Ed, M., Marshall, S. W., & Ph, D. (2007). Measurement of head impacts in collegiate football players: an investigation of positional and event-type differences. *Neurosurgery*, *61*(6), 1229–1235. https://doi.org/10.1227/01.NEU.0000280147.37163.30

Mihalik, J. P., Guskiewicz, K. M., Jeffries, J. A., Greenwald, R. M., & Marshall, S. W. (2008). Characteristics of head impacts sustained by youth ice hockey players. *Proceedings of the Institution of Mechanical Engineers, Part P: Journal of Sports Engineering and Technology*, *222*(1), 45–52. https://doi.org/10.1243/17543371JSET4

Mondello, S., Guedes, V. A., Lai, C., Jeromin, A., Bazarian, J. J., & Gill, J. M. (2020). Sex Differences in Circulating T-Tau Trajectories After Sports-Concussion and Correlation With Outcome. *Frontiers in Neurology*, *11*, 1–8. https://doi.org/10.3389/fneur.2020.00651

Mondello, S., Jeromin, A., Buki, A., Bullock, R., Czeiter, E., Kovacs, N., Barzo, P., Schmid, K., Tortella, F., Wang, K. K., & Hayes, R. L. (2012). Glial neuronal ratio: A novel index for differentiating injury type in patients with severe traumatic brain injury. *Journal of Neurotrauma*, *29*(6), 1096–1104. https://doi.org/10.1089/neu.2011.2092

Mondello, S., Sandner, V., Goli, M., Czeiter, E., Amrein, K., Kochanek, P. M., Gautam, S., Cho, B. G., Morgan, R., Nehme, A., Fiumara, G., Eid, A. H., Barsa, C., Haidar, M. A., Buki, A., Kobeissy, F. H., & Mechref, Y. (2022). Exploring serum glycome patterns after moderate to severe traumatic brain injury: A prospective pilot study. *EClinicalMedicine*, *50*. https://doi.org/10.1016/j.eclinm.2022.101494

Mondello, S., Shear, D. A., Bramlett, H. M., Dixon, C. E., Schmid, K. E., Dietrich, W. D., Wang, K. K. W., Hayes, R. L., Glushakova, O., Catania, M., Richieri, S. P., Povlishock, J. T., Tortella, F. C., & Kochanek, P. M. (2016). Insight into Pre-Clinical Models of Traumatic Brain Injury Using Circulating Brain Damage Biomarkers: Operation Brain Trauma Therapy. *Journal of Neurotrauma*, *33*(6), 595–605. https://doi.org/10.1089/neu.2015.4132

Mondello, S., Sorinola, A., Synnot, A., Donoghue, E., Wang, K. K. W., Diaz-arrastia, R., Steyerberg, E. W., Menon, D. K., Maas, A. I. R., & Buki, A. (2021). *Blood-Based Protein Biomarkers for the Management of Traumatic Brain Injuries in Adults Presenting to Emergency Departments with Mild Brain Injury : A Living Systematic Review and Meta-*

Analysis. *1106*, 1086–1106. https://doi.org/10.1089/neu.2017.5182

Montenigro, P. H., Alosco, M. L., Martin, B. M., Daneshvar, D. H., Mez, J., Chaisson, C. E., Nowinski, C. J., Au, R., McKee, A. C., Cantu, R. C., McClean, M. D., Stern, R. A., & Tripodis, Y. (2017). Cumulative Head Impact Exposure Predicts Later-Life Depression, Apathy, Executive Dysfunction, and Cognitive Impairment in Former High School and College Football Players. *Journal of Neurotrauma*, *34*(2), 328–340. https://doi.org/10.1089/neu.2016.4413

Montenigro, P. H., Baugh, C. M., Daneshvar, D. H., Mez, J., Budson, A. E., Au, R., Katz, D. I., Cantu, R. C., & Stern, R. A. (2014). Clinical subtypes of chronic traumatic encephalopathy: Literature review and proposed research diagnostic criteria for traumatic encephalopathy syndrome. *Alzheimer's Research and Therapy*, *6*(5–8), 1–17. https://doi.org/10.1186/s13195-014-0068-z

Muñoz, E. R., Caccese, J. B., Wilson, B. E., Shuler, K. T., Santos, F. V., Cabán, C. T., Jeka, J. J., Langford, D., & Hudson, M. B. (2021). Effects of purposeful soccer heading on circulating small extracellular vesicle concentration and cargo. *Journal of Sport and Health Science*, *10*(2), 122–130. https://doi.org/10.1016/j.jshs.2020.11.006

Muraoka, S., DeLeo, A. M., Yang, Z., Tatebe, H., Yukawa-Takamatsu, K., Ikezu, S., Tokuda, T., Issadore, D., Stern, R. A., & Ikezu, T. (2021). Proteomic profiling of extracellular vesicles separated from plasma of former National Football League players at risk for chronic traumatic encephalopathy. *Aging and Disease*, *12*(6), 1363–1375. https://doi.org/10.14336/AD.2020.0908

Muraoka, S., Jedrychowski, M. P., Tatebe, H., DeLeo, A. M., Ikezu, S., Tokuda, T., Gygi, S. P., Stern, R. A., & Ikezu, T. (2019). Proteomic Profiling of Extracellular Vesicles Isolated From Cerebrospinal Fluid of Former National Football League Players at Risk for Chronic Traumatic Encephalopathy. *Frontiers in Neuroscience*, *13*, 1–12. https://doi.org/10.3389/fnins.2019.01059

Mussack, T., Dvorak, J., Graf-Baumann, T., & Jochum, M. (2003). Serum S-100B protein levels in young amateur soccer players after controlled heading and normal exercise. *European Journal of Medical Research*, *8*(10), 457–464.

Myer, G. D., Barber Foss, K., Thomas, S., Galloway, R., Dicesare, C. A., Dudley, J., Gadd, B., Leach, J., Smith, D., Gubanich, P., Meehan, W. P., Altaye, M., Lavin, P., & Yuan, W.

(2019). Altered brain microstructure in association with repetitive subconcussive head impacts and the potential protective effect of jugular vein compression: A longitudinal study of female soccer athletes. *British Journal of Sports Medicine*, *53*(24), 1539–1551. https://doi.org/10.1136/bjsports-2018-099571

Myer, G. D., Yuan, W., Barber Foss, K. D., Thomas, S., Smith, D., Leach, J., Kiefer, A. W., Dicesare, C., Adams, J., Gubanich, P. J., Kitchen, K., Schneider, D. K., Braswell, D., Krueger, D., & Altaye, M. (2016). Analysis of head impact exposure and brain microstructure response in a season-long application of a jugular vein compression collar: A prospective, neuroimaging investigation in American football. *British Journal of Sports Medicine*, *50*(20), 1276–1285. https://doi.org/10.1136/bjsports-2016-096134

Naunheim, R. S., Ryden, A., Standeven, J., Genin, G., Lewis, L., Thompson, P., & Bayly, P. (2003). Does soccer headgear attenuate the impact when heading a soccer ball? *Academic Emergency Medicine*, *10*(1), 85–90. https://doi.org/10.1197/aemj.10.1.85

Naunheim, R. S., Standeven, J., Richter, C., & Lewis, L. M. (2000). *Comparison of Impact Data in Hockey , Football , and Soccer*. *48*(5), 48–51.

Neselius, S., Brisby, H., Theodorsson, A., Blennow, K., Zetterberg, H., & Marcusson, J. (2012). CSF-biomarkers in olympic boxing: Diagnosis and effects of repetitive head trauma. *PLoS ONE*, *7*(4), 1–8. https://doi.org/10.1371/journal.pone.0033606

Neselius, S., Zetterberg, H., Blennow, K., Marcusson, J., & Brisby, H. (2013a). Increased CSF levels of phosphorylated neurofilament heavy protein following bout in amateur boxers. *PLoS ONE*, *8*(11), 1–5. https://doi.org/10.1371/journal.pone.0081249

Neselius, S., Zetterberg, H., Blennow, K., Randall, J., Wilson, D., Marcusson, J., & Brisby, H. (2013b). Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma. *Brain Injury*, *27*(4), 425–433. https://doi.org/10.3109/02699052.2012.750752

Newcombe, V. F. J., Ashton, N. J., Posti, J. P., Glocker, B., Manktelow, A., Chatfield, D. A., Winzeck, S., Needham, E., Correia, M. M., Williams, G. B., Simrén, J., Takala, R. S. K., Katila, A. J., Maanpää, H. R., Tallus, J., Frantzén, J., Blennow, K., Tenovuo, O., Zetterberg, H., & Menon, D. K. (2022). Post-acute blood biomarkers and disease progression in traumatic brain injury. *Brain*, *145*(6), 2064–2076. https://doi.org/10.1093/brain/awac126

Nichols, E., Steinmetz, J. D., Vollset, S. E., Fukutaki, K., Chalek, J., Abd-Allah, F., Abdoli, A., Abualhasan, A., Abu-Gharbieh, E., Akram, T. T., Al Hamad, H., Alahdab, F., Alanezi, F. M., Alipour, V., Almustanyir, S., Amu, H., Ansari, I., Arabloo, J., Ashraf, T., … Vos, T. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*, *7*(2). https://doi.org/10.1016/S2468-2667(21)00249-8

Niedfeldt, M. W. (2011). Head injuries, heading, and the use of headgear in soccer. *Current Sports Medicine Reports*, *10*(6), 324–329. https://doi.org/10.1249/JSR.0b013e318237be53

Nowak, M. K., Bevilacqua, Z. W., Ejima, K., Huibregtse, M. E., Chen, Z., Mickleborough, T. D., Newman, S. D., & Kawata, K. (2020). Neuro-Ophthalmologic Response to Repetitive Subconcussive Head Impacts: A Randomized Clinical Trial. *JAMA Ophthalmology*, *138*(4), 350–357. https://doi.org/10.1001/jamaophthalmol.2019.6128

Nowak, M. K., Ejima, K., Quinn, P. D., Bazarian, J. J., Mickleborough, T. D., Harezlak, J., Newman, S. D., & Kawata, K. (2022). ADHD May Associate With Reduced Tolerance to Acute Subconcussive Head Impacts: A Pilot Case-Control Intervention Study. *Journal of Attention Disorders*, *26*(1), 125–139. https://doi.org/10.1177/1087054720969977

Nozaki, D., Kawashima, N., Aramaki, Y., Akai, M., Nakazawa, K., Nakajima, Y., & Yano, H. (2003). Sustained muscle contractions maintained by autonomous neuronal activity within the human spinal cord. *Journal of Neurophysiology*, *90*(4). https://doi.org/10.1152/jn.00200.2003

Ntikas, M., Binkofski, F., Shah, N. J., & Ietswaart, M. (2022). Repeated Sub-Concussive Impacts and the Negative Effects of Contact Sports on Cognition and Brain Integrity. *International Journal of Environmental Research and Public Health*, *19*(12). https://doi.org/10.3390/ijerph19127098

O'Connell, B., Wilson, F., Boyle, N., O'Dwyer, T., Denvir, K., Farrell, G., & Kelly, Á. M. (2018). Effects of match play and training on circulating S100B concentration in professional rugby players. *Brain Injury*, *32*(13–14), 1811–1816. https://doi.org/10.1080/02699052.2018.1532112

O'Connor, K. L., Peeters, T., Szymanski, S., & Broglio, S. P. (2017). Individual Impact Magnitude vs. Cumulative Magnitude for Estimating Concussion Odds. *Annals of*

Biomedical Engineering, *45*(8), 1985–1992. https://doi.org/10.1007/s10439-017-1843-3

O'Keeffe, E., Kelly, E., Liu, Y., Giordano, C., Wallace, E., Hynes, M., Tiernan, S., Meagher, A., Greene, C., Hughes, S., Burke, T., Kealy, J., Doyle, N., Hay, A., Farrell, M., Grant, G. A., Friedman, A., Veksler, R., Molloy, M. G., … Campbell, M. (2020). Dynamic Blood-Brain Barrier Regulation in Mild Traumatic Brain Injury. *Journal of Neurotrauma*, *37*(2), 347–356. https://doi.org/10.1089/neu.2019.6483

Obmiński, Z., Hübner-Wożniak, E., & Stanisław, Ł. (2009). Hormonal and Metabolic Blood Status in Boxers After a 3-Round Match. *Polish Journal of Sport & Tourism*, *16*(4), 221–224.

Oliver, J. M., Anzalone, A. J., Stone, J. D., Turner, S. M., Blueitt, D., Garrison, J. C., Askow, A. T., Luedke, J. A., & Jagim, A. R. (2019). Fluctuations in blood biomarkers of head trauma in NCAA football athletes over the course of a season. *Journal of Neurosurgery*, *130*(5), 1655–1662. https://doi.org/10.3171/2017.12.JNS172035

Oliver, J. M., Jones, M. T., Anzalone, A. J., Kirk, K. M., Gable, D. A., Repshas, J. T., Johnson, T. A., Höglund, K., Blennow, K., & Zetterberg, H. (2017). A Season of American Football Is Not Associated with Changes in Plasma Tau. *Journal of Neurotrauma*, *34*(23), 3295–3300. https://doi.org/10.1089/neu.2017.5064

Oliver, J. M., Jones, M. T., Kirk, K. M., Gable, D. A., Repshas, J. T., Johnson, T. A., Andréasson, U., Norgren, N., Blennow, K., & Zetterberg, H. (2016). Serum Neurofilament Light in American Football Athletes over the Course of a Season. *Journal of Neurotrauma*, *33*(19), 1784–1789. https://doi.org/10.1089/neu.2015.4295

Omalu, B. I., DeKosky, S. T., Hamilton, R. L., Minster, R. L., Kamboh, M. I., Shakir, A. M., & Wecht, C. H. (2006). Chronic traumatic encephalopathy in a National Football League player: Part II. *Neurosurgery*, *59*(5), 1086–1092. https://doi.org/10.1227/01.NEU.0000245601.69451.27

Omalu, B. I., DeKosky, S. T., Minster, R. L., Kamboh, M. I., Hamilton, R. L., & Wecht, C. H. (2005). Chronic traumatic encephalopathy in a National Football League player. *Neurosurgery*, *57*(1), 128–133. https://doi.org/10.1227/01.NEU.0000163407.92769.ED

Otto, M., Holthusen, S., Bahn, E., Sohnchen, N., Wiltfang, J., Geese, R., Fischer, A., & Reimers, C. D. (2000). Boxing and running lead to a rise in serum levels of S-100B

protein. *International Journal of Sports Medicine*, *21*(8), 551–555. https://doi.org/10.1055/s-2000-8480

Oztasyonar, Y. (2017). Interaction between different sports branches such as taekwondo, box, athletes and serum brain derived neurotrophic factor levels. *Journal of Sports Medicine and Physical Fitness*, *57*(4), 457–460. https://doi.org/10.23736/S0022-4707.16.06070-X

Peek, K., Elliott, J. M., & Orr, R. (2020). Higher neck strength is associated with lower head acceleration during purposeful heading in soccer: A systematic review. *Journal of Science and Medicine in Sport*, *23*(5), 453–462. https://doi.org/10.1016/j.jsams.2019.11.004

Peek, K., McKay, M., Fu, A., Meyer, T., Oxenham, V., Esopenko, C., Caccese, J., & Andersen, J. (2021). The effect of ball characteristics on head acceleration during purposeful heading in male and female youth football players. *Science and Medicine in Football*, *5*(3). https://doi.org/10.1080/24733938.2021.1897657

Peng, W., Kobeissy, F., Mondello, S., Barsa, C., & Mechref, Y. (2022). MS-based glycomics: An analytical tool to assess nervous system diseases. *Frontiers in Neuroscience, 16*. https://doi.org/10.3389/fnins.2022.1000179

Petzold, A. (2015). Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease. *Brain Research, 1600*. https://doi.org/10.1016/j.brainres.2014.12.027

Pin, E., Petricoin, E. F., Cortes, N., Bowman, T. G., Andersson, E., Uhlén, M., Nilsson, P., & Caswell, S. V. (2021). Immunoglobulin A Autoreactivity toward Brain Enriched and Apoptosis-Regulating Proteins in Saliva of Athletes after Acute Concussion and Subconcussive Impacts. *Journal of Neurotrauma*, *38*(17), 2373–2383. https://doi.org/10.1089/neu.2020.7375

Pluhar, E., McCracken, C., Griffith, K. L., Christino, M. A., Sugimoto, D., & Meehan, W. P. (2019). Team sport athletes may be less likely to suffer anxiety or depression than individual sport athletes. *Journal of Sports Science and Medicine*, *18*(3), 490–496.

Puvenna, V., Brennan, C., Shaw, G., Yang, C., Marchi, N., Bazarian, J. J., Merchant-Borna, K., & Janigro, D. (2014). Significance of ubiquitin carboxy-terminal hydrolase L1 elevations in athletes after sub-concussive head hits. *PLoS ONE*, *9*(5), 1–9. https://doi.org/10.1371/journal.pone.0096296

Recommendations for sensor locations in hip or upper leg muscles. (n.d.). Retrieved February

24, 2024, from http://seniam.org/quadricepsfemorisrectusfemoris.html

Richardson, J. T. E. (2011). Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review, 6*(2). https://doi.org/10.1016/j.edurev.2010.12.001

Rogatzki, M. J., Keuler, S. A., Harris, A. E., Ringgenberg, S. W., Breckenridge, R. E., White, J. L., & Baker, J. S. (2018). Response of protein S100B to playing American football, lifting weights, and treadmill running. *Scandinavian Journal of Medicine and Science in Sports*, *28*(12), 2505–2514. https://doi.org/10.1111/sms.13297

Rogatzki, M. J., Soja, S. E., McCabe, C. A., Breckenridge, R. E., White, J. L., & Baker, J. S. (2016). Biomarkers of brain injury following an American football game: A pilot study. *International Journal of Immunopathology and Pharmacology*, *29*(3), 450–457. https://doi.org/10.1177/0394632016657091

Roser, P., Wehrhahn, T., Krogmann, H., Riedel, N., Marshall, R. P., Gille, J., Flitsch, J., & Aberle, J. (2018). Somatotrope Pituitary Function in Professional Soccer Players. *Experimental and Clinical Endocrinology and Diabetes*, *126*(5), 306–308. https://doi.org/10.1055/s-0043-119876

Rubin, L. H., Tierney, R. T., Kawata, K., Wesley, L., Lee, J. H., Blennow, K., Zetterberg, H., & Langford, D. (2019). NFL blood levels are moderated by subconcussive impacts in a cohort of college football players. *Brain Injury*, *33*(4), 456–462. https://doi.org/10.1080/02699052.2019.1565895

Ruiz-Ocaña, A., Pérez, F., Stoilova, V., Picalló, M., & Lecat, M. (2023). *Professional Football Report 2023*. https://digitalhb.fifa.com/m/2a5dc95026d9cf8a/original/FIFA-Professional-Football-Report-2023.pdf

Russell, E. R., Mackay, D. F., Lyall, D., Stewart, K., MacLean, J. A., Robson, J., Pell, J. P., & Stewart, W. (2022). Neurodegenerative disease risk among former international rugby union players. *Journal of Neurology, Neurosurgery & Psychiatry*, *93*(12), 1262–1268. https://doi.org/10.1136/jnnp-2022-329675

Sandmo, S. B., Filipcik, P., Cente, M., Hanes, J., Andersen, T. E., Straume-Naesheim, T. M., & Bahr, R. (2020). Neurofilament light and tau in serum after head-impact exposure in soccer. *Brain Injury*, *34*(5), 602–609. https://doi.org/10.1080/02699052.2020.1725129

Scott, E., Kidgell, D. J., Frazer, A. K., & Pearce, A. J. (2020). The Neurophysiological Responses of Concussive Impacts: A Systematic Review and Meta-Analysis of Transcranial Magnetic Stimulation Studies. *Frontiers in Human Neuroscience, 14*. https://doi.org/10.3389/fnhum.2020.00306

Shahim, P., Zetterberg, H., Tegner, Y., & Blennow, K. (2017). Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. *Neurology*, *88*(19), 1788–1794. https://doi.org/10.1212/WNL.0000000000003912

Siebner, H. R., Dressnandt, J., Auer, C., & Conrad, B. (1998). Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle and Nerve*, *21*(9). https://doi.org/10.1002/(SICI)1097-4598(199809)21:9<1209::AID-MUS15>3.0.CO;2-M

Smith, D. H., Johnson, V. E., Trojanowski, J. Q., & Stewart, W. (2019). Chronic traumatic encephalopathy — confusion and controversies. *Nature Reviews Neurology, 15*(3). https://doi.org/10.1038/s41582-018-0114-8

Soriano, S., Curry, K., Sadrameli, S. S., Wang, Q., Nute, M., Reeves, E., Kabir, R., Wiese, J., Criswell, A., Schodrof, S., Britz, G. W., Gadhia, R., Podell, K., Treangen, T., & Villapol, S. (2022). Alterations to the gut microbiome after sport-related concussion in a collegiate football players cohort: A pilot study. *Brain, Behavior, & Immunity - Health*, *21*. https://doi.org/10.1016/j.bbih.2022.100438

Stålnacke, B. M., Ohlsson, A., Tegner, Y., & Sojka, P. (2006). Serum concentrations of two biochemical markers of brain tissue damage S-100B and neurone specific enolase are increased in elite female soccer players after a competitive game. *British Journal of Sports Medicine*, *40*(4), 313–316. https://doi.org/10.1136/bjsm.2005.021584

Stålnacke, B. M., & Sojka, P. (2008). Repeatedly heading a soccer ball does not increase serum levels of S-100B, a biochemical marker of brain tissue damage: An experimental Study. *Biomarker Insights*, *2008*(3), 87–91. https://doi.org/10.4137/bmi.s359

Stålnacke, B. M., Tegner, Y., & Sojka, P. (2003). Playing ice hockey and basketball increases serum levels of S-100B in elite players: A pilot study. *Clinical Journal of Sport Medicine*, *13*(5), 292–302. https://doi.org/10.1097/00042752-200309000-00004

Stålnacke, B. M., Tegner, Y., & Sojka, P. (2004). Playing soccer increases serum

concentrations of the biochemical markers of brain damage S-100B and neuron-specific enolase in elite players: A pilot study. *Brain Injury*, *18*(9), 899–909. https://doi.org/10.1080/02699050410001671865

Stein, T. D., Alvarez, V. E., & McKee, A. C. (2015). Concussion in Chronic Traumatic Encephalopathy. *Current Pain and Headache Reports*, *19*(10). https://doi.org/10.1007/s11916-015-0522-z

Stephen, S. J., Hasman, L., Goldenberg, M., Merchant-Borna, K., Kawata, K., Mannix, R., & Bazarian, J. J. (2022). Short-Term Neurologic Manifestations of Repetitive Head Impacts among Athletes: A Scoping Review. *Journal of Head Trauma Rehabilitation*, *37*(5), 318–325. https://doi.org/10.1097/HTR.0000000000000767

Stern, R. A., Tripodis, Y., Baugh, C. M., Fritts, N. G., Martin, B. M., Chaisson, C., Cantu, R. C., Joyce, J. A., Shah, S., Ikezu, T., Zhang, J., Gercel-Taylor, C., & Taylor, D. D. (2016). Preliminary study of plasma exosomal tau as a potential biomarker for chronic traumatic encephalopathy. *Journal of Alzheimer's Disease*, *51*(4), 1099–1109. https://doi.org/10.3233/JAD-151028

Sterne, J. A., Hernán, M. A., Reeves, B. C., Savović, J., Berkman, N. D., Viswanathan, M., Henry, D., Altman, D. G., Ansari, M. T., Boutron, I., Carpenter, J. R., Chan, A. W., Churchill, R., Deeks, J. J., Hróbjartsson, A., Kirkham, J., Jüni, P., Loke, Y. K., Pigott, T. D., … Higgins, J. P. (2016). ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ (Online)*, *355*. https://doi.org/10.1136/bmj.i4919

Stewart, A., Marfell-Jones, M., Olds, T., & De Ridder, H. (2011). *International standards for anthropometric assessment* (Third edit). International Society for the Advancement of Kinanthropometry.

Straume-Naesheim, T. M., Andersen, T. E., Dvorak, J., & Bahr, R. (2005). Effects of heading exposure and previous concussions on neuropsychological performance among Norwegian elite footballers. *British Journal of Sports Medicine*, *39*, 70–77. https://doi.org/10.1136/bjsm.2005.019646

Straume-Naesheim, T. M., Andersen, T. E., Jochum, M., Dvorak, J., & Bahr, R. (2008). Minor head trauma in soccer and serum levels of S100B. *Neurosurgery*, *62*(6), 1297-1305. https://doi.org/10.1227/01.neu.0000333301.34189.3d

Svaldi, D. O., McCuen, E. C., Joshi, C., Robinson, M. E., Nho, Y., Hannemann, R., Nauman, E. A., Leverenz, L. J., & Talavage, T. M. (2017). Cerebrovascular reactivity changes in asymptomatic female athletes attributable to high school soccer participation. *Brain Imaging and Behavior*, *11*(1), 98–112. https://doi.org/10.1007/s11682-016-9509-6

Symons, G. F., Clough, M., O'Brien, W. T., Ernest, J., Salberg, S., Costello, D., Sun, M., Brady, R. D., McDonald, S. J., Wright, D. K., White, O., Abel, L., O'Brien, T. J., Mccullough, J., Aniceto, R., Lin, I.-H., Agoston, D. V, Fielding, J., Mychasiuk, R., & Shultz, S. R. (2020). Shortened telomeres and serum protein biomarker abnormalities in collision sport athletes regardless of concussion history and sex. *Journal of Concussion*, *4*. https://doi.org/10.1177/2059700220975609

Tanriverdi, F., De Bellis, A., Battaglia, M., Bellastella, G., Bizzarro, A., Sinisi, A. A., Bellastella, A., Unluhizarci, K., Selcuklu, A., Casanueva, F. F., & Kelestimur, F. (2010). Investigation of antihypothalamus and antipituitary antibodies in amateur boxers: Is chronic repetitive head trauma-induced pituitary dysfunction associated with autoimmunity? *European Journal of Endocrinology*, *162*(5), 861–867. https://doi.org/10.1530/EJE-09-1024

Tanriverdi, F., Unluhizarci, K., Coksevim, B., Selcuklu, A., Casanueva, F. F., & Kelestimur, F. (2007a). Kickboxing sport as a new cause of traumatic brain injury-mediated hypopituitarism. *Clinical Endocrinology*, *66*(3), 360–366. https://doi.org/10.1111/j.1365-2265.2006.02737.x

Tanriverdi, F., Unluhizarci, K., Kocyigit, I., Tuna, I. S., Karaca, Z., Durak, A. C., Selcuklu, A., Casanueva, F. F., & Kelestimur, F. (2008). Brief communication: Pituitary volume and function in competing and retired male boxers. *Annals of Internal Medicine*, *148*(11), 827–831. https://doi.org/10.7326/0003-4819-148-11-200806030-00005

Tanriverdi, F., Unluhizarci, K., Selcuklu, A., Casanueva, F. F., & Kelestimur, F. (2007b). Transient hypogonadotropic hypogonadism in an amateur kickboxer after head trauma. *Journal of Endocrinological Investigation*, *30*(2), 150–152. https://doi.org/10.1007/BF03347414

The Football Association. (2020). Heading Guidance. *The Football Association*, 1–13. http://www.thefa.com/news/2020/feb/24/updated-heading-guidance-announcement-240220

Tierney, G., Power, J., & Simms, C. (2020). Force experienced by the head during heading is influenced more by speed than the mechanical properties of the football. *Scandinavian Journal of Medicine and Science in Sports*, *31*(1), 124–131. https://doi.org/10.1111/sms.13816

Tierney, R. T., Higgins, M., Caswell, S. V., Brady, J., McHardy, K., Driban, J. B., & Darvish, K. (2008). Sex differences in head acceleration during heading while wearing soccer headgear. *Journal of Athletic Training*, *43*(6), 578–584. https://doi.org/10.4085/1062-6050-43.6.578

Tricco, A. C., Lillie, E., Zarin, W., O'Brien, K. K., Colquhoun, H., Levac, D., Moher, D., Peters, M. D. J., Horsley, T., Weeks, L., Hempel, S., Akl, E. A., Chang, C., McGowan, J., Stewart, L., Hartling, L., Aldcroft, A., Wilson, M. G., Garritty, C., … Straus, S. E. (2018). PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. *Annals of Internal Medicine, 169*(7), 467–473. American College of Physicians. https://doi.org/10.7326/M18-0850

Tysvaer, A. T., & Lochen, E. A. (1991). Soccer injuries to the brain: A neuropsychologic study of former soccer players. *American Journal of Sports Medicine*, *19*(1), 56–60. https://doi.org/10.1177/036354659101900109

Tysvaer, A. T., Storli, O. V., & Bachen, N. I. (1989). Soccer injuries to the brain. A neurologic and electroencephalographic study of former players. *Acta Neurologica Scandinavica*, *80*(2). https://doi.org/10.1111/j.1600-0404.1989.tb03858.x

Ueda, P., Pasternak, B., Lim, C.-E., Neovius, M., Kader, M., Forssblad, M., Ludvigsson, J. F., & Svanström, H. (2023). Neurodegenerative disease among male elite football (soccer) players in Sweden: a cohort study. *The Lancet Public Health*, *8*(4), 256–265. https://doi.org/10.1016/s2468-2667(23)00027-0

Undén, J., Ingebrigtsen, T., & Romner, B. (2013). Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: An evidence and consensus-based update. *BMC Medicine*, *11*(1). https://doi.org/10.1186/1741-7015-11-50

Wallace, C., Smirl, J. D., Zetterberg, H., Blennow, K., Bryk, K., Burma, J., Dierijck, J., Wright, A. D., & Van Donkelaar, P. (2018). Heading in soccer increases serum neurofilament light protein and SCAT3 symptom metrics. *BMJ Open Sport and Exercise Medicine*, *4*(1), 1–5. https://doi.org/10.1136/bmjsem-2018-000433

Walter, A. E., Wilkes, J. R., Arnett, P. A., Miller, S. J., Sebastianelli, W., Seidenberg, P., & Slobounov, S. M. (2022). The accumulation of subconcussive impacts on cognitive, imaging, and biomarker outcomes in child and college-aged athletes: a systematic review. *Brain Imaging & Behavior*, *16*(1), 503–517. https://doi.org/10.1007/s11682-021-00489-6

Werhahn, K. J., Kunesch, E., Noachtar, S., Benecke, R., & Classen, J. (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *Journal of Physiology*, *517*(2). https://doi.org/10.1111/j.1469-7793.1999.0591t.x

Whitehouse, D. P., Vile, A. R., Adatia, K., Herlekar, R., Roy, A. S., Mondello, S., Czeiter, E., Amrein, K., Büki, A., Maas, A. I. R., Menon, D. K., & Newcombe, V. F. J. (2022). Blood Biomarkers and Structural Imaging Correlations Post-Traumatic Brain Injury: A Systematic Review. *Neurosurgery*, *90*(2). https://doi.org/10.1227/NEU.0000000000001776

Wilcox, B. J., Beckwith, J. G., Greenwald, R. M., Raukar, N. P., Chu, J. J., McAllister, T. W., Flashman, L. A., Maerlender, A. C., Duhaime, A. C., & Crisco, J. J. (2015). Biomechanics of head impacts associated with diagnosed concussion in female collegiate ice hockey players. *Journal of Biomechanics*, *48*(10), 2201–2204. https://doi.org/10.1016/j.jbiomech.2015.04.005

Wilson, L., Stewart, W., Dams-O'Connor, K., Diaz-Arrastia, R., Horton, L., Menon, D. K., & Polinder, S. (2017). The chronic and evolving neurological consequences of traumatic brain injury. *The Lancet Neurology*, *16*(10), 813–825. https://doi.org/10.1016/S1474-4422(17)30279-X

Wirsching, A., Chen, Z., Bevilacqua, Z. W., Huibregtse, M. E., & Kawata, K. (2019). Association of Acute Increase in Plasma Neurofilament Light with Repetitive Subconcussive Head Impacts: A Pilot Randomized Control Trial. *Journal of Neurotrauma*, *36*(4), 548–553. https://doi.org/10.1089/neu.2018.5836

Withnall, C., Shewchenko, N., Wonnacott, M., & Dvorak, J. (2005). Effectiveness of headgear in football. *British Journal of Sports Medicine*, *39*, 39–48. https://doi.org/10.1136/bjsm.2005.019174

Witol, A. D., & Webbe, F. M. (2003). Soccer heading frequency predicts neuropsychological deficits*. *Archives of Clinical Neuropsychology*, *18*(4), 397–417.

https://doi.org/10.1016/S0887-6177(02)00151-8

World Medical Association. (2013). *WMA Declaration of Helsinki: ethical principles for medical research involving human subjects*. *310*(20), 2191–2194. http://www.wma.net/en/30publications/10policies/b3/index.html

Yuan, W., Diekfuss, J. A., Barber Foss, K. D., Dudley, J. A., Leach, J. L., Narad, M. E., Dicesare, C. A., Bonnette, S., Epstein, J. N., Logan, K., Altaye, M., & Myer, G. D. (2021). High School Sports-Related Concussion and the Effect of a Jugular Vein Compression Collar: A Prospective Longitudinal Investigation of Neuroimaging and Neurofunctional Outcomes. *Journal of Neurotrauma*, *38*(20), 2811–2821. https://doi.org/10.1089/neu.2021.0141

Yue, J. K., Yuh, E. L., Korley, F. K., Winkler, E. A., Sun, X., Puffer, R. C., Deng, H., Choy, W., Chandra, A., Taylor, S. R., Ferguson, A. R., Huie, J. R., Rabinowitz, M., Puccio, A. M., Mukherjee, P., Vassar, M. J., Wang, K. K. W., Diaz-Arrastia, R., Okonkwo, D. O., … Zafonte, R. (2019). Association between plasma GFAP concentrations and MRI abnormalities in patients with CT-negative traumatic brain injury in the TRACK-TBI cohort: a prospective multicentre study. *The Lancet Neurology*, *18*(10). https://doi.org/10.1016/S1474-4422(19)30282-0

Zetterberg, H., Hietala, M. A., Jonsson, M., Andreasen, N., Styrud, E., Karlsson, I., Edman, Ä., Popa, C., Rasulzada, A., Wahlund, L. O., Mehta, P. D., Rosengren, L., Blennow, K., & Wallin, A. (2006). Neurochemical aftermath of amateur boxing. *Archives of Neurology*, *63*(9), 1277–1280. https://doi.org/10.1001/archneur.63.9.1277

Zetterberg, H., Jonsson, M., Rasulzada, A., Popa, C., Styrud, E., Hietala, M. A., Rosengren, L., Wallin, A., & Blennow, K. (2007). No neurochemical evidence for brain injury caused by heading in soccer. *British Journal of Sports Medicine*, *41*(9), 574–577. https://doi.org/10.1136/bjsm.2007.037143

Zetterberg, H., Smith, D. H., & Blennow, K. (2013). Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nature Reviews. Neurology*, *9*(4), 201–210. https://doi.org/10.1038/nrneurol.2013.9

Zetterberg, H., Tanriverdi, F., Unluhizarci, K., Selcuklu, A., Kelestimur, F., & Blennow, K. (2009). Sustained release of neuron-specific enolase to serum in amateur boxers. *Brain Injury*, *23*(9), 723–726. https://doi.org/10.1080/02699050903120399

Zewdie, E., & Kirton, A. (2016). TMS Basics: Single and Paired Pulse Neurophysiology. In *Pediatric Brain Stimulation: Mapping and Modulating The Developing Brain*. https://doi.org/10.1016/B978-0-12-802001-2.00001-1

Zhang, L., Yang, K. H., & King, A. I. (2004). A Proposed Injury Threshold for Mild Traumatic Brain Injury. *Journal of Biomechanical Engineering*, *126*(2), 226–236. https://doi.org/10.1115/1.1691446

Zonner, S. W., Ejima, K., Bevilacqua, Z. W., Huibregtse, M. E., Charleston, C., Fulgar, C., & Kawata, K. (2019). Association of increased serum S100B levels with high school football subconcussive head impacts. *Frontiers in Neurology*, *10*, 1–10. https://doi.org/10.3389/fneur.2019.00327

Appendices

Appendix A: Biofluid Marker Data (Chapter 3)

Table A.1. Mean biofluid marker levels with standard deviation and range for experiment one (soccer study) and two (sparring study).

		Baseline	0 h	24 hrs	48 hrs	2 wks
S100B (ug/L)	**Exp. 1**	0.035 ± 0.018 (0.011–0.080)	0.034 ± 0.016 (0.012–0.075)	0.034 ± 0.017 (0.012–0.075)	0.033 ± 0.019 (0.013–0.094)	0.037 ± 0.017 (0.012–0.083)
	Exp. 2	0.049 ± 0.022 (0.023–0.100)	0.088 ± 0.046 (0.029–0.213)	0.052 ± 0.024 (0.025–0.110)	N/A	N/A
GFAP (pg/mL)	**Exp. 1**	69.16 ± 21.61 (34.33–122.76)	68.93 ± 21.38 (30.29–104.16)	74.50 ± 28.84 (33.09–146.18)	67.59 ± 22.41 (30.40–104.63)	64.03 ± 23.62 (29.40–128.85)
	Exp. 2	59.62 ± 15.90 (32.96–83.66)	63.75 ± 22.49 (22.65–122.37)	71.47 ± 35.78 (19.96–181.87)	N/A	N/A
NfL (pg/mL)	**Exp. 1**	7.42 ± 3.26 (2.50–15.09)	6.58 ± 2.76 (2.28–12.29)	7.71 ± 2.83 (3.47–14.65)	7.58 ± 3.28 (2.97–16.16)	6.51 ± 2.41 (2.38–12.77)
	Exp. 2	7.69 ± 2.13 (4.47–12.60)	8.42 ± 4.20 (3.37–21.57)	7.76 ± 2.91 (4.23–14.45)	N/A	N/A
UCHL-1 (pg/mL)	**Exp. 1**	15.39 ± 7.53 (5.31–31.61)	16.87 ± 8.67 (2.78–38.88)	14.78 ± 8.77 (3.32–35.70)	17.05 ± 9.56 (6.12–42.89)	14.70 ± 8.82 (2.10–33.80)
	Exp. 2	18.59 ± 12.50 (5.08–47.33)	28.57 ± 19.12 (2.60–80.90)	14.68 ± 6.57 (4.03–29.34)	N/A	N/A
Tau (pg/mL)	**Exp. 1**	2.95 ± 1.80 (0.05–7.56)	2.80 ± 1.76 (0.15–7.85)	2.77 ± 1.72 (0.24–7.53)	2.81 ± 1.72 (0.14–6.66)	2.87 ± 2.02 (0.46–7.51)
	Exp. 2	2.94 ± 1.56 (0.97–5.86)	5.24 ± 2.69 (1.10–9.75)	3.82 ± 1.86 (0.94–6.58)	N/A	N/A
P-tau181 (pg/mL)	**Exp. 1**	5.56 ± 2.67 (0.63–11.94)	6.63 ± 4.02 (0.30–16.31)	5.50 ± 3.22 (0.47–13.19)	6.11 ± 3.70 (0.14–15.73)	5.11 ± 2.39 (0.39–8.46)
	Exp. 2	5.49 ± 1.34 (3.58–7.93)	8.43 ± 2.36 (4.31–13.05)	5.74 ± 2.49 (2.71–12.20)	N/A	N/A

Appendix B: Sample Size Calculation

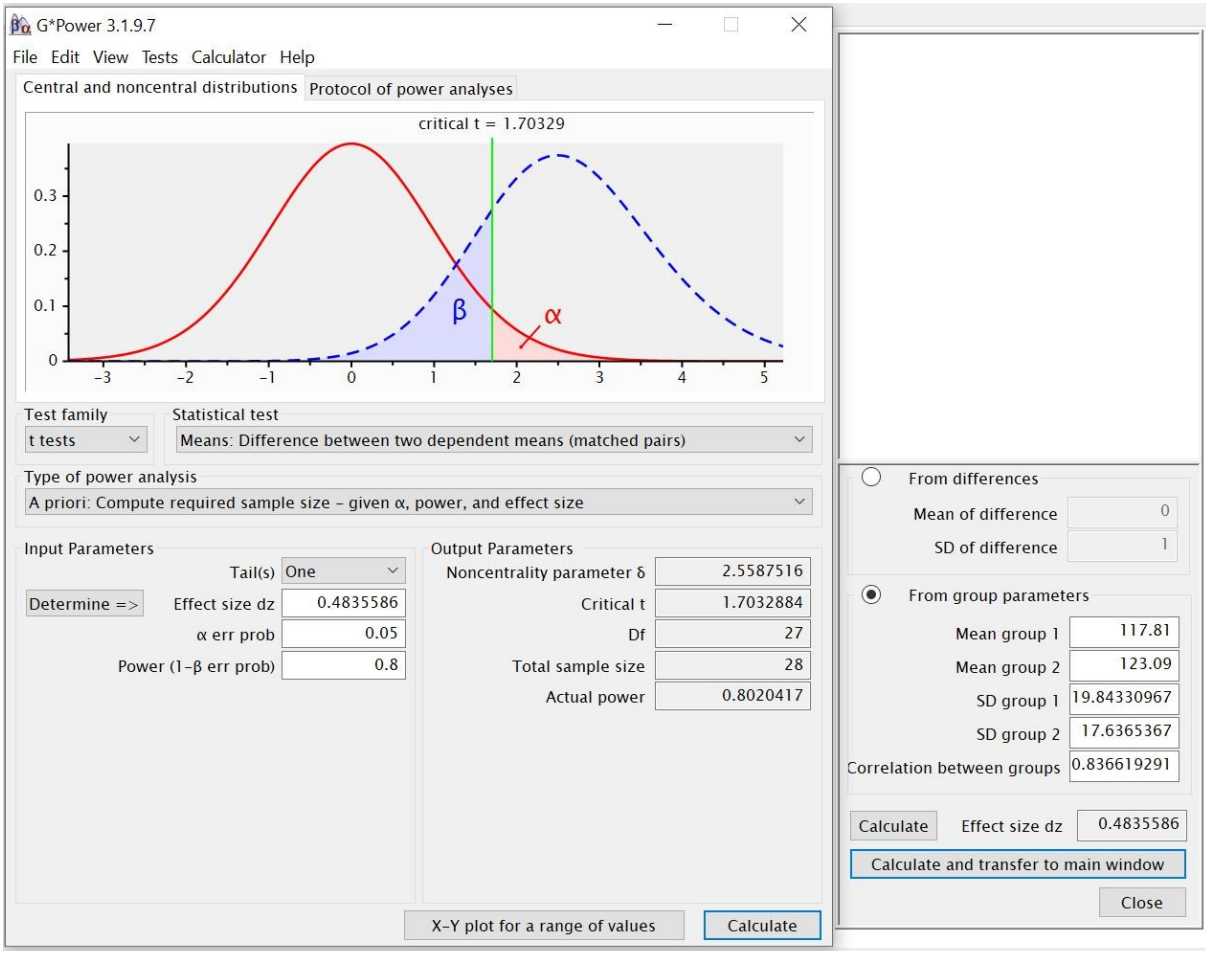


Figure A.1. Sample size calculation based on Di Virgilio et al. (2016) data.

Appendix C: Transcranial Magnetic Stimulation Data Extraction Protocol and Results

Protocol

Two raters (L-ML and AM[4]) extracted the timestamp for silent period (SP) offset for each trial. L-ML also extracted the timestamp for the offset of the trigger (TMS stimulation). SP duration was defined as the time between the end of the trigger and resumption of continuous voluntary EMG data (Figure C.1). Breakthrough EMG activity was included in the SP duration (since it is unlikely to reflect cortical activity) as recommended by Hupfeld et al. (2020). In cases where (complete) EMG silence did not occur and/or could not be distinguished from gradual resumption of voluntary EMG activity the signals were excluded. Furthermore, only the duration of the first SP was recorded in trials with secondary silent period also present. If the primary SP was not distinguishable from the secondary SP, then the signal was excluded.

Difference of ≥ 5 ms in SP offset between raters was considered a discrepancy and was reviewed by a third rater (TDV[5]). L-ML's extracted data were used in the analysis when the difference between L-ML's and AM's extracted data were ≤ 4 ms.

L-ML and AM also extracted the peak-to-peak amplitude of motor evoked potentials (MEP) for the later calculation of SP:MEP ratio. Both raters (L-ML and AM) also extracted the timestamp for MEP onset. The purpose of both L-ML and AM extracting MEP peak-to-peak amplitude data was to address ambiguity surrounding atypical signals such as signals without a clear MEP. Any discrepancies in MEP amplitude were reviewed. Extracting the onset of the MEPs helped review discrepancies in MEP amplitude data.

Prior to the start of the data extraction L-ML and AM performed a pilot extraction to ensure consistency in the extraction methods between the raters. For the pilot, L-ML and AM extracted data for 72 signals. Any disagreements in individual signals (≥ 5 ms) between L-ML and AM in the pilot data extraction were discussed with TDV with the aim to reach a consensus on how to extract data consistently across raters.

All raters (L-ML, AM and TDV) were blinded to the participant, condition and sampling time information. TDV was also blinded to the rater information (L-ML or AM) during the consensus process.

[4] Ali Muqtadir (AM) – a PhD candidate at University of Stirling, Psychology Division
[5] Dr Thomas Di Virgilio (TDV) – a researcher and lecturer at University of Stirling, Physiology, Exercise and Nutrition Research Group

Data were extracted using the AcqKnowledge software (version 3.9.1.6, BIOPAC Systems, Inc., Goleta, CA, USA). EMG signals were not rectified. The scale (horizontal and vertical) of the EMG signal was standardised within the files when extracting the data (approximately 0.25 seconds per division).

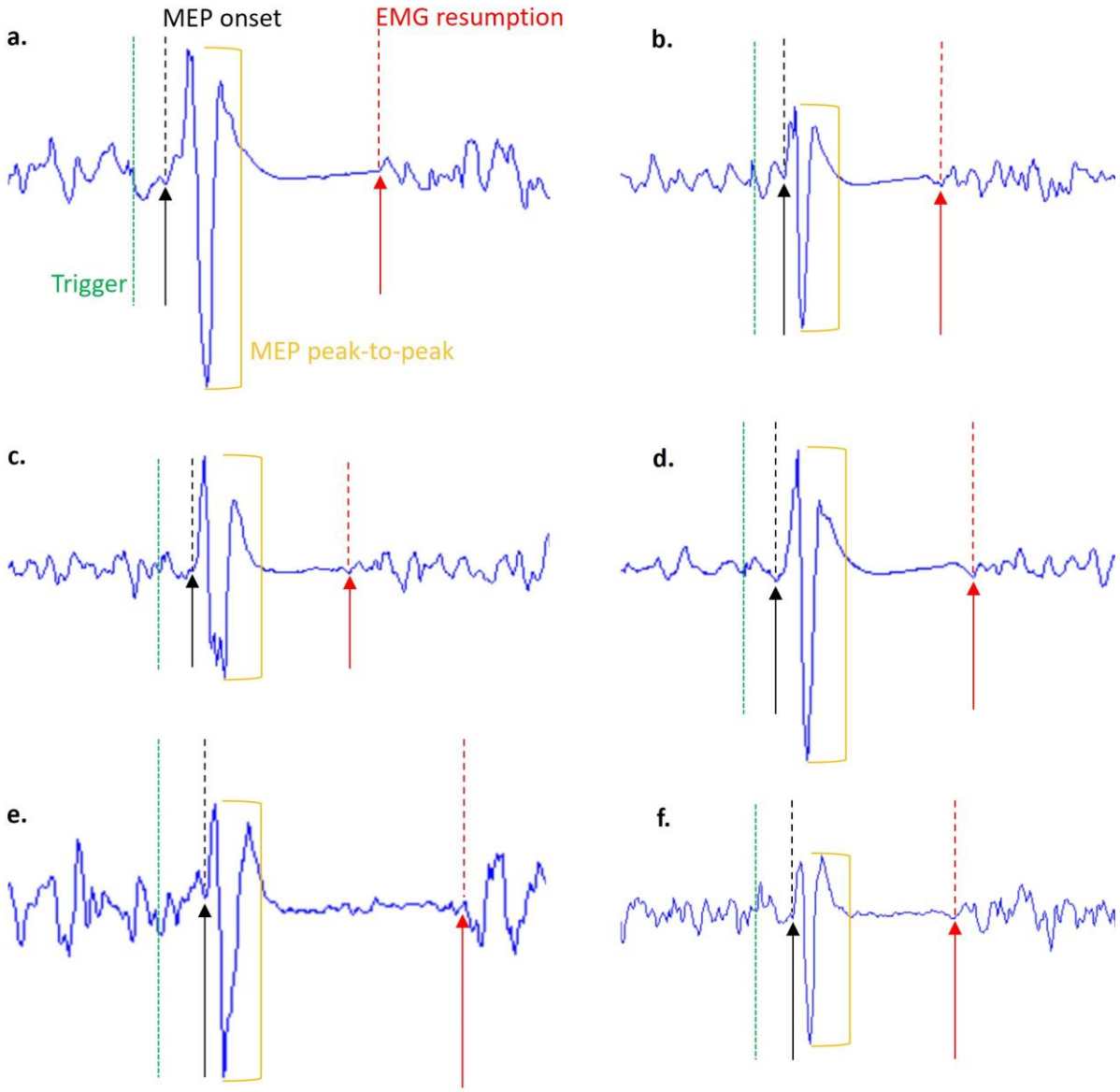


Figure C.1. Six independent examples of onset of MEP and offset of SP (a.to f.). TMS trigger is indicated in green, MEP onset is marked with back, MEP peak-to peak amplitude is shown in yellow and the resumption of continuous voluntary EMG signal is displayed in red.

Results

Silent Period Data

L-ML and AM extracted data for 1064 rectus femoris signals, of which 355 (33.4%) SP offset timestamps were discrepant (≥ 5 ms) between the reviewers. TDV reviewed 358 signals in total (33.6% of the entire data; the three extra signals were reviewed by mistake as they were not discrepant). Of the 358 signals TDV assigned an identical value to either reviewer one or two to 92 (25.7%) signals, and a value that was < 5 ms different to reviewer one or two to 199 (55.6%) signals. Further, 35 (9.8%) signals were assigned a value that was ≥ 5 ms different to reviewer one and two by TDV and these signals did not need further discussion upon review. Finally, 32 (8.9%) signals were assigned a value ≥ 5 ms different to reviewer one and two by TDV and these signals needed further consensus between the reviewers (i.e., signals were atypical).

It total, 29 (2.7%) signals were considered not analysable (end of the silent period and resumption of uninterrupted EMG were not clear or silent period was not 'silent') as a result of the consensus process.

Intraclass correlation coefficient (ICC) was calculated between L-ML and AM for SP duration assessment for signals that were considered analysable by both reviewers (1028 out of 1064 signals, 96.6%). ICC estimates were calculated using a two-way mixed model and absolute agreement type. ICC between raters one and two was 0.861 (95% CI = 0.812–0.894), indicating good reliability (where ICC < 0.40 = poor, 0.4–0.59 = fair, 0.6–0.74 = good and 0.75–1 = excellent reliability; Cicchetti, 1994).

Peak-to-Peak Amplitude Data

Of the 1064 signals for which MEP amplitude data was extracted 35 (3.3%) were discrepant (i.e., not identical values) between reviewer one and two. Twelve (34%) of the 35 discrepant signals did not need reviewing as those signals were considered not analysable during the SP offset consensus process and were excluded from the dataset. Remaining 23 peak-to-peak amplitude discrepancies were reviewed by L-ML and when necessary, by TDV (six signals were reviewed by L-ML alone and 17 by L-ML and TDV together). One of the signals reviewed by L-ML alone was discrepant since it contained a typo and therefore did not need reviewing by TDV. The remaining five signals reviewed by L-ML alone had data available from only one of the two raters (either L-ML or AM) since either of the reviewers had considered the signal not analysable. However, as a results of the SP offset consensus process those

five signals were considered analysable and as such the amplitude data available from either of the reviewers was put forward for the analysis.

Appendix D: Chapter 4 Data

Table D.1. Inhibition (silent period duration), inhibition to excitability ratio (silent period to motor evoked potential ratio) and muscular fatigue/participant effort (maximum voluntary contraction force) mean ± *SD* data.

			Baseline	0 h	1 h
SP (ms)	**Experiment 1**	**20 kicks (control)**	103.9 ± 13.0	101.4 ± 13.9	104.8 ± 13.1
		10 headers	103.7 ± 15.5	104.0 ± 18.1	104.8 ± 17.8
		20 headers	105.3 ± 16.3	102.9 ± 13.3	104.6 ± 14.3
	Experiment 2	**20 kicks (control)**	104.3 ± 13.3	102.0 ± 14.1	104.8 ± 13.5
		20 headers	104.4 ± 16.5	102.6 ± 13.5	105.0 ± 14.4
		20 headers w HG	104.0 ± 15.6	103.9 ± 14.4	105.1 ± 15.4
SP:MEP	**Experiment 1**	**20 kicks (control)**	21.6 ± 8.9	22.8 ± 10.5	24.3 ± 10.4
		10 headers	21.0 ± 10.7	21.3 ± 9.7	23.3 ± 11.4
		20 headers	21.0 ± 7.4	20.9 ± 7.3	22.0 ± 8.3
	Experiment 2	**20 kicks (control)**	21.8 ± 9.2	23.0 ± 11.0	24.4 ± 10.9
		20 headers	21.0 ± 7.6	21.0 ± 7.6	22.1 ± 8.5
		20 headers w HG	22.1 ± 10.1	23.2 ± 9.7	24.1 ± 10.2
MVC (N)	**Experiment 1**	**20 kicks (control)**	311.8 ± 111.4	302.3 ± 109.3	297.5 ± 115.0
		10 headers	308.7 ± 108.5	317.0 ± 121.5	307.8 ± 122.4
		20 headers	309.7 ± 114.8	315.3 ± 104.2	310.7 ± 104.2
	Experiment 2	**20 kicks (control)**	310.7 ± 114.2	304.8 ± 112.9	300.5 ± 118.1
		20 headers	312.4 ± 118.5	318.9 ± 107.0	312.8 ± 107.7
		20 headers w HG	311.5 ± 115.5	316.1 ± 115.7	310.5 ± 121.6

w HG – with headgear; SP – silent period; MEP – motor evoked potential; MVC – maximum voluntary contraction

Table D.2. Mean motor unit firing rate and motor unit firing rate CV% data (mean ± *SD*).

			Baseline	0 h	1 h
Firing rate (pps)	**Experiment 1**	**20 kicks (control)**	11.46 ± 2.82	12.00 ± 3.87	12.47 ± 3.93
		10 headers	11.66 ± 3.44	11.94 ± 3.94	11.22 ± 2.71
		20 headers	11.88 ± 4.44	11.50 ± 2.83	11.53 ± 2.88
	Experiment 2	**20 kicks (control)**	10.95 ± 2.80	12.04 ± 3.81	12.33 ± 3.86
		20 headers	11.95 ± 4.44	11.65 ± 2.70	11.47 ± 2.71
		20 headers w HG	11.66 ± 3.25	11.42 ± 3.25	12.29 ± 3.30
Firing rate CV%	**Experiment 1**	**20 kicks (control)**	38.37 ± 11.33	36.68 ± 11.15	35.36 ± 10.45
		10 headers	36.02 ± 7.25	34.54 ± 10.58	38.65 ± 12.45
		20 headers	36.61 ± 15.21	37.74 ± 14.43	35.77 ± 13.25
	Experiment 2	**20 kicks (control)**	39.58 ± 10.54	36.19 ± 12.33	34.52 ± 10.78
		20 headers	36.00 ± 15.73	36.28 ± 13.16	36.06 ± 12.88
		20 headers w HG	38.46 ± 8.85	38.50 ± 9.48	33.69 ± 11.84

w HG – with headgear; pps – pulses per second; CV% – coefficient of variation

Table D.3. Results for cognitive task performed during gait trials (mean ± *SD*).

		Baseline	0 h	Δ
Experiment one	**20 kicks (control)**	1.04 ± 0.08	1.04 ± 0.05	-0.01 ± 0.06
	10 headers	1.06 ± 0.08	1.05 ± 0.06	-0.01 ± 0.07
	20 headers	1.05 ± 0.09	1.04 ± 0.07	-0.01 ± 0.05
Experiment two	**20 kicks (control)**	1.04 ± 0.09	1.03 ± 0.05	-0.01 ± 0.06
	20 headers	1.05 ± 0.10	1.04 ± 0.07	0.02 ± 0.09
	20 headers with HG	1.04 ± 0.09	1.03 ± 0.04	-0.01 ± 0.05

Ratio of total number of answers divided by number of correct responses (lower ratio indicates better performance). Δ – difference between post heading/kicking and baseline performance (negative change denotes improvement in performance). HG – headgear

Table D.4. Gait temporal variables mean data for undivided and divided attention trials (mean ± *SD*).

			20 kicks (control)		10 headers		20 headers		20 headers with HG	
			Undivided	**Divided**	**Undivided**	**Divided**	**Undivided**	**Divided**	**Undivided**	**Divided**
Walking speed (m/s)	**Exp. 1**	Baseline	1.03 ± 0.09	0.96 ± 0.10	1.04 ± 0.09	0.97 ± 0.11	1.04 ± 0.10	0.96 ± 0.11	N/A	N/A
		0 h	1.03 ± 0.11	0.96 ± 0.11	1.03 ± 0.10	0.96 ± 0.10	1.04 ± 0.09	0.96 ± 0.10	N/A	N/A
	Exp. 2	Baseline	1.03 ± 0.09	0.97 ± 0.10	N/A	N/A	1.04 ± 0.11	0.96 ± 0.12	1.02 ± 0.11	0.95 ± 0.12
		0 h	1.03 ± 0.11	0.96 ± 0.11	N/A	N/A	1.04 ± 0.10	0.96 ± 0.11	1.02 ± 0.12	0.95 ± 0.11
Stride (s)	**Exp. 1**	Baseline	1.24 ± 0.10	1.29 ± 0.12	1.23 ± 0.09	1.29 ± 0.13	1.24 ± 0.10	1.30 ± 0.12	N/A	N/A
		0 h	1.25 ± 0.12	1.30 ± 0.12	1.24 ± 0.10	1.29 ± 0.12	1.25 ± 0.09	1.30 ± 0.12	N/A	N/A
	Exp. 2	Baseline	1.23 ± 0.10	1.28 ± 0.13	N/A	N/A	1.24 ± 0.10	1.29 ± 0.14	1.25 ± 0.11	1.29 ± 0.15
		0 h	1.24 ± 0.12	1.29 ± 0.13	N/A	N/A	1.24 ± 0.10	1.30 ± 0.13	1.24 ± 0.12	1.28 ± 0.13
Step (s)	**Exp. 1**	Baseline	0.62 ± 0.05	0.64 ± 0.06	0.62 ± 0.05	0.64 ± 0.06	0.62 ± 0.05	0.65 ± 0.06	N/A	N/A
		0 h	0.62 ± 0.06	0.65 ± 0.06	0.62 ± 0.05	0.65 ± 0.06	0.62 ± 0.05	0.65 ± 0.06	N/A	N/A
	Exp. 2	Baseline	0.61 ± 0.05	0.64 ± 0.06	N/A	N/A	0.62 ± 0.05	0.65 ± 0.07	0.62 ± 0.06	0.64 ± 0.07
		0 h	0.62 ± 0.06	0.64 ± 0.07	N/A	N/A	0.62 ± 0.05	0.65 ± 0.07	0.62 ± 0.06	0.64 ± 0.07
Stance (s)	**Exp. 1**	Baseline	0.75 ± 0.06	0.77 ± 0.07	0.74 ± 0.06	0.77 ± 0.09	0.75 ± 0.07	0.78 ± 0.09	N/A	N/A
		0 h	0.75 ± 0.08	0.78 ± 0.08	0.75 ± 0.07	0.78 ± 0.08	0.75 ± 0.06	0.78 ± 0.08	N/A	N/A
	Exp. 2	Baseline	0.74 ± 0.07	0.77 ± 0.08	N/A	N/A	0.74 ± 0.07	0.78 ± 0.10	0.75 ± 0.07	0.78 ± 0.09
		0 h	0.75 ± 0.08	0.78 ± 0.09	N/A	N/A	0.75 ± 0.06	0.78 ± 0.09	0.75 ± 0.07	0.78 ± 0.09

Stance (% GC)	**Exp. 1**	Baseline	60.2 ± 1.2	60.1 ± 1.4	59.9 ± 1.5	60.1 ± 1.7	60.1 ± 1.4	60.2 ± 1.5	N/A	N/A
		0 h	60.5 ± 1.6	60.4 ± 1.5	60.5 ± 1.5	60.5 ± 1.7	60.0 ± 1.7	60.2 ± 1.8	N/A	N/A
	Exp. 2	Baseline	60.1 ± 1.2	60.1 ± 1.3	N/A	N/A	59.9 ± 1.1	60.2 ± 1.3	60.0 ± 1.1	60.2 ± 1.3
		0 h	60.6 ± 1.6	60.5 ± 1.4	N/A	N/A	60.2 ± 1.5	60.3 ± 1.7	60.3 ± 1.2	60.5 ± 1.4
Swing (s)	**Exp. 1**	Baseline	0.49 ± 0.04	0.51 ± 0.05	0.49 ± 0.04	0.51 ± 0.05	0.50 ± 0.04	0.52 ± 0.05	N/A	N/A
		0 h	0.49 ± 0.04	0.51 ± 0.05	0.49 ± 0.04	0.51 ± 0.05	0.50 ± 0.04	0.52 ± 0.05	N/A	N/A
	Exp. 2	Baseline	0.49 ± 0.04	0.51 ± 0.05	N/A	N/A	0.50 ± 0.04	0.52 ± 0.06	0.50 ± 0.04	0.51 ± 0.06
		0 h	0.49 ± 0.05	0.51 ± 0.05	N/A	N/A	0.49 ± 0.04	0.51 ± 0.05	0.49 ± 0.04	0.51 ± 0.05
Swing (% GC)	**Exp. 1**	Baseline	39.8 ± 1.2	39.9 ± 1.4	40.1 ± 1.5	39.9 ± 1.7	39.9 ± 1.4	39.8 ± 1.5	N/A	N/A
		0 h	39.5 ± 1.6	39.6 ± 1.5	39.5 ± 1.5	39.5 ± 1.7	40.0 ± 1.7	39.8 ± 1.8	N/A	N/A
	Exp. 2	Baseline	39.9 ± 1.2	39.9 ± 1.3	N/A	N/A	40.1 ± 1.1	39.8 ± 1.3	40.0 ± 1.1	39.8 ± 1.3
		0 h	39.4 ± 1.6	39.5 ± 1.4	N/A	N/A	39.8 ± 1.5	39.7 ± 1.7	39.7 ± 1.2	39.5 ± 1.4
Single leg support (% GC)	**Exp. 1**	Baseline	39.8 ± 1.2	39.9 ± 1.4	40.1 ± 1.6	39.9 ± 1.7	39.9 ± 1.4	39.7 ± 1.5	N/A	N/A
		0 h	39.5 ± 1.6	39.6 ± 1.5	39.5 ± 1.5	39.5 ± 1.7	40.0 ± 1.7	39.8 ± 1.9	N/A	N/A
	Exp. 2	Baseline	39.9 ± 1.2	39.9 ± 1.3	N/A	N/A	40.1 ± 1.1	39.8 ± 1.3	40.0 ± 1.1	39.8 ± 1.3
		0 h	39.4 ± 1.6	39.5 ± 1.4	N/A	N/A	39.8 ± 1.5	39.6 ± 1.7	39.7 ± 1.3	39.5 ± 1.3
Double leg support (% GC)	**Exp. 1**	Baseline	20.3 ± 2.5	20.2 ± 2.8	19.8 ± 3.1	20.1 ± 3.5	20.1 ± 2.9	20.5 ± 2.9	N/A	N/A
		0 h	21.0 ± 3.2	20.8 ± 3.0	21.0 ± 2.9	21.0 ± 3.4	20.1 ± 3.4	20.5 ± 3.7	N/A	N/A
	Exp. 2	Baseline	20.1 ± 2.3	20.1 ± 2.6	N/A	N/A	19.9 ± 2.3	20.3 ± 2.6	20.0 ± 2.3	20.4 ± 2.6

		0 h	21.2 ± 3.1	21.0 ± 2.8	N/A	N/A	20.4 ± 3.0	20.7 ± 3.3	20.5 ± 2.5	21.0 ± 2.7

HG – headgear; GC – gait cycle

Table D.5. Gait temporal variables CV% data for undivided and divided attention trials (mean ± *SD*).

			20 kicks (control)		10 headers		20 headers		20 headers with HG	
			Undivided	**Divided**	**Undivided**	**Divided**	**Undivided**	**Divided**	**Undivided**	**Divided**
Walking speed (m/s) (log)	**Exp. 1**	Baseline	0.52 ± 0.16	0.49 ± 0.18	0.50 ± 0.16	0.52 ± 0.17	0.44 ± 0.15	0.52 ± 0.13	N/A	N/A
		0 h	0.46 ± 0.19	0.50 ± 0.15	0.50 ± 0.19	0.53 ± 0.17	0.46 ± 0.15	0.54 ± 0.13	N/A	N/A
	Exp. 2	Baseline	0.51 ± 0.16	0.48 ± 0.18	N/A	N/A	0.43 ± 0.15	0.50 ± 0.13	0.46 ± 0.12	0.49 ± 0.14
		0 h	0.45 ± 0.19	0.48 ± 0.13	N/A	N/A	0.45 ± 0.14	0.51 ± 0.10	0.46 ± 0.10	0.50 ± 0.19
Stride (s)	**Exp. 1**	Baseline	3.84 ± 1.20	4.05 ± 1.11	3.85 ± 1.29	4.13 ± 1.29	3.79 ± 1.26	4.15 ± 1.22	N/A	N/A
		0 h	3.76 ± 1.31	4.17 ± 1.48	3.71 ± 1.27	4.20 ± 1.26	3.86 ± 1.26	4.30 ± 1.31	N/A	N/A
	Exp. 2	Baseline	3.78 ± 1.26	4.10 ± 1.08	N/A	N/A	3.82 ± 1.31	4.24 ± 1.23	3.89 ± 1.42	4.24 ± 1.16
		0 h	3.82 ± 1.39	4.29 ± 1.52	N/A	N/A	3.95 ± 1.28	4.39 ± 1.36	3.89 ± 1.33	4.32 ± 1.06
Step (s)	**Exp. 1**	Baseline	5.30 ± 1.28	5.56 ± 1.20	5.35 ± 1.25	5.67 ± 1.20	5.16 ± 1.45	5.60 ± 1.30	N/A	N/A
		0 h	5.57 ± 1.71	5.64 ± 1.47	5.37 ± 1.57	5.75 ± 1.48	5.25 ± 1.40	5.76 ± 1.54	N/A	N/A
	Exp. 2	Baseline	5.28 ± 1.33	5.68 ± 1.14	N/A	N/A	5.21 ± 1.52	5.71 ± 1.35	5.38 ± 1.56	5.87 ± 1.31
		0 h	5.68 ± 1.77	5.76 ± 1.46	N/A	N/A	5.37 ± 1.44	5.83 ± 1.61	5.49 ± 1.85	5.98 ± 1.67
Stance (s)	**Exp. 1**	Baseline	4.94 ± 1.18	5.28 ± 1.10	5.08 ± 1.40	5.39 ± 1.33	4.89 ± 1.53	5.36 ± 1.35	N/A	N/A
		0 h	5.19 ± 1.66	5.37 ± 1.58	4.96 ± 1.44	5.30 ± 1.34	4.79 ± 1.50	5.34 ± 1.44	N/A	N/A
	Exp. 2	Baseline	4.87 ± 1.22	5.36 ± 1.05	N/A	N/A	4.85 ± 1.55	5.46 ± 1.40	4.98 ± 1.54	5.41 ± 1.17
		0 h	5.25 ± 1.67	5.49 ± 1.59	N/A	N/A	4.91 ± 1.52	5.48 ± 1.46	5.26 ± 1.66	5.61 ± 1.31

Stance (% GC)	**Exp. 1**	Baseline	3.04 ± 0.60	3.20 ± 0.64	3.16 ± 0.66	3.33 ± 0.77	2.92 ± 0.85	3.21 ± 0.80	N/A	N/A
		0 h	3.43 ± 1.39	3.18 ± 0.71	3.18 ± 0.81	3.24 ± 0.67	2.91 ± 0.72	2.96 ± 0.61	N/A	N/A
	Exp. 2	Baseline	3.01 ± 0.56	3.25 ± 0.60	N/A	N/A	2.84 ± 0.75	3.24 ± 0.81	2.95 ± 0.75	3.22 ± 0.72
		0 h	3.43 ± 1.22	3.18 ± 0.59	N/A	N/A	2.95 ± 0.76	2.99 ± 0.63	3.30 ± 0.97	3.47 ± 1.11
Swing (s)	**Exp. 1**	Baseline	6.12 ± 1.36	6.37 ± 1.21	6.19 ± 1.33	6.61 ± 1.47	5.84 ± 1.62	6.44 ± 1.41	N/A	N/A
		0 h	6.71 ± 2.14	6.45 ± 1.44	6.26 ± 1.70	6.67 ± 1.65	6.02 ± 1.60	6.18 ± 1.36	N/A	N/A
	Exp. 2	Baseline	6.02 ± 1.43	6.41 ± 1.20	N/A	N/A	5.73 ± 1.60	6.48 ± 1.47	5.96 ± 1.65	6.50 ± 1.59
		0 h	6.70 ± 2.07	6.49 ± 1.43	N/A	N/A	6.13 ± 1.67	6.27 ± 1.42	6.29 ± 1.71	6.96 ± 1.98
Swing (% GC)	**Exp. 1**	Baseline	4.60 ± 0.99	4.84 ± 1.03	4.75 ± 1.12	5.03 ± 1.27	4.40 ± 1.32	4.86 ± 1.18	N/A	N/A
		0 h	5.29 ± 2.22	4.83 ± 1.00	4.90 ± 1.26	5.03 ± 1.25	4.40 ± 1.18	4.47 ± 0.86	N/A	N/A
	Exp. 2	Baseline	4.53 ± 0.91	4.89 ± 0.91	N/A	N/A	4.23 ± 1.07	4.87 ± 1.11	4.42 ± 1.10	4.85 ± 1.00
		0 h	5.31 ± 2.02	4.85 ± 0.82	N/A	N/A	4.48 ± 1.22	4.55 ± 0.90	4.99 ± 1.42	5.44 ± 1.74
Single leg support (% GC)	**Exp. 1**	Baseline	4.88 ± 1.04	4.99 ± 1.02	4.94 ± 1.01	5.21 ± 1.22	4.60 ± 1.23	5.04 ± 1.10	N/A	N/A
		0 h	5.55 ± 2.10	5.00 ± 0.98	5.24 ± 1.53	5.28 ± 1.32	4.69 ± 1.13	4.62 ± 0.84	N/A	N/A
	Exp. 2	Baseline	4.80 ± 0.95	5.04 ± 0.91	N/A	N/A	4.40 ± 0.91	5.04 ± 1.05	4.60 ± 1.07	4.99 ± 0.92
		0 h	5.55 ± 1.92	5.01 ± 0.75	N/A	N/A	4.76 ± 1.17	4.70 ± 0.87	5.20 ± 1.40	5.49 ± 1.68
Double leg support (% GC)	**Exp. 1**	Baseline	8.39 ± 1.83	7.87 ± 1.63	8.22 ± 1.58	8.24 ± 1.95	7.85 ± 1.37	7.78 ± 1.50	N/A	N/A
		0 h	8.43 ± 2.01	7.44 ± 1.48	8.53 ± 2.21	7.92 ± 2.06	8.05 ± 1.69	7.79 ± 1.77	N/A	N/A
	Exp. 2	Baseline	8.34 ± 1.75	7.67 ± 1.02	N/A	N/A	7.55 ± 1.03	7.68 ± 1.27	7.98 ± 1.44	7.79 ± 1.22

		0 h	8.41 ± 1.83	7.56 ± 1.49	N/A	N/A	7.95 ± 1.68	7.55 ± 1.55	9.07 ± 3.06	8.09 ± 1.98

HG – headgear; CV% – coefficient of variation; GC – gait cycle

Appendix E: Follow-up Univariate Analysis Results (Chapter 4)

Table E.1. Temporal gait variables that were significantly different between divided and undivided attention trials.

Experiment One (Dose Response Study)
Mean Data Results: Stride (s): $F(1,23) = 21.198$, $p < 0.001$, $\eta^2 = 0.480$ Step (s): $F(1,23) = 21.124$, $p < 0.001$, $\eta^2 = 0.479$ Stance (s): $F(1,23) = 18.183$, $p < 0.001$, $\eta^2 = 0.442$ Swing (s): $F(1,23) = 22.329$, $p < 0.001$, $\eta^2 = 0.493$
CV% Data Results: Stride (s): $F(1,23) = 6.367$, $p = 0.019$, $\eta^2 = 0.217$ Step (s): $F(1,23) = 6.609$, $p = 0.017$, $\eta^2 = 0.223$ Stance (s): $F(1,23) = 6.532$, $p = 0.018$, $\eta^2 = 0.221$
Experiment Two (Headgear Study)
CV% Data Results: Stride (s): $F(1,20) = 5.142$, $p = 0.035$, $\eta^2 = 0.205$ Step (s): $F(1,20) = 5.341$, $p = 0.032$, $\eta^2 = 0.211$ Stance (s): $F(1,20) = 6.712$, $p = 0.017$, $\eta^2 = 0.251$ Double support (s): $F(1,20) = 15.336$, $p = 0.016$, $\eta^2 = 0.256$