Neurovascular implications of life-long exposure to contact in rugby union; the link to cognition

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Abstract

Introduction: Recurrent contact and concussion in sport represents a significant and growing public health concern which may contribute to long-term neurologic sequela in later-life. To what extent this relates to an accelerated decline in cerebral perfusion, a major risk factor for cognitive impairment and neurodegeneration remains to be explored.

Aims: This thesis sought to: 1) determine the molecular, cerebrovascular and cognitive signatures of professional rugby union players over a single season, stratified by frequency of contact events, playing position and concussion risk, and 2) determine the chronic molecular, cerebrovascular and cognitive signatures of formerly concussed retired rugby players exposed to recurrent contact.

Hypothesis: It was hypothesised that compared to controls, formerly concussed rugby union players would present with elevated oxidative-nitrosative (OXNOS) stress, suppressed cerebrovascular function and a decline in cognition.

Methodology: *Study 1* **–** A longitudinal study was conducted across one rugby union season. Participants were divided into two separate groups. Twenty-one professional rugby union players aged 25 ± 4 years with 3 ± 2 previous concussions incurred over 16 ± 4 years were compared with 17 sex, age-, physical activity- and education-matched controls, with no participation in contact sports or concussion history. Data were collected at three time points: 1) pre-season, 2) in-season, and 3) post-season. During pre- and post-season, venous concentrations of the ascorbate radical ($A^{\bullet-}$) and total bioactive nitric oxide (NO – nitrite [NO_2^-] and S-nitrosothiols [RSNO]) were obtained. Transcranial Doppler (TCD) ultrasonography was used to determine cerebral perfusion via middle cerebral artery velocity (MCAv). The range of cerebrovascular reactivity to changes in end tidal carbon dioxide ($CVR_{CO2RANGE}$) was assessed using hypercapnia ($CVR_{CO2HYPER}$) and hypocapnia ($CVR_{CO2HYPO}$). Cognition was assessed via neuropsychometric tests and screened for mild cognitive impairment (MCI) using the Montreal Cognitive Assessment (MoCA). In-season notational analysis observed all match events throughout each game of the season with all incurred injuries noted. ***Study 2*** **–** Twenty retired rugby players aged 64 ± 5 years with 3 ± 3 concussions incurred over 22 ± 7 years were compared to 21 sex, age-, cardiorespiratory fitness (CRF)- and education-matched controls with no participation in contact sports or concussion history. Venous concentrations of $A^{\bullet-}$, and total bioactive NO were obtained. Cerebral perfusion was determined via MCAv. Cerebrovascular reactivity was assessed in response to

hypercapnia/hypocapnia. Cognition was assessed via neuropsychometric tests and screened for MCI using the MoCA.

Results: *Study 1* – Elevated OXNOS was apparent in players during pre-season. Players had lower $CVR_{CO2RANGE}$, memory, fine-motor coordination and executive function compared to controls. The concussion incidence rate corresponded to 10 concussions per 1000 match hours throughout the season. When players were divided into forwards (n = 13) and backs (n = 8), forwards were subject to more contact events per game, most notably for collisions, jackals and tackles. During post-season, elevated nitrosative stress remained apparent in players compared to controls, despite no differences in oxidative stress. Similarly, $CVR_{CO2RANGE}$ decreased by 18% across the season in players. Players confirmed reductions in memory, fine-motor coordination and executive function compared to controls. *Study 2* – Retired players had lower total NO bioactivity, despite an absence of elevated oxidative stress compared to controls. Cerebral perfusion was lower in players at rest and throughout hyper/hypocapnia, however no differences in CVR_{CO2} were detected between-groups. Prolonged concussion symptomology was apparent in players during completion of a Sports Concussion Assessment. Mild cognitive impairment was identified in players, including a reduction in executive function and fine-motor coordination of the non-dominant hand.

Discussion: Recurrent contact and concussion history accelerates the age-related decline in cognition among young and aged rugby union players. The findings of this thesis indicate that molecular, cerebral haemodynamic and cognitive decline are apparent among current professional rugby union players. While subtle impairments can be identified over the course of a playing season among current players, greater physiological decline can be observed following retirement from sport.

Conclusion: Methods to lower recurrent contact and concussion incidence should remain a priority of the sports-medicine community with more dedicated focus towards prevention, molecular-diagnostic capabilities and longitudinal observations of contact sport athletes across the globe.

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Abbreviations

$A^{\bullet-}$: Ascorbate free radical

ADP: Adenosine diphosphate

AMPAR: 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid receptor

ANOVA: Analysis of variance

ASL: Arterial spin labelling

ATP: Adenosine triphosphate

AU: Arbitrary units

BBB: Blood brain barrier

BP: Blood pressure

CBF: Cerebral blood flow

CDO_2: Cerebral oxygen delivery

CI: Confidence interval

CO_2: Carbon dioxide

CRF: Cardiorespiratory fitness

CTE: Chronic traumatic encephalopathy

CVCi: Cerebrovascular conductance

CVR: Cerebrovascular reactivity

$CVRCO_2$: Cerebrovascular reactivity to changes in end-tidal carbon dioxide

$CVRCO_{2HYPER}$: Cerebrovascular reactivity in response to hypercapnia

$CVRCO_{2RANGE:}$ Cerebrovascular reactivity range

$CVRCO_{2HYPO:}$ Cerebrovascular reactivity to changes in response to hypocapnia

CVRi: Cerebrovascular resistance

CW: Continuous wave

DPF: Differential path length factor

DST: Digit Span Test

DSST: Digit Symbol Substitution Test

ECG: Electrocardiogram

eNOS: endothelial nitric oxide synthase

EPR: Electron paramagnetic resonance

GDPR: General Data Protection Regulation

GFAP: Glial fibrillary acidic protein

GP: General practitioner

GPD: Grooved Pegboard Dexterity Test using dominant hand

GPND: Grooved Pegboard Dexterity Test using non-dominant hand

Hb: Haemoglobin

HIA: Head Injury Assessment

HHb: Deoxyhaemoglobin

HR: Heart rate

imPACT: Immediate Post-Concussion Assessment and Cognitive Testing

K^+: Potassium

KD: King Devick

LOC: Loss of consciousness

MAP: Mean arterial pressure

MCA: Middle cerebral artery

MCAv: Middle cerebral artery velocity

MCI: Mild cognitive impairment

MoCA: Montreal Cognitive Assessment

mTBI: Mild traumatic brain injury

Na^+: Sodium

NAA: N-acetyl-asparate

$NaNO_2$: Sodium nitrite

NaOH: Sodium hydroxide

NMDA: N-methyl-D-asparate

NO: Nitric oxide

NO_2: Nitrogen dioxide

NO_2^-: Nitrite

NO^-_3: Nitrate

NOA: Nitric oxide analyser

NOS: Nitric oxide synthase

NSE: Neuron-specific enolase

O_2: Oxygen

$O_2^{\cdot}$: Superoxide

O_2Hb: Oxyhaemoglobin

O_3: Ozone

OBC: Ozone-based chemiluminescence

·OH: Hydroxyl radical

ONOO-: Peroxynitrite

OXNOS: Oxidative-nitrosative stress

PET_{CO2}: End-tidal carbon dioxide

$PaCO_2$: Partial pressure of arterial carbon dioxide

PCS: Post-concussion syndrome

PI: Pulsatility index

P-tau: Phosphorylated tau

Q: Cardiac output

RAVLT: Rey Auditory Verbal Learning Test

RAVLT-A: Rey Auditory Verbal Learning Test A

RAVLT-A1:A5: Sum of Rey Auditory Verbal Learning Test A1-A5 trials

RAVLT-A6: Rey Auditory Verbal Learning Test A6 trial

RAVLT-A6-A5: Rey Auditory Verbal Learning Test A6 trial minus A5 trial

RAVLT-B: Rey Auditory Verbal Learning Test B

RBC: Red blood cell

RDB: Repetition of Digits Backwards Test

RDF: Repetition of Digits Forwards Test

RDT: Repetition of Digits Total

RER: Respiratory exchange ratio

RFU: England Rugby

ROS: Reactive oxygen species

RPM: Revolutions per minute

RSNO: S-nitrosothiols

S100β: S100 calcium binding protein β

SaO_2: Arterial oxygen saturation

SD: Standard deviation

sncRNA: Small non-coding ribonucleic acid

SRC: Sports-related concussion

SCAT: Sports Concussion Assessment Tool

SCAT5: Sports Concussion Assessment Tool 5^{th} edition

TCD: Transcranial Doppler

TDP-43: TAR DNA-binding protein 43

THb: Total haemoglobin

TMT: Trail Making Test

TMT-A: Trail Making Test A

TMT-B: Trail Making Test B

T-tau: Total tau

UCH-L1: Ubiquitin C-terminal hydrolase L1

$\dot{V}O_2$: Oxygen consumption

$\dot{V}CO_2$: Carbon dioxide consumption

WAIS-III: Wechsler Adult Intelligence Scale

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Chapter 1
General Introduction

1.1. Introduction

Since biblical times, concussion has been recognised as a form of brain injury (McCrory and Berkovic, 2001). In ancient Greece, medical literature referred to a brain injury as '*commotio cerebri'* meaning 'brain locomotion'. In the 16th century, Bernal Diaz del Castillo used the terms "concussion", "commotion" and "shaking of the brain" when recording the death of a patient who had sustained a blow to the head without damage to the skull. Later, the French surgeon Jean Louis Petit identified a type of 'contre-coup' injury described as 'concussion' (Flamm, 1997), and by the 20th century, the deleterious effects of concussion were becoming increasingly apparent in boxing athletes who were regularly exposed to blunt head trauma (Trotter, 1925). Presently, concussion or sports-related concussion (SRC) is defined as "*a traumatic brain injury induced by biomechanical forces"* (McCrory et al., 2017), whereby short-lived neurological impairment are apparent as a result of functional disturbance to the brain. Subsequently, concussion is regularly accompanied by neurological symptoms including headaches, amnesia and balance deficits (among others), that evolve over the course of minutes, hours or days following injury (McCrory et al., 2017).

Concussion has represented a significant public health concern within the community in recent years, as widespread media coverage has brought its adverse neurological effects to the attention of the general public. This has been further exacerbated since the long-term implications of concussion are slowly becoming apparent in retired athletes. For instance, those with three or more concussions have a fivefold prevalence of mild cognitive impairment (MCI, Guskiewicz et al., 2005). There is also evidence that repetitive head impacts lead to the development of chronic traumatic encephalopathy (CTE, Gardner et al., 2014a). CTE has been observed in boxing, American football, ice-hockey, soccer and wrestling (McKee et al., 2009) and most recently detected in a retired rugby union player (Stewart et al., 2016).

Following the advent of professionalism in 1995, modern day rugby has become more physically demanding with improvements in player skill, size, strength, power and cardiorespiratory fitness, as well as the time that the ball is in play (Sedeaud et al., 2012, Hendricks et al., 2012). Consequently, elite-level players may be exposed to over 11,000 contact events per-season comprised of tackles, collisions, mauls, rucks and scrums which elevate concussion risk (Fuller et al., 2007a). Indeed, the number of observed tackles has quadrupled and cause 52% of all injuries (Cross et al., 2017, England Rugby, 2020). Concussion has now become the most common injury in rugby union (England Rugby, 2019)

with 1 in 10 athletes likely to encounter prolonged symptoms which can persist for weeks, months or years, depending on the severity of the injury (McCrory et al., 2017). Consequently, the incidence rate (IR) of concussion is now comparable with other contact sports, including American football and ice hockey (Koh et al., 2003, Clay et al., 2013). This raises concern for rugby union's 8.5 million global participants (World Rugby, 2016), given that the adverse effects of concussion are becoming increasingly apparent, but the underlying mechanisms remain to be established.

Moreover, recurrent contact or 'sub-concussion' has generated growing interest in the scientific community given the links to neurodegeneration in later-life (VanItallie, 2019). Unlike concussion, recurrent contact represents a traumatic event whereby conventional detection methods for concussion (i.e. structural imaging, behaviroal/neurological/cognitive assessments) offer no clinical significance when observing a patient's molecular, cerebral haemodynamic and cognitive signatures (Rawlings et al., 2020). These apparently invisible injuries therefore represent a potentially lethal long-term threat to the health and well-being of most athletes who encounter recurrent contact in sport.

The current thesis describes a suite of experimental studies that take a functionally integrated translational approach to establish the molecular, cerebral haemodynamic and cognitive signatures of formerly concussed retired rugby players exposed to recurrent contact. Chapter 2 (Literature Review) provides a critical summary of the current understanding of concussion from a physiological and epidemiological viewpoint (Figure 1). This includes the identification of 'gaps' in our knowledge that collectively serve to inform the experimental studies that form this thesis. Chapter 4 describes a comparative investigation to determine the implications of recurrent impact and concussion incurred over the course of a season in young rugby union players. Young professional rugby union players were compared to age and physical activity-matched, non-contact sport controls absent of concussion history. Using an identical balanced design, this was further extended in aged, retired rugby players to determine how former recurrent contact and concussion history affects the 'normal' ageing process (Chapter 5). Chapter 6 hosts a general discussion surrounding the findings of the research, including areas to consider for the future. Publications and presentation awards arising from this thesis are located in the appendices.

Chapter 2
Literature Review

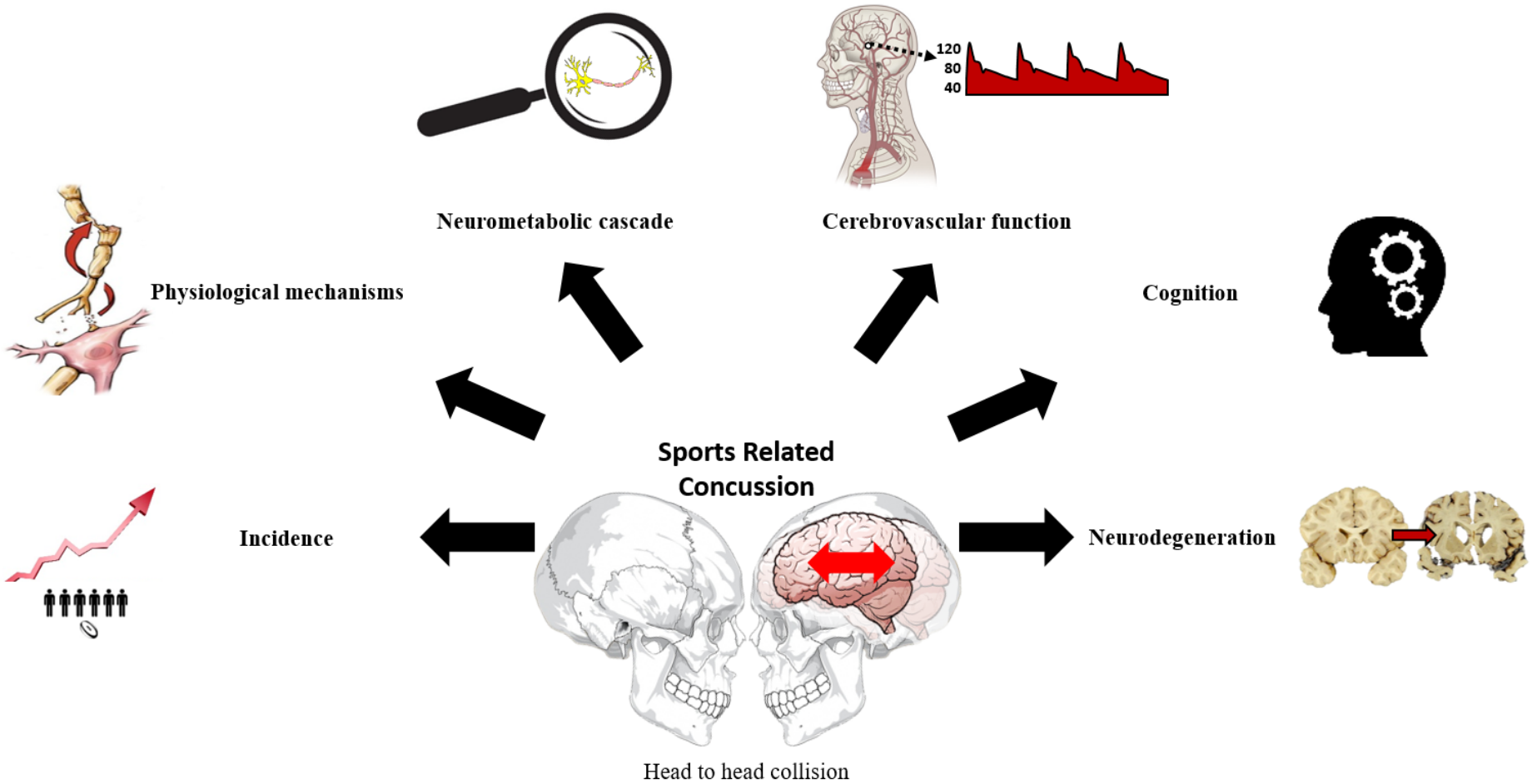

Figure 1: Summary of research topics reviewed in the present thesis. Prior to the literature review, select fields of concussion research were chosen comprised of concussion incidence; physiological mechanisms and the neurometabolic cascade of concussion, cerebrovascular function, cognition and neurodegeneration.

2.1. Incidence

2.1.1. Historical Rugby

The incidence rate of concussion in rugby union has been documented since the 1950's. Sparks (1981), observed over half a million hours of schoolboy rugby in 30 seasons, which equated to 1.03 concussions per 1000 hours. However, due to primitive reporting techniques, injuries were commonly reported as percentages by others. Schoolboy rugby injuries were later reported by Nathan (1983) and Roux and Goedeke (1987). Concussion accounted for 22% and 12% of all injuries, in 31 games and 3,350 games respectively. Although rare, further studies observing senior first-class players were undertaken, with concussion contributing to 5.7% of all injuries reported among 6,000 participants (Durkin, 1977). Our research group performed a retrospective analysis of concussion incidence among 708 community and elite level rugby players between 1982-1984, to report an incidence of 0.62 concussions per 1000 hours (equating for 6% of all injuries, Owens et al., 2019).

Preceding the recognition of rugby union as a professional sport in 1995, it is likely that concussion was underreported during the 'amateur' years of the game. This was due to misconceptions and varied understanding of concussion among coaches, players and parents (Gardner et al., 2014b), notwithstanding the limitations of primitive injury reporting methods. Therefore, interpretative caution must be exercised when evaluating concussion incidence during the amateur years of rugby union.

2.1.2. Modern Rugby

Following 1995, the professional status of rugby union enabled an improved means of injury reporting (Fuller et al., 2007b). The findings of the most recent systematic review and meta-analysis investigating concussion incidence in rugby union was 1.19 per 1,000 match hours at elite level, 2.08 per 1,000 match hours at amateur level and 0.62 per 1,000 match hours at schoolboy level (Gardner et al., 2014b). More recently, a systematic review by Prien et al. (2018) that evaluated concussion incidence in team sports from 2000 – 2017 revealed concussion incidence in rugby (0.02 – 8.83 concussions per 1000 hours) had surpassed that of other sports, including American football, ice hockey, and others. Although these observations are useful from a general perspective, it does not reflect the nature of individual teams, leagues or championships. While data for concussion incidence at the lower levels of play exists, the changing nature of concussion incidence is best captured consistently at the elite level. The Injury Surveillance Report constructed by England Rugby (RFU) is one of the longest running

injury reporting outlets at present. During the 2018-19 season, the RFU reported a concussion incidence of 20.4 per 1000 match hours at elite level (England Rugby, 2020), surpassing the combined findings of Gardner et al. (2014b). Figure 2 illustrates the gradual increase in concussion incidence since 2002 at elite level. While the 2017-2018 season saw the first decline in concussion incidence since 2009, concussion incidence has risen yet again and now represents 20% of all injuries reported within the game (England Rugby, 2020).

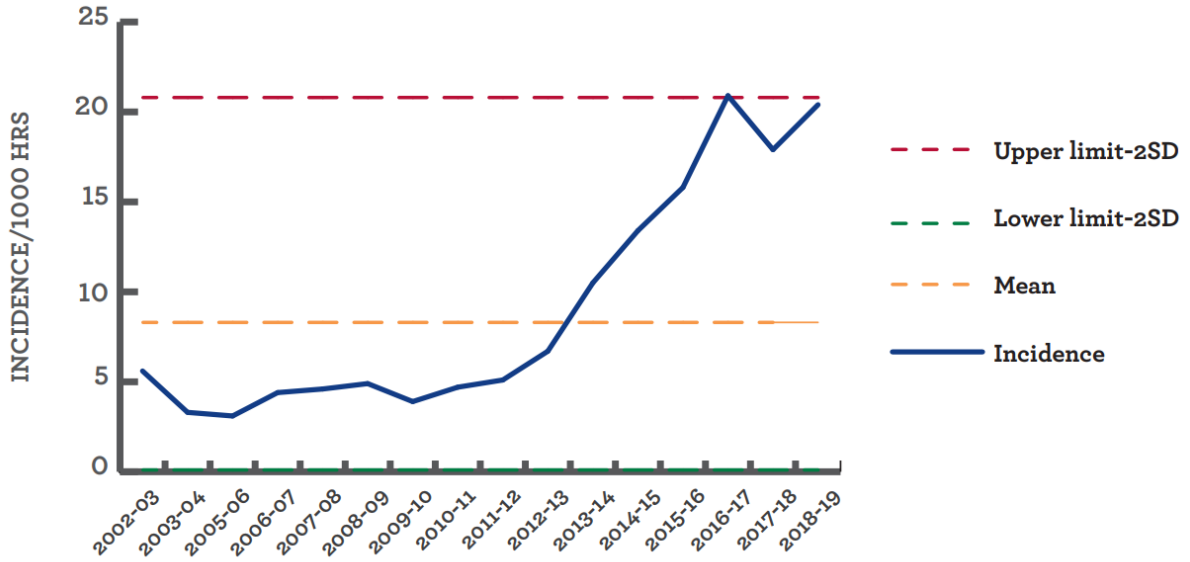

Figure 2: Outline of the increase in concussion incidence rate from all English professional rugby union clubs from 2002-2019 collected as a part of the England Professional Rugby Injury Surveillance Project. During the 2018-19 season, concussion incidence was 20.4 per 1000 match hours. Upper and lower limits represent expected season-to-season incidence variation. Sourced from England Rugby (2020).

Furthermore, concussion severity has gradually increased and is attributable to improved reporting standards (Figure 3, England Rugby, 2020). Professional rugby players are more likely than not to sustain concussion within 25 games and have a 60% greater risk of subsequent injury (Cross et al., 2016, Rafferty et al., 2018). Therefore, the increase in injury severity observed in recent years may be present in an attempt to prevent subsequent injury risk.

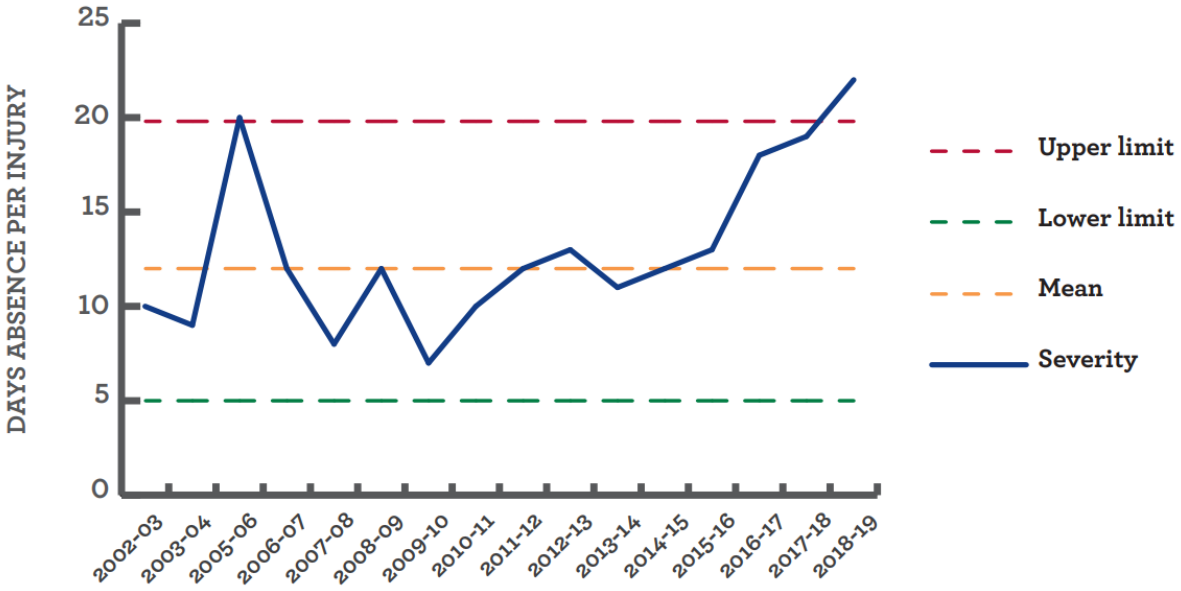

Figure 3: Outline of the increase in concussion severity in all English professional rugby union clubs from 2002-2019 collected as a part of the England Professional Rugby Injury Surveillance Project. The average severity of match concussion during the 2018-19 season was 22 days. . Sourced from England Rugby (2020).

2.1.3. Player position

Playing position and concussion incidence is widely argued. Several studies have investigated the differences between forwards and backs, given that roles among these positional groups are disparate and favour heavier forwards and lighter backs (Brooks et al., 2005a, Quarrie and Hopkins, 2008, Sedeaud et al., 2012). Some have observed greater concussion incidence in forwards (Fuller et al., 2013, Cross et al., 2016, Cosgrave and Williams, 2019) and propose that these athletes are heavier, which subsequently increase impact forces (Eaves and Hughes, 2003). Others speculate higher incidence in forwards since the positional demands are associated with greater match events, including tackles, scrums, and rucks which subsequently increase the risk of injury (Reardon et al., 2017).

Conversely, some argue that backs are at greater risk of concussion (Brooks et al., 2005a, Brooks et al., 2005b, Fuller et al., 2008, Kemp et al., 2008, Fuller et al., 2013). Naturally, backs are more athletic, lighter and reach higher speeds throughout play (Sedeaud et al., 2012). It is plausible that entry forces into contact events are greater and are unfavourable if the opposing team player is a heavier forward in particular (Quarrie and Hopkins, 2008). Therefore, the differences between positional influence on concussion incidence are not clearly defined (Cross et al., 2016).

2.1.4. Match events

Although several discrepancies exist while reporting concussion risk factors, match events and concussion incidence rates are more clearly established. The tackle is most associated with concussion incidence, effecting both ball carrier and tackler (Brooks et al., 2005a, Fuller et al., 2008, Fuller et al., 2013, Gardner et al., 2014b, Fuller et al., 2015, England Rugby, 2019, England Rugby, 2020). Most recently, it is apparent that the player being tackled is most prone to concussion, with the tackler less frequently injured at a professional level (Figure 4, England Rugby, 2020). However, the extent by which the tackler or ball carrier is at higher risk of concussion varies between years of investigation (Brooks et al., 2005a, Fuller et al., 2008, Fuller et al., 2013, England Rugby, 2018, England Rugby, 2019). Tackling is one of the most frequent match events and varying body positions during impact are attributed to the changing relationship between tackler and ball carrier incidence across years (England Rugby, 2020). Further, legislation changes to tackle height and technique in recent years have failed to reduce concussion incidence. It appears that the risk of concussion is elevated in the event that a tackler accelerates into the tackle, or the tackler travels at higher speed relative to the ball carrier (Cross et al., 2017). The risk of concussion is further elevated if head-to-head contact is made between the tackler and ball carrier, which often occurs when tackles are made above the line of the armpit, subsequent to an upright tackle position (Cross et al., 2017, Tucker et al., 2017). Conversely, concussion incidence is reduced in the event a ball carrier is bent at the waist during the tackle (Tucker et al., 2017). However, interventions to implement changes to tackle style in professional rugby were halted early due to a 67% rise in concussion incidence at European Championship level (BBC Sport, 2019). Therefore, tackling remains the primary cause of concern for concussion incidence in rugby union.

Further match events which contribute towards concussion incidence in rugby union include collisions, rucks/jackals, scrums, mauls and lineouts (England Rugby, 2019). The incidence of injuries related specifically to these match events vary between playing standards and year of data collection (Gardner et al., 2014b). Changes to game legislation are able to influence concussion incidence of some match events, as forwards encounter higher frequencies of scrums, lineouts and rucks relative to backs (Brooks et al., 2005a, Sedeaud et al., 2012). However, the variation in incidence as a result of match events throughout recent observations highlight similar risk to positional groups as discussed previously (England Rugby, 2020). Indeed, increased risk of concussion can be attributed to foul play which has accounted for up to 6% of injuries (Brooks et al., 2005a). Although, emphasis remains on identifying effective

risk reduction strategies for both ball carrier and tackler at present (Cross et al., 2017, Rafferty et al., 2018).

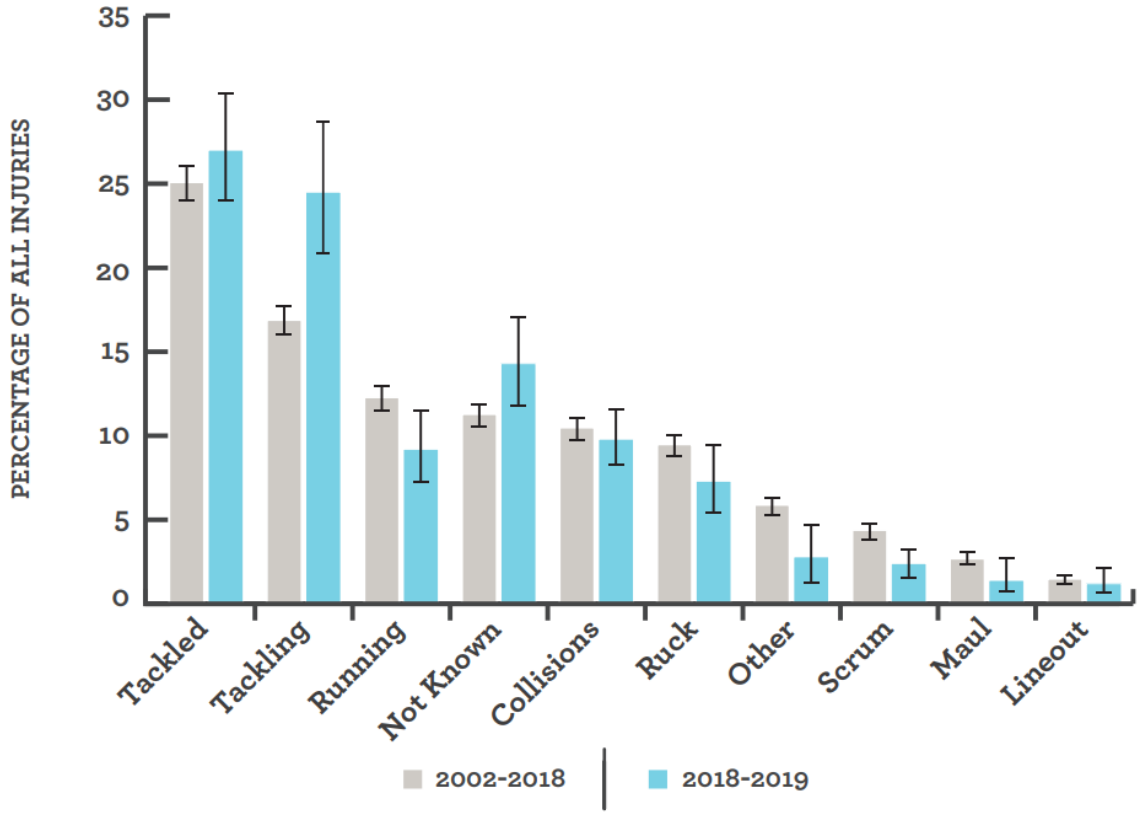

Figure 4: Relative comparison of elite rugby union match events between 2002-2018 and 2018-2019 in all English professional rugby union clubs collected as a part of the England Professional Rugby Injury Surveillance Project. 52% of injuries in the 2018-19 season were the result of tackling. Being tackled accounted for 27% of injuries and tackling accounted for 25%. Concussion was the most common injury following a tackle which represented 16% of all injuries to the ball carrier and 36% of all injuries to the tackler. Tackled defined as a collision where player in possession of the ball is grounded by the opposing players arms; Tackling defined as a collision where opposing player uses arms to ground player in possession of the ball; Collision defined as opposing player grounds player in possession of the ball without use of arms; Ruck defined as a loose scrum formed around a player with the ball on the ground, Scrum defined as players packing closely together with their heads down and attempting to gain possession of the ball, Maul defined as a loose scrum formed around a player with the ball off the ground, Lineout defined as a formation of parallel lines of opposing forwards at right angles to the touchline when the ball is thrown in. Sourced from (England Rugby, 2020).

2.1.5. Environmental

Environmental factors offer valuable information in relation to concussion incidence in rugby. Artificial turf as opposed to conventional grass has shown to decrease head injury incidence (England Rugby, 2019). While this is a novel observation, the limited number of studies comparing playing surfaces limits the ability to differentiate concussion incidence between artificial and conventional surfaces at present. The concussion incidence is higher while playing on wet and heavily saturated pitches (0.59 per 1000 hours) versus firm and dry pitches (0.35 per 1000 hours, Lee and Garraway, 2000). However, the observations of others argue the contrary (Durkin, 1977, Chalmers et al., 2012), with some research groups identifying no differences in injury incidence and ground condition (Takemura et al., 2007). Injury during quarter of play has also been investigated, which revealed that injuries are most often sustained during the third (40-60 minutes) and fourth quarters (60-80 minutes) of play and are likely attributed to player warm up after half-time and fatigue in the closing minutes of play (Bathgate et al., 2002, Roberts et al., 2017, Brooks et al., 2005a).

To summarise, concussion incidence in rugby union has substantially risen since observations began and is now comparable with other contact sports, including American football and ice hockey. Given this observation and the established literature surrounding the deleterious effects of concussion in the latter sports, concerns alight as to whether rugby players, both past and present, may share the same unfortunate decline in cognition and risk of neurodegenerative disease. For the remainder of the present review, attention was focused towards better understanding the mechanisms postulated in the literature that underpin the development of neurological dysfunction.

2.2. Mechanism of injury

Two forms of trauma are described following concussion known as primary and secondary damage (Cornelius et al., 2013). Primary damage refers to the immediate trauma sustained to the cerebral tissue following concussion and is due to linear and rotational head acceleration, which causes the brain to rapidly shift within the skull and produce strain patterns in cerebral tissue (Ommaya and Gennarelli, 1974). Concussion is a diffuse axonal injury (Figure 5) capable of inducing varying symptoms, which commonly resolve within days or weeks (Guskiewicz and Mihalik, 2011). The thalamus and brain stem are regions which elicit high shear stress following simulated head-to-head field collisions replicated from American football using a human head model (Figure 6, Zhang et al., 2004). High shear stress in the

thalamus and brain stem correspond with abnormal fractional anisotropy in concussed collegiate athletes, indicative of a reduction in white matter tracts (Churchill et al., 2020).

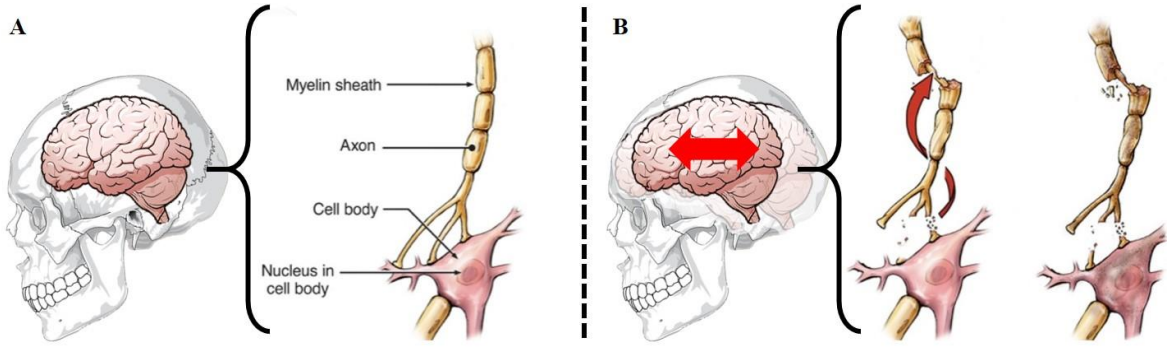

Figure 5: Non-injured brain with structure of intact neuron (**A**). Concussion is a diffuse axonal injury, which produces torsional rotation in axons, which lead to mechanical shear and loss of neuronal networks within brain tissue (**B**). Adapted from Mozlin (2015).

Some have explored the quantitative force threshold required to induce concussion and used varied techniques including computer, animal, cadaver and surrogate models (Viano et al., 1989, Ono and Kanno, 1996, Viano and Lovsund, 1999). Others have suggested that force ranges of 65-106*g* are required to induce concussive injury (Guskiewicz and Mihalik, 2011, Zhang et al., 2004). With advancements in wearable microtechnology, MacLeod et al. (2018) identified that the average collision forces in elite-level rugby were 19*g*, surpassing the 18*g* threshold that triggers the medical warning light used in Formula 1 racing requiring drivers to undergo a medical examination (International Federation of Automobiles, 2020). Nonetheless, the acceleration force required to induce concussion remains elusive as controversy surrounds linear and rotational forces. Rotational acceleration commonly induces loss of consciousness (LOC) due to shearing of axons in the brainstem as opposed to linear acceleration, which influence peripheral areas of the cerebral tissue that are less likely to cause LOC (Ommaya and Gennarelli, 1974).

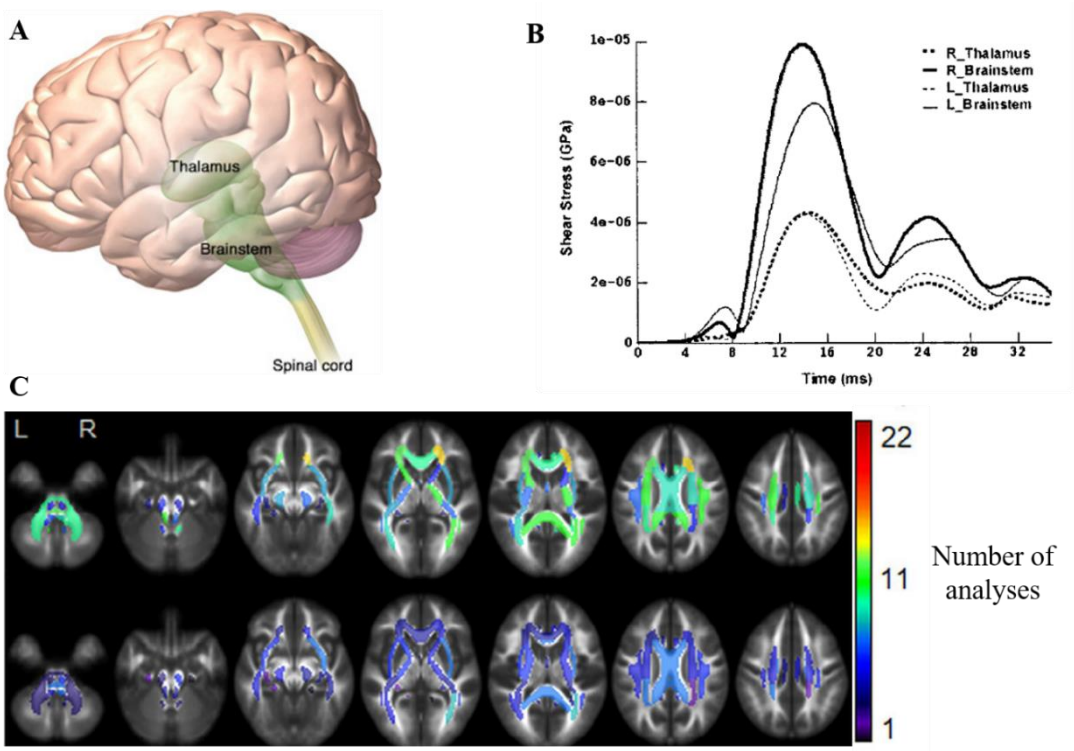

Figure 6: The thalamus and brain stem (**A**) are brain regions which elicit high shear stress following 12 simulated head-to-head field collisions replicated from American football using the Wayne State Brain Injury Model (**B**). High shear stress in the thalamus and brain stem correspond with abnormal fractional anisotropy in 12 concussed collegiate athletes, indicative of a loss of white matter tracts (**C**). Values presented as mean ± SD. Adapted from Zhang et al. (2004) and Churchill et al. (2020).

The speculation that a force threshold exists for concussion remains speculative since the brain is resilient to multiple impacts without concussion diagnosis, as demonstrated in studies by Guskiewicz and Mihalik (2011) and Schnebel et al. (2007). These studies revealed that collegiate football players were able to sustain an average of 950-1000 head impacts over the course of a typical season with low frequencies of medically diagnosed concussion (n = 2). Zhang et al. (2004) has identified force probabilities for concussion and determined impacts of 66g, 82g and 106g are likely to result in diagnosis in 25%, 50% and 80% of cases. These probabilities have been challenged by Guskiewicz et al. (2007b) and McCrea et al. (2003) since impacts observed in collegiate American football players exceeding 80g only inflicted concussion in 0.35% of cases, while impacts exceeding 90g without self-reported symptoms failed to predict concussion. As force thresholds have not proven to be an effective method for diagnosing concussion (Romeu-Mejia et al., 2019), others have explored the unique sequence of neurometabolic events that arise from the primary injury.

2.2.1. Neurometabolic cascade

The secondary phase of concussion is known as the neurometabolic cascade (Giza and Hovda, 2001, Cornelius et al., 2013). Four events are consistently reported after concussion, namely altered membrane conductivity, altered glucose metabolism, altered protein and axonal function, including changes to cerebral blood flow (CBF, Banks and Domínguez, 2019). Figure 7 illustrates the neurometabolic sequence of events incurred after concussion while Figure 8 depicts a timeline of metabolic changes.

Following acceleration of the brain tissue, neuronal cell membrane disruption and axonal shearing are apparent, which alter membrane electrical charges and cause unregulated influxes of excitatory amino acids including glutamate. This precedes sodium (Na^+) influx and potassium (K^+) efflux resulting in depolarisation of the cell membrane. Glutamatergic receptors including N-methyl-D-asparate (NMDA, Katayama et al., 1990) activate and calcium concentration increases intracellularly due to simultaneous potassium efflux (Casson, 2006), while 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid receptor (AMPAR) encourages continued depolarisation with entry of sodium (Cornelius et al., 2013). Intracellular calcium is toxic when consistently elevated, resulting in degradation of microtubules and neurofilaments (Dominguez, 2004), subsequent to calpain-mediated proteolysis of cytoskeletal proteins (Banks and Domínguez, 2019). Moreover, neurofilament stability is compromised and leads to structural collapse, followed by axonal disconnection (Scott and Manavis, 1994).

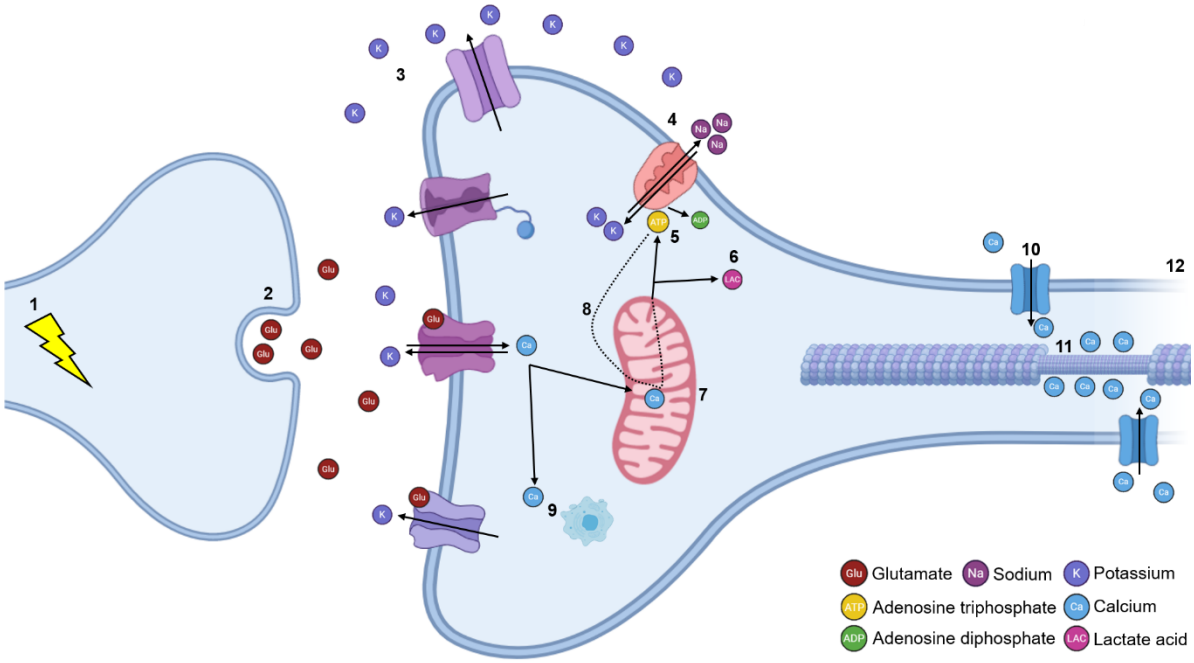

Figure 7: Proposed mechanism for the neurometabolic cascade of concussion. Depolarisation of pre-synaptic neuron causes initiation of action potential (**1**). The release of excitatory neurotransmitters from the pre-synaptic neuron (**2**) bind with receptors of the post-synaptic neuron to generate potassium efflux (**3**). Restoration of homeostasis is achieved by increased activity of sodium-potassium-ATPase pumps (**4**) via hyperglycolysis (**5**) which causes lactate accumulation (**6**). Calcium influx and sequestration in mitochondria causes impaired oxidative metabolism (**7**) and decreased adenosine triphosphate (ATP) production (**8**). Calcium accumulation causes activation of calpain which initiates apoptosis (**9**). Disruption in the axon initiates calcium influx (10) and phosphorylation which causes neurofilament compaction (**11**) and microtubule disassembly to generate axonal swelling and eventual axotomy (**12**). Recent evidence indicates that a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability adversely impacts the structural integrity, molecular signalling and flow autoregulation across the neurovascular unit, which are all established biomarkers underlying cognitive decline and dementia. Adapted from Giza and Hovda (2001) and Bailey et al. (2020).

Due to hyperactivation of ionic pumps immediately following concussion, glucose metabolism can increase by up to 46% and persist for four hours in the rodent model (Yoshino et al., 1991). This occurs due to the rapid ionic flux as sodium-potassium pumps attempt to re-regulate the ionic balance, which call upon glucose storage in the mitochondria to produce ATP. Subsequently, stored glucose is depleted and relies upon glycolysis (Banks and Domínguez, 2019). This sequence of events are detrimental since lactic acid is produced as a bi-product of glycolysis and cause an accumulation of fluid, tissue swelling, impaired neuronal transport and

cell death (Giza and Hovda, 2001, Vagnozzi et al., 2010, Signoretti et al., 2011, Banks and Domínguez, 2019).

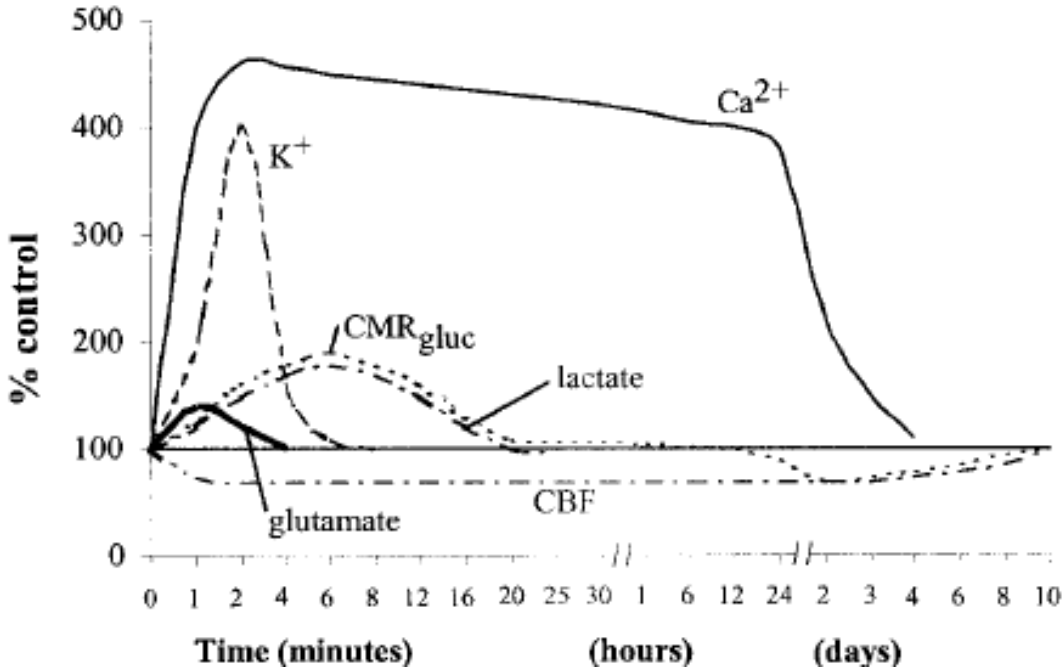

Figure 8: Timeline of post-concussive metabolic and cerebral hemodynamic alterations between 0 minutes to 10 days in the rodent model. Values presented as mean percentage change from control; K^+, potassium; Ca^{2+}, calcium; CMRgluc, oxidative glucose metabolism; CBF, cerebral blood flow. Sourced from Giza and Hovda (2000).

Further, the high intracellular concentration of calcium that accumulates in the mitochondria increases oxidative stress and results in mitochondrial dysfunction (Xiong et al., 1997) including decreased expression of cytochrome oxidase (Hovda et al., 1991). Observations of the depletion and recovery of N-acetyl-aspartate (NAA, Belli et al., 2006, Signoretti et al., 2004) and adenine nucleotides (Vagnozzi et al., 2007) after single or recurrent concussion offer a potential diagnostic tool for confirmation of normalised neurometabolism. This is because NAA mirrors the depletion and recovery of ATP and adenosine diphosphate (ADP) after concussion, thereby depicting tissue energy state and mitochondrial function following injury (Figure 9, Holshouser et al., 2005, Vagnozzi et al., 2007).

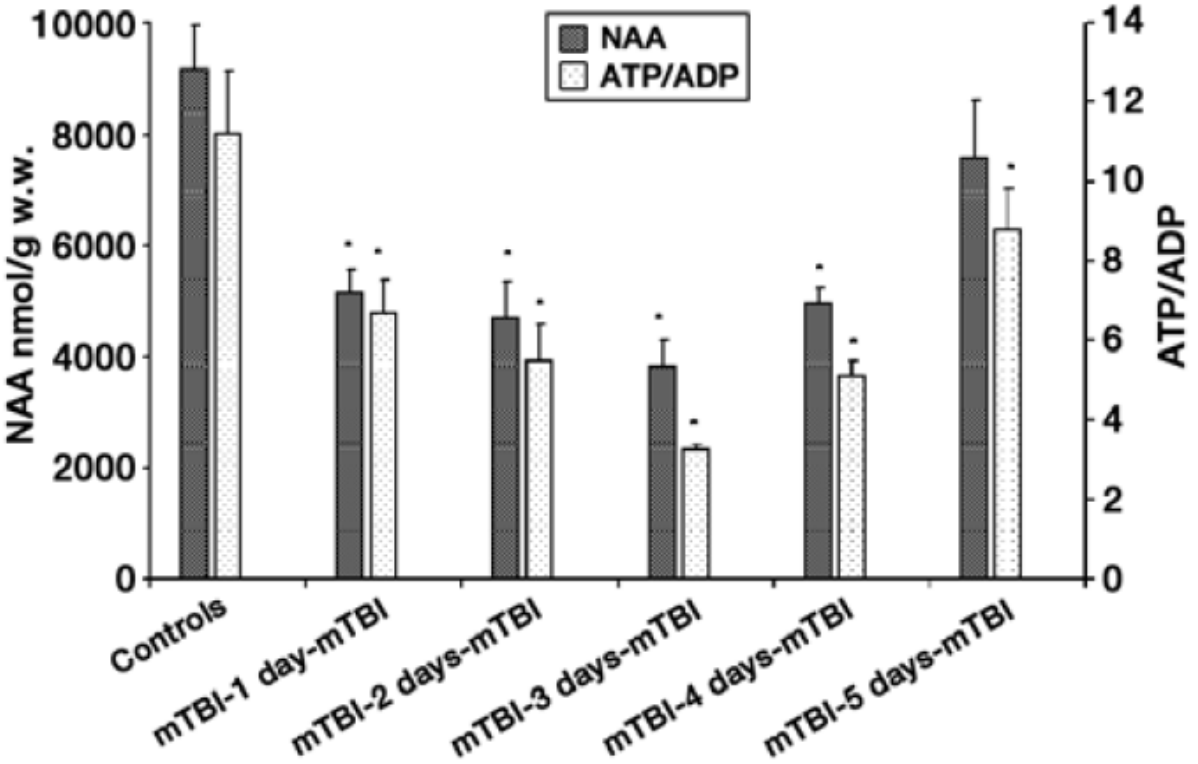

Figure 9: Comparison of N-acetyl-aspartate concentration and ATP/ADP ratios from rat hemisphere extracts that were subjected to repeat mild traumatic brain injury between 1-5 days or Sham injury (controls). Values of six different animals presented per timepoint as mean ± SD; *, $P < 0.05$ *vs.* control; NAA, N-acetyl-asparate; ATP, adenosine triphosphate; ADP, adenosine diphosphate. Sourced from Vagnozzi et al. (2007).

2.2.2. Oxidative-nitrosative stress (OXNOS)

Free radicals mediate cell signalling and contribute to other key functions such as regulating the activity of transcription factors and gene expression (Richardson et al., 2007). Free radicals are highly oxidising and serve as potential aggregates to disturb cerebral homeostasis (Smith et al., 1994). Concussion and indeed contact events may produce several oxidising substrates, namely the superoxide ($O_2^{\cdot}$) and hydroxyl (·OH) radicals (Hall et al., 2004, Tavazzi et al., 2007, Cornelius et al., 2013). With increased free radical concentration, oxidative stress may become apparent. This reflects an imbalance between the manifestation of reactive oxygen species (ROS) and antioxidant defences (Cornelius et al., 2013). Cerebral oxidative metabolism increases after brain injury, which promotes haemoglobin-mediated oxidative stress via the Fenton and Haber-Weiss reactions (Sadrzadeh and Eaton, 1988, Das et al., 2015, Vagnozzi et al., 2007). Iron released from haemoglobin may undergo several reactions, which allow ferrous iron (Fe^{2+}) to combine with hydrogen peroxide (H_2O_2) to form ferric iron (Fe^{3+}). ·OH is

concomitantly produced which reacts with H_2O_2 to form $O_2^{\cdot}$ (Fenton reaction), which may further react with H_2O_2 to form ·OH (Haber-Weiss reaction, Das et al., 2015).

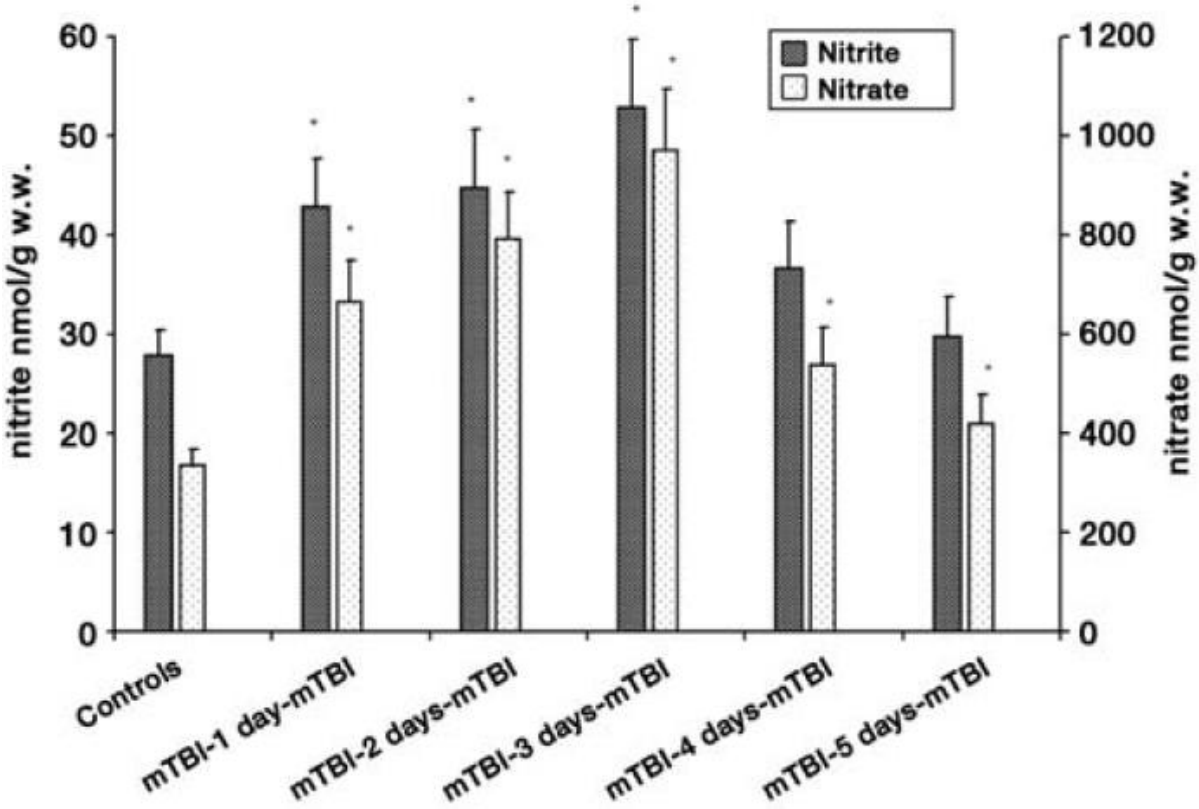

Figure 10: Nitrite and nitrate concentration of rat cerebral hemispheres subjected to repeat mild traumatic brain injury between 1-5 days after first injury or Sham injury (controls). Values of six different animals presented per timepoint as mean ± SD; *, $P < 0.05$ *vs.* control; mTBI, mild traumatic brain injury. Sourced from Tavazzi et al. (2007).

Concussion promotes an increase in nitrate (NO^-_3) and nitrite (NO^-_2) concentration after single and recurrent concussion (Figure 10, Tavazzi et al., 2007). Alongside neurometabolic alterations to the cerebral vasculature, concussion generates acidosis and promotes calcium dependent nitric oxide synthase (NOS) activity (Tavazzi et al., 2007, Zweier et al., 2010). Combined, these mechanisms increase nitric oxide bioactivity. NO is a reactive nitrogen species which governs vascular endothelial function (Lavi et al., 2003). However, NO can be scavenged by $O_2^{\cdot}$ to generate peroxynitrite (ONOO-, Avila et al., 2008). This process reflects OXNOS whereby a free radical generated reduction in NO bioactivity occurs (Bailey et al., 2019). Oxidative-nitrosative stress is associated with a decline in the vasoreactivity of blood vessels and can cause cerebral hypoperfusion (Bailey et al., 2019). Consequently, increased OXNOS has been ascribed to accelerated vascular ageing and neurodegeneration (Van Der Loo et al., 2000, Dorszewska et al., 2014, Everett et al., 2014). Despite these mechanisms being

widely accepted, the acute and chronic alterations in OXNOS and its implication towards accelerated cerebrovascular and cognitive impairment have not been established in contact sport athletes.

2.3. Cerebrovascular function

The brain demands 20% of the body's energy expenditure at rest and is only capable of survival for 4-6 minutes without oxygen (O_2, Squire et al., 2012). Therefore, the function of the cerebral vasculature and regulation of CBF are vitally important. However, the neurometabolic cascade and presence of increased OXNOS resulting from recurrent contact and concussion promote cerebrovascular dysfunction, which is an independent risk factor for the development of MCI and neurodegeneration (Wolters et al., 2017).

2.3.1. Cerebral blood flow

Several non-invasive methods to determine global and regional CBF exist, including magnetic resonance imaging and duplex ultrasonography (Schöning et al., 1994, Wang et al., 2019). Transcranial Doppler (TCD) ultrasonography has also been used to determine the flow velocity of the middle cerebral artery (MCA) as an indirect measure of CBF (Bishop et al., 1986, Larsen et al., 1994, Purkayastha and Sorond, 2012). Following concussion, both short (<1-month) and long term (>12-months) reductions in CBF have been documented (Meier et al., 2015, Grossman et al., 2013). Several mechanisms may explain hypoperfusion after head contact and concussion. These relate to the integrity of the microvasculature (Park et al., 2009), including reduced capillary density (Meier et al., 2015), diminished oxygen demand of functionally impaired neurons (Zhang et al., 2004), and lesions to white matter that cause a dysregulation in blood vessel permeability (Anderson et al., 1996). Indeed, the neurometabolic cascade may also promote hypoperfusion. While mitochondrial hypermetabolism promotes OXNOS and limits the vasoactive capabilities of the microvasculature (Ellis et al., 2016, Giza and Hovda, 2001), mitochondrial dysfunction occurs following intracellular calcium accumulation and causes neuronal apoptosis (Giza and Hovda, 2001, Giza and Hovda, 2014). Alterations to middle cerebral artery velocity (MCAv) have been observed immediately after brain injury (McQuire et al., 1998), while Len et al. (2011), Bailey et al. (2013a) and Len et al. (2013) found no differences in resting MCAv 2-5 days after concussion when comparing concussed and non-concussed athletes. Despite instances where global CBF is considered normal, regional CBF can be impaired at injury sites (Ellis et al., 2016). Hypoperfusion can arise due

to structural damage whereby the vascular beds of functionally impaired neurons redistribute blood in favour of vascular beds with greater vasodilatory reserve (Ellis et al., 2016).

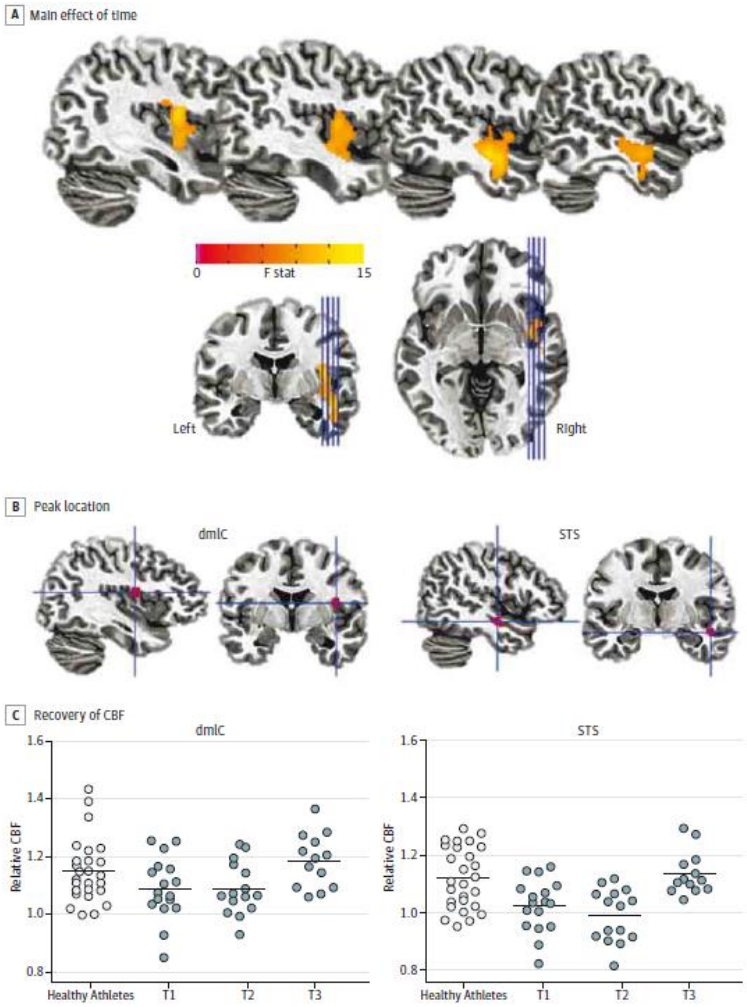

Figure 11: Region-specific reductions in cerebral blood flow among 17 collegiate American football players 1-week post-concussion compared to 27 non-concussed controls (**A**). Athletes were observed with regional CBF impairments in the right dorsal midinsular cortex (dmIC) and right superior temporal sulcus (STS, **B**). Cerebral blood flow in 17 concussed athletes was lower one-day (T1) and one-week (T2) after concussion relative to 27 non-concussed athletes with no differences apparent between groups 1-month (T3) post-injury (**C**). Values presented as mean ± SD. Sourced from Meier et al. (2015).

The thalamus elicits the greatest shear stress in computational models of concussion, which correspond to a 50% reduction in regional perfusion and impaired cognition 23 days after injury (Grossman et al., 2013). It is uncertain how long cerebral hypoperfusion may persist after concussion. Grossman et al. (2013) observed regional CBF impairments among mild traumatic brain injury (mTBI) patients via arterial spin labelling (ASL) 369 days after injury. Meier et al. (2015) observed similar regional CBF impairments using ASL among concussed collegiate

American football players one-week and one-month post-injury. Most athletes were characterised by decreased CBF one-week post-injury, followed by a full recovery. Athletes that presented with prolonged CBF deficits one-month post-injury were characterised by poorer clinical outcome and greater symptomology (Figure 11). Therefore, reductions in global and regional perfusion can be detected after concussion at rest. It appears that the measurement of CBF in the weeks and months after concussion is subjective on the method of assessment, given that regional reductions lasting 12 months post-injury have only been detected via MRI previously. However, the true extent to which long-term CBF deficits prevail locally after concussion remains unclear.

2.3.2. Cerebrovascular reactivity (CVR)

Cerebral blood flow is regulated by cerebral perfusion pressure, neurometabolic activity, and autonomic nervous stimuli (Busija and Heistad, 1984). Athletes that are apparently asymptomatic days after a concussion at rest may present with headache, dizziness and migraines during exercise. There is evidence that this is a consequence of impaired CVR which plays a significant role in CBF regulation (Gardner et al., 2015). Impaired CVR increases a one's susceptibility to neurodegenerative diseases in later-life, therefore examining cerebrovascular response to physiological stressors after a concussion are important for detecting impairments that are not apparent at rest, in effect, forcing the signal out of the noise (Sweeney et al., 2018, Churchill et al., 2019). Cerebrovascular reactivity to changes in end-tidal carbon dioxide (CVR_{CO2}) is commonly used to assess cerebrovascular function and reflects the ability of the vascular bed to vasodilate or constrict in response to fluctuations in the partial pressure of arterial carbon dioxide ($PaCO_2$, Ainslie and Duffin, 2009). Hypercapnia reflects an increase in $PaCO_2$ concentration which increases CBF, while hypocapnia reflects a decrease in $PaCO_2$ to reduce CBF (Bailey et al., 2013a). Cerebral blood flow and the ventilatory response to changes in $PaCO_2$ are tightly linked. Therefore CBF regulation is important for stabilised breathing and maintenance of central pH under alterations of chemical stimuli (Ainslie and Duffin, 2009). Increased carbon dioxide (CO_2) concentration promotes vasodilation in smooth muscle of all cerebral vessels, but particularly small vessels. Conversely, decreased CO_2 concentration promotes vasoconstriction which is uniform regardless of vessel size (Ainslie and Duffin, 2009). CO_2 concentration concomitantly alters pH via opening of ATP-sensitive potassium voltage-gated channels, which can cause acidosis induced cerebral vasodilation (Kinoshita and Katusic, 1997). Complimentary mechanisms exist via shear stress induced NO release which further promote vasodilation and increase CBF

(Smith et al., 1997). Acidosis and increased NO metabolite concentration are apparent after concussion (Giza and Hovda, 2014, Giza and Hovda, 2001, Tavazzi et al., 2007). Given that NO plays a role in cerebrovascular regulation (Ainslie and Duffin, 2009), CVR_{CO2} may also be effected by brain injury.

To date, three studies addressing CVR_{CO2} among concussed participants have been performed by Len et al. (2011), Bailey et al. (2013a) and Len et al. (2013). Among a mixed cohort of 10 hockey and basketball athletes, Len et al. (2011) observed suppressed CVR_{CO2} in response to hyper/hypocapnia in comparison to non-concussed controls four days post-injury (Figure 12). Later, Len et al. (2013) performed an observation of the time course of CVR_{CO2} following concussion among a mixed cohort of 20 hockey, basketball and extreme sports athletes two days, four days and eight days after concussion. Suppressed CVR_{CO2} was noted during repeated apnoeas two days post- concussion in comparison to baseline. However, no impairments were noted for any other time-point, thus concluding adequate recovery of the cerebral vasculature's compliance to alterations in CO_2 within four days (Len et al., 2013).

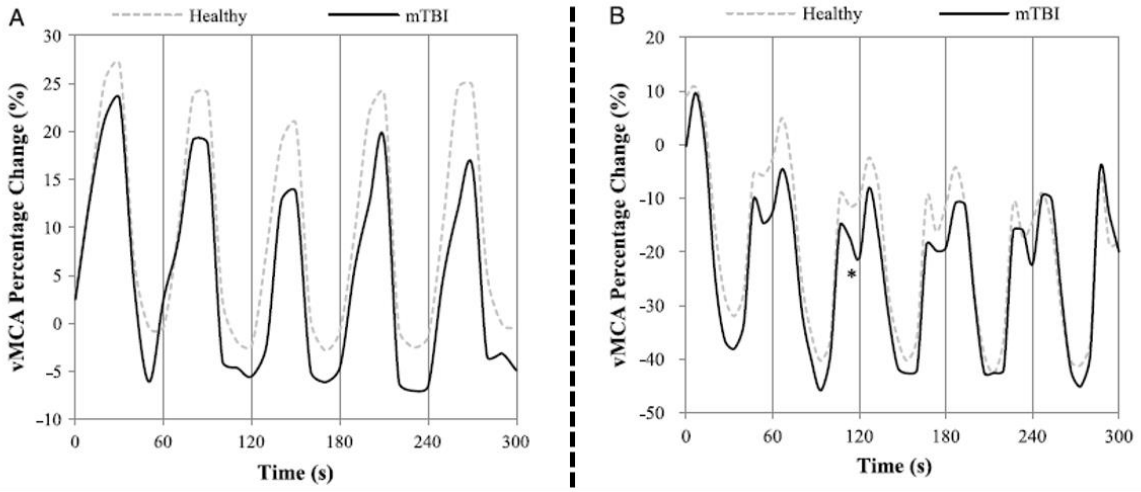

Figure 12: The percentage change in middle cerebral artery velocity (MCAv) during repeated 20-second apneoeas (hypercapnia, **A**) and controlled hyperventilation (hypocapnia) was lower in 10 concussed collegiate athletes compared to non-concussed controls (**B**). Values presented as mean ± SD; *, $P < 0.05$ *vs.* control. vMCA, Middle cerebral artery velocity, mTBI, mild traumatic brain injury. Adapted from Len et al. (2011).

Bailey et al. (2013a) sought to determine CVR_{CO2} among a cohort of professional boxers within three days of a sparring bout in comparison with a non-concussed control group. Resting MCAv was not different between groups. However, CVR_{CO2} in response to hyper/hypocapnia and range ($CVR_{CO2RANGE}$) in boxers was lower relative to controls (Figure 13).

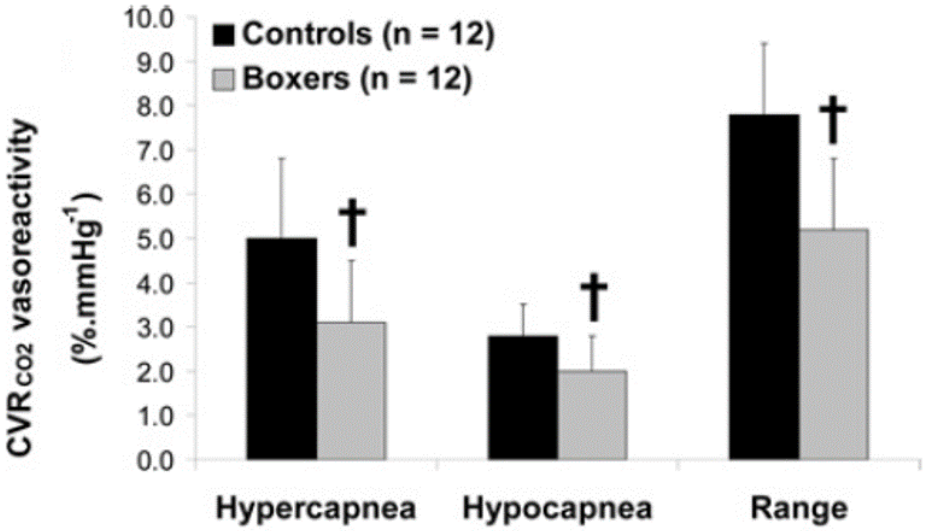

Figure 13: Cerebrovascular reactivity to changes in carbon dioxide (CVR_{CO2}) comparing 12 boxers after a sparring bout (<72 hours) and 12 non-concussed controls; †, $P < 0.05$ *vs.* control. Values presented as mean ± SD. Sourced from Bailey et al. (2013a).

It is therefore apparent that CVR_{CO2} is suppressed following concussion and repetitive head trauma (Gardner et al., 2015). Cerebral vasoreactivity is partly dependant on NO release from the vascular endothelium (Gardner et al., 2015). However, increased OXNOS arising from concussion may reduce NO bioactivity and compromise the ability of the cerebral blood vessels to vasodilate (Tavazzi et al., 2007). Presently, CVR_{CO2} has not been observed in rugby union players and uncertainty remains as to whether these athletes present with impaired cerebral vascular function that may increase the risk of cognitive decline, stroke and mortality (Ainslie and Duffin, 2009).

2.4. Cognition

Impaired cognition has often been associated with neurological disorders including MCI, particularly in ageing athletes with three or more concussions (Guskiewicz et al., 2005). Cognitive impairment after concussion is commonly short-lived, lasting between 7-10 days (Belanger and Vanderploeg, 2005, Belanger et al., 2010, Cross et al., 2016) and aligns with the recovery of metabolic and cerebrovascular function discussed earlier (section 2.2.1-2.3.2). Moreover, athletes with the least elapsed time since concussion present with the worst cognitive scores and all cognitive domains may be impaired (Taylor et al., 2018). The extent to which specific domains are affected varies depending on the anatomical location and severity of injury (Belanger and Vanderploeg, 2005). Cognitive assessments are often used to draw

comparisons between concussed and non-concussed cohorts, however baseline cognition must be determined in order allow diagnostic potential (Gardner et al., 2012).

Cognition following concussion in past and present rugby players has been documented by several research groups. Among a mixed cohort of 124 high school and national level rugby players with concussion history, Shuttleworth-Edwards et al. (2008) observed decreased Digit Symbol Substitution Test (DSST), Trail Making Test (TMT) A (TMT-A) and B (TMT-B) scores relative to 102 non-concussed controls, indicative of impaired visuomotor coordination. Similarly, Shuttleworth-Edwards and Radloff (2008) demonstrated that non-concussed university rugby players performed worse throughout Immediate Post-Concussion Assessment and Cognitive Testing (imPACT), TMT-A and TMT-B relative to non-contact sport controls across a competitive season. Learning effects are common among cognitive assessments (Marley et al., 2017). However, concussed athletes have previously failed to elicit learning effects across a competitive season relative to non-concussed controls (Shuttleworth-Edwards et al., 2014). Subsequently, the lack of practice effects may offer unconventional diagnostic potential. Gardner et al. (2010) observed that rugby players with three or more concussions elicited reduced motor processing speed during completion of the Wechsler Adult Intelligence Scale processing speed index and imPACT. King et al. (2012) developed the King Devick (KD) Tool to assess saccadic rhythm for rapid-number naming. Among 37 amateur rugby players, KD completion time increased on the day of concussion indicative of impaired saccadic function, attention and language ability (King et al., 2013). Using the Sports Concussion Assessment Tool (SCAT), which assesses immediate memory, attention and delayed recall, over 50% of elite players were cognitively impaired 24 hours after concussion. These impairments resolved in 85% of players within 7 days and 99% of players within 14 days (Figure 14B, Cross et al., 2016).

Concussion history does not correspond with post-concussive syndrome (PCS) likelihood or a decline in cognition among active young players (Thornton et al., 2008). However, retired players elicit a dose-response associated with concussion history, memory deficits and PCS (Thornton et al., 2008). Hume et al. (2017) observed that a cohort of retired rugby players (n = 302) presented with reduced attention, processing speed and executive function relative to a non-contact sport group (n = 65) using the CNS Vital Signs test. A further decline in cognition was observed when players were grouped by playing standard, which revealed that a subdivision of elite retired players (n = 103) performed worse relative to both community level players (n = 198) and non-contact sport controls (Hume et al., 2017). Similar impairments

were noted by McMillan et al. (2017) who concluded that 52 elite players performed worse in tests of verbal learning via the Rey Auditory Verbal Learning Test (RAVLT) and fine motor coordination via the Grooved Pegboard Dexterity Test, relative to non-contact sport controls. While McMillan et al. (2017) noted that the decline in cognition was 'detectable but non-clinical', Decq et al. (2016) revealed that a separate cohort of retired rugby players (n = 239) presented with MCI via the Modified Telephone Interview for Cognitive Status.

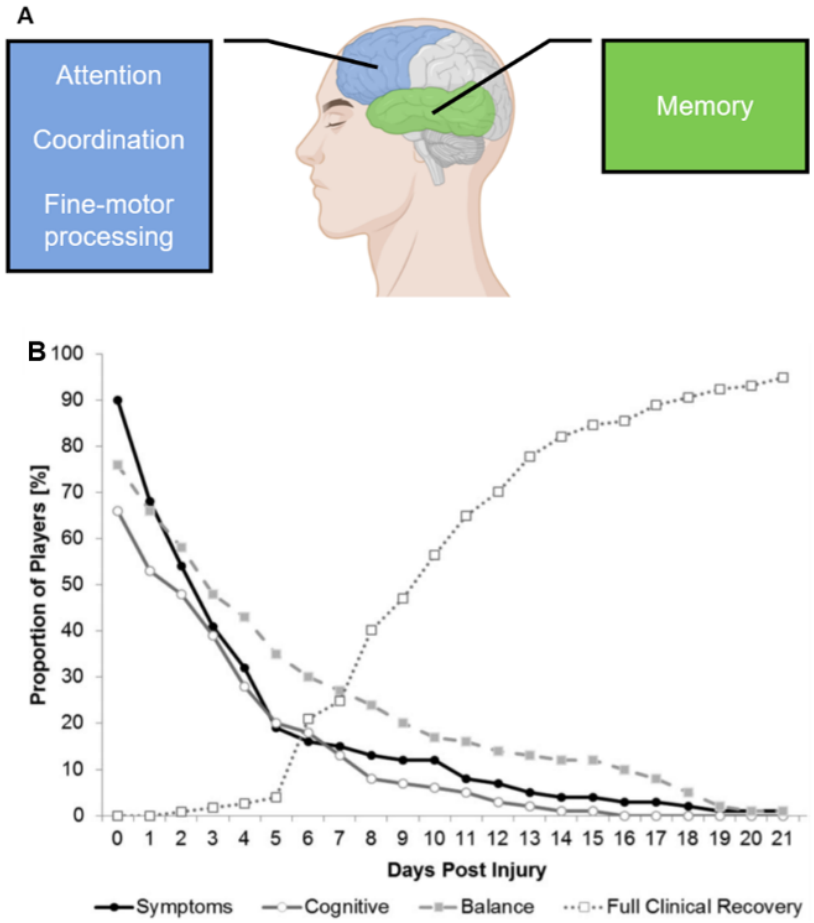

Figure 14: Frontotemporal cognitive impairments are most commonly reported among current and retired rugby players with concussion history (**A**). Mean percentage of 100 professional rugby union players exhibiting symptoms, cognitive deficit and impaired balance over the first 21 days post-concussion (**B**) Adapted from (Cross et al., 2016).

It is therefore apparent that cognitive consequences exist following recurrent contact and concussion in rugby union. While cognition among current rugby players may be commonly resolved within 7-10 days after concussion, there is evidence that cumulative exposure to prior recurrent contact and concussion may accelerate a player's trajectory towards cognitive impairment.

2.5. Neurodegeneration

Cognitive impairment increases one's susceptibility to neurodegeneration. Annually, 10-20% of patients diagnosed with MCI develop Alzheimer's disease compared to 1-2% of non-MCI patients (Tierney et al., 1996, Meyer et al., 2002). However retired contact sport athletes are at an increased risk of developing CTE, a form of neurodegeneration unique to individuals with history of repetitive head contact (McKee et al., 2016). The current neuropathological criteria for the diagnosis for CTE are collectively defined by the National Institute of Neurological Disorders and Stroke, and the National Institute of Biomedical Imaging and Bioengineering (McKee et al., 2016). CTE diagnosis can be confirmed via legions of phosphorylated tau (p-tau) aggregates, which form irregular spatial patterns at depths of the cortical sulci populating neurons, astrocytes and cell processes around the small blood vessels (Figure 15A).

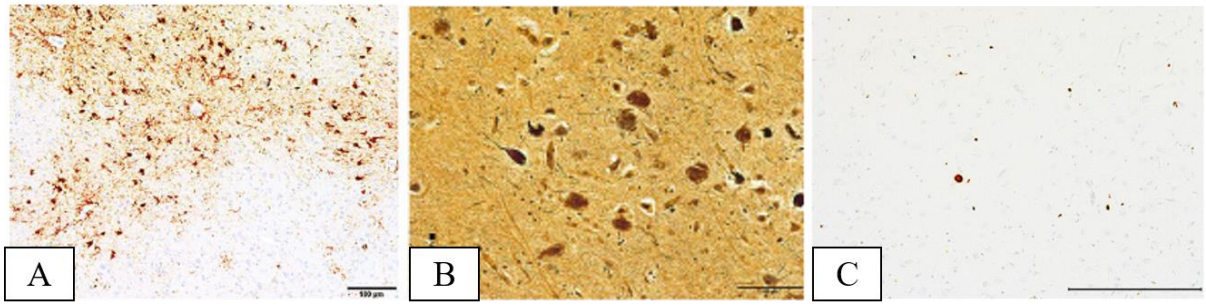

Figure 15: Microscopic images captured during brain autopsy of a deceased CTE patient demonstrating rounded perivascular P-tau lesion pathology in the sulci (**A**), including neurofibrillary tangles (**B**), and TDP-43 inclusions and dot-like neurites the CA1 region of the hippocampus (**C**). Adapted from McKee et al. (2016).

CTE may also be diagnosed in the presence of other pathologies, which include neurofibrillary tangles (Figure 15B), β-amyloid plaques and TAR DNA-binding protein (TDP)-43 formation (Figure 15C). CTE pathology commonly presents 10-20 years following retirement from contact sports (Geddes et al., 1996, McKee et al., 2009) with tau pathology that stems around the Papez circuit (i.e. continuum of hippocampal formation, Papez, 1995, Eggers, 2007). Consequently, neuronal loss leads to brain atrophy which is associated with a subset of behavioural changes and functional disorders including depression and suicide tendency (Omalu et al., 2006). CTE has been confirmed in sporting athletes from boxing, soccer and ice hockey, but is most prolific in American footballers (Gardner et al., 2014a).

Some research groups have confirmed CTE pathology in up to 99% of brains donated by deceased National Football League players (Mez et al., 2017). Despite comparable concussion incidence rates in rugby union and American football (Prien et al., 2018), fewer cases of CTE have been confirmed in rugby union players. Stewart et al. (2016) postulated that CTE may be an under-recognised consequence of participation in rugby union when confirming the first known case of the unique neurodegenerative disease in a deceased 57-year-old rugby player. Later, Lee et al. (2019) performed brain autopsies in four deceased players diagnosed with dementia and confirmed CTE pathology among three. Common macroscopic observations confirmed atrophy of the frontal and temporal lobes, including the hippocampus (Figure 16). Microscopic inspection revealed perivascular p-tau lesions, neurites and thorn shaped astrocytes clustered around cortical vessels and TDP-43 deposition (Stewart et al., 2016, Lee et al., 2019).

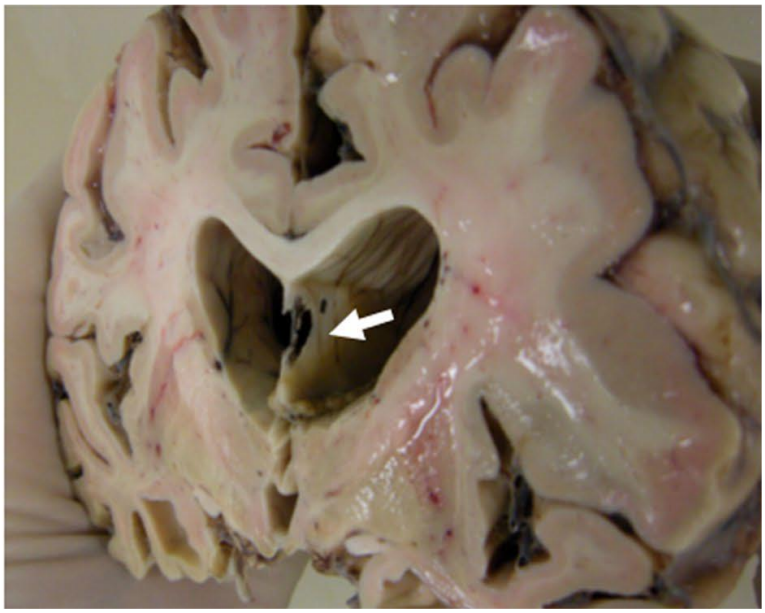

Figure 16: Representative macroscopic image from a deceased 70-year-old rugby union player with altered behavioural traits and cognitive disturbance over the course of 18 years. Sectioning of the brain through the mammillary bodies revealed ventriculomegally, consistent with cerebral atrophy and fenestration of the septum pellucidum (white arrow). Sourced from Lee et al. (2019)

CTE has further been confirmed in variants of rugby union, including rugby league and Australian Rules (McKee et al., 2014, Buckland et al., 2019). Combined, the growing body of literature argues that CTE may be an under-recognised consequence of rugby (Lee et al., 2019), however the mechanisms responsible for the development of the disease remain largely unknown, given that only a minority of athletes present with these unique behavioural and pathological alterations.

2.6. Summary

The understanding and governance of concussion has progressed exponentially during the 21st century. It is apparent that prior exposure to recurrent contact and concussion may promote an accelerated decline in cognition and elevated risk of neurodegeneration. Several mechanisms may work synergistically, namely mechanical trauma, altered neurometabolism, elevated OXNOS and suppressed cerebrovascular function. Furthermore, participation in rugby union is at an all-time high, with 8.5 million players engaging with the game globally. Concussion in rugby union is comparable with that of other contact sports including American football and ice hockey. However, unlike other contact sports, to what extent concussion in rugby union precedes MCI and neurodegeneration remains to be established.

2.7. Knowledge gaps

While there is a diverse literature base detailing the detrimental effects of concussion across various sports, less is known about these effects in rugby players. Moreover, the extent to which recurrent (non-concussive) impact may increase a rugby players trajectory towards neurological complications is unknown. Molecular profiling among rugby players has not been established and likewise, observations of CBF and cerebrovascular function are lacking. There is a potential that these collective mechanisms contribute to an accelerated decline in cognition. However, there is a lack of longitudinal studies in young players which seek to address the cumulative effect of recurrent contact and concussion on cognition across one playing season. Additionally, there is no cross-sectional study which seeks to address the chronic effects of recurrent contact in retired rugby players, whereby a combination of concussion history and an age-related decline in general health serve to further increase the risk of cognitive impairment and neurodegeneration. This thesis seeks to bridge these knowledge gaps.

2.8. Objective, aims and hypotheses

Two functionally integrated translational research studies formed the basis of the present thesis. The overarching objective of the research was to examine changes in molecular, cerebral haemodynamic and cognitive function across the human adult lifespan following exposure to recurrent contact & concussion. The overarching hypothesis was that rugby players exposed to recurrent contact with concussion history would present with elevated OXNOS, suppressed cerebrovascular function and a decline in cognition, which would be especially pronounced in the retired rugby players given the combination of ageing and physical inactivity (Figure 17).

2.8.1. Study 1 - Professional (young) players study

Aim: To determine the molecular, cerebrovascular and cognitive signatures of professional rugby union players over a single season, stratified by frequency of contact events, playing position and concussion risk.

Hypothesis: Compared to controls, current rugby union players would present with elevated OXNOS, suppressed cerebrovascular function and a decline in cognition with subtle alterations in these metrics detected over one season.

2.8.2. Study 2 - Retired (aged) players study

Aim: To determine the chronic molecular, cerebrovascular and cognitive signatures of retired rugby players with concussion history.

Hypothesis: Compared to controls, retired rugby union players would present with elevated OXNOS, suppressed cerebrovascular function and cognition.

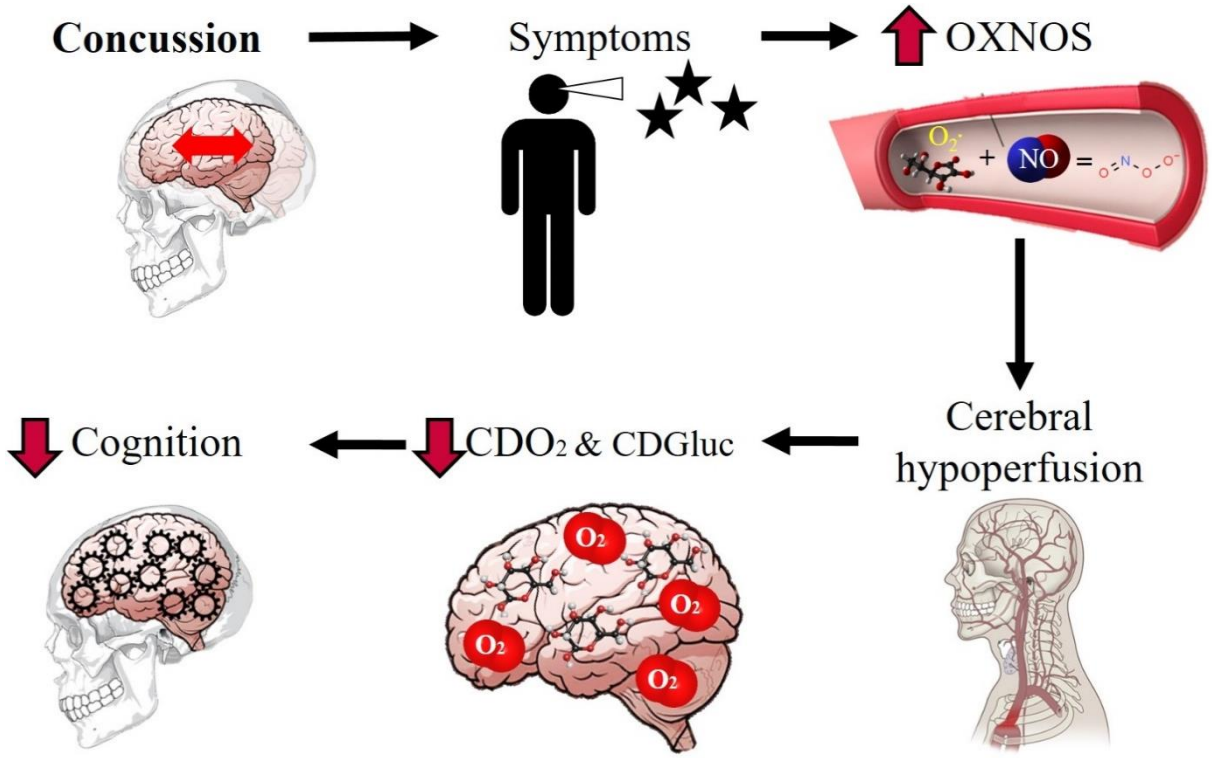

Figure 17: Schematic overview of the simplified hypothesis proposed in this thesis. OXNOS, oxidative-nitrosative stress; CDO_2, cerebral delivery of oxygen, CDGluc, cerebral delivery of glucose; O_2, oxygen.

Chapter 3
General Methodology

3.1. Ethical approval

Two research studies were conducted to form the basis of the present thesis. Ethical approval was granted by the University of South Wales ethics committee and assigned separate approval numbers (Study 1: 0617LSETOE0, Appendix II, Study 2: 2017TO1102, Appendix III). Verbal and written informed consent was obtained from all participants following a thorough explanation of the study measurements via participant information sheets. Participants were further informed of their right to withdraw consent of participation in the study at any point, without providing explanation. Anonymity was ensured via unique participant codes assigned via a random generator. All experimental procedures conformed to the standards set by the Declaration of Helsinki of the World Medical Association, except for registration in a database (Williams, 2008). From its introduction in 2018, data were collected under the General Data Protection Regulation (GDPR, European Commission, 2018).

3.2. Inclusion/Exclusion criteria

Participant eligibility for Study 1 and 2 was contingent on stringent inclusion and exclusion criteria.

Inclusion

In the first instance, participant eligibility was assessed via a telephone screening questionnaire. Male participants aged 18 years or older were eligible to participate in Study 1. For assignment to the 'young' rugby group, players were required to be of professional status and contracted to a full season with the team to attain follow up measures. For study 2, male participants aged between 60 – 80 years were eligible to participate. For assignment to the 'aged' rugby group, retired players were required to have sustained at least one self-reported concussion throughout their playing career. A control group was actively recruited to both studies who were matched for age, sex, fitness and education. Controls had not participated in contact sports (outside of any educational curriculum) and had no prior history of concussion.

Exclusion

Participants were excluded if they were suffering from cardiovascular (ischaemic heart disease, hypertension), cerebrovascular (stroke, transient ischaemia), or respiratory disease (chronic obstructive pulmonary disorder, bronchitis). Participants were excluded if they were smokers or recreational drug users (cannabis, cocaine, MDMA etc.), or if they could not confirm registration with a General practitioner (GP). For Study 2, participants were clinically screened via a 12-lead functional diagnostic electrocardiogram (ECG) at rest, during and following an

incremental exercise test under clinical supervision (outlined in section 3.8). Participants were excluded if any electrical/symptomatic abnormalities presented consistent with the guidelines set by the American College of Sports Medicine (American College of Sports Medicine, 2014).

3.3. Participants

3.3.1. Power calculations

Prospective power calculations were determined using commercially available software (nQuery, Statistical Solutions, Cork, Ireland). The primary end-outcome variable for both studies was cognition. Cohort sizes were determined using the concussion-related effect size on cognition observed by Bailey et al. (2013a). Using two-sided independent samples t-tests assuming power of > 0.95 at $P < 0.05$, pooled effect size of 1.67 and standard deviation (SD) of 6.86, it was estimated that 32 participants, divided into two equal groups of 16 (concussed vs. controls for each study) would be required to detect differences in cognition. Assuming a conservative 30% attrition due to drop-out/technical complications during each of the study periods, this was inflated to 21 participants per group.

3.3.2. Study 1 - Professional (young) players study

Forty-five males were recruited into two sub-groups. Twenty-three professional rugby union players aged 25 ± 4 years with 3 ± 2 previous concussions incurred over 16 ± 4 years were compared with 17 sex, age-, physical activity- and education-matched controls, with no participation in contact sports or concussion history. The final analysis comprised of 21 players (13 forwards/8 backs) and 17 controls. Table 1 outlines participant demographics (section 4.2).

3.3.3. Study 2 - Retired (aged) players study

Forty-four males were recruited into the study and divided into two sub-groups. Twenty-two retired rugby players aged 64 ± 5 years with 3 ± 3 concussions incurred over 22 ± 7 years were compared to 22 age-, CRF- and education-matched controls with no participation in contact sports or concussion history. Forty-one participants were included in the final analysis comprised of 20 retired players and 21 controls. Table 8 outlines participant demographics (section 5.3).

3.4. Experimental design

For Studies 1 and 2, all participants participants refrained from physical activity, caffeine and alcohol, followed a low nitrate/nitrite diet and had completed a 12 hour overnight fast prior to experimentation (Wang et al., 1997, Bailey et al., 2017). Immediately upon entry to the

laboratory, the investigator provided an explanation for all laboratory measurements scheduled to take place using the participant information sheet before written consent was obtained from the participants.

Study 1 – Professional (young) players study

A longitudinal study design was implemented, with measurements obtained at three time-points; pre-season, in-season and post-season. Controls attended the Neurovascular Research Laboratory at the University of South Wales and rugby players attended the Vale Training Pavilion before (pre-season) and after (post-season) the 2017-18 rugby union season. Data collection comprised of anthropometry, haematological measurements, cerebrovascular function and cognition. During the in-season, notational analysis monitored players throughout every game of the season to log match events. Control participants pursued habitual life during this period. An overview of the study is presented in Figure 30 and a detailed description of measurements outlined in section 3.5 to 3.10.

Study 2 - Retired (aged) players study

A single-comparison, cross-sectional design was implemented over the course of two laboratory visits at the Neurovascular Research Laboratory. During visit one, participant anthropometrics were collected, followed by a routine assessment of general health performed by a GP specialising in Sports Medicine (Dr Gareth Jones) and a venous blood sample for haematologic assessment. Physical screening was conducted using an ECG whilst undergoing an incremental exercise test to exhaustion on a semi-recumbent cycle to assess CRF. During visit two, cognitive assessments were performed, followed by a baseline SCAT5 and measurements of cerebrovascular function. An overview of the study is presented in Figure 35 and a detailed description of measurements outlined in section 3.5 to 3.9.

3.5. Anthropometrics

3.5.1. Stature

Participant stature was determined during each laboratory visit. Participants removed any footwear including socks before measurement using a stadiometer (Seca, Cardiokinetics, UK). Data were recorded to the nearest 0.1 cm.

3.5.2. Mass

Body mass was determined using a weighing scale (Seca, Cardiokinetics, UK). Participants removed any unnecessary outer clothing before measurement. Body mass was recorded to the nearest 0.1 kg.

3.5.3. Body mass index (BMI)

Body mass index provides estimates of weight range based from participant height (Cole et al., 1995). BMI was calculated for all participants using the following equation:

$$\text{BMI } (kg.m^2) = \text{mass } (kg) \div \text{stature } (m^2)$$

Participants were classified in accordance to output score as <18.5 representing underweight, 18.5 – 24.9 representing normal weight range, 25 – 29.9 representing overweight and 30 – 39.9 representing obesity (Cole et al., 1995).

3.5.4. Body composition

Bioelectrical impedance analysis (Tanita BC-418MA, Japan) was used to estimate body fat derived from multi-frequency electrical currents (Kyle et al., 2004a). Procedures were carried out in accordance to recommended guidelines (Kyle et al., 2004b). Briefly, participants removed outer clothing and footwear, an estimation of remaining clothes weight was entered (0.5 – 1.0 kg) and gender selection was sub-categorised into one of two categories ("athletic male" was selected if participants completed 10 hours or more of moderate to vigorous physical activity per week and "normal male" was selected if this criteria was not met). Stature and age was inputted into the system before participants stood motionless with bare feet on the weighing platform, ensuring adequate contact with the electrodes. Impedance analysis was conducted in an upright standing position following confirmation of mass stabilisation on the electrodes.

3.6. Clinical consultation (Study 2 - Retired [aged] players study only)

A clinical consultation was carried out via Dr Gareth Jones. The clinician was provided with a copy of the medical background of participants that was obtained from the telephone screening questionnaire and supplementary GP medical records if provided. Participants were taken to a private room with the clinician to conduct a brief medical consultation.

To begin, the clinician confirmed information provided by the participant during the prior telephone screening questionnaire. Following confirmation, family history (particularly of parent and/or siblings) of any major diseases and/or conditions were noted. Participants were

asked to confirm any chest pain at rest, in response, or prior to exercise in the previous three months preceding experimental visits, including any indications of dyspnoea, syncope, palpitations or cerebrovascular symptoms aligned to transient ischaemic episodes, or peripheral vascular symptoms indicative of claudication. If participants were prescribed medication, a review was conducted with regard to any recent changes in dosage and reasoning.

A brief physical examination was then conducted. Blood pressure (BP) and heart rate (HR) was recorded after 10 minutes of supine rest. Participants were then asked to sit in an upright position and the clinician screened for heart murmurs and irregular heart sounds using a stethoscope (Littmann Classic III, 3M, Bracknell, UK). Brief respiratory observations were completed for the nose and throat, followed by percussion and auscultation of the chest. A 12 lead ECG was then fitted (Ultima CardiO_2 ECG, Mortara instruments, Stirling, UK) to determine the rhythm, rate, and electrical axis at rest and in response to incremental exercise.

3.6.1. 12 lead ECG

A 12 lead ECG (Welch Allyn, Buckinghamshire, UK) was fitted to all participants of Study 2 for observations at rest and in response to an incremental exercise test, under the supervision of a clinician (Dr Gareth Jones) and cardiovascular physiologist with over 50 years' experience conducting functional diagnostic exercise tests (Professor Bruce Davies).

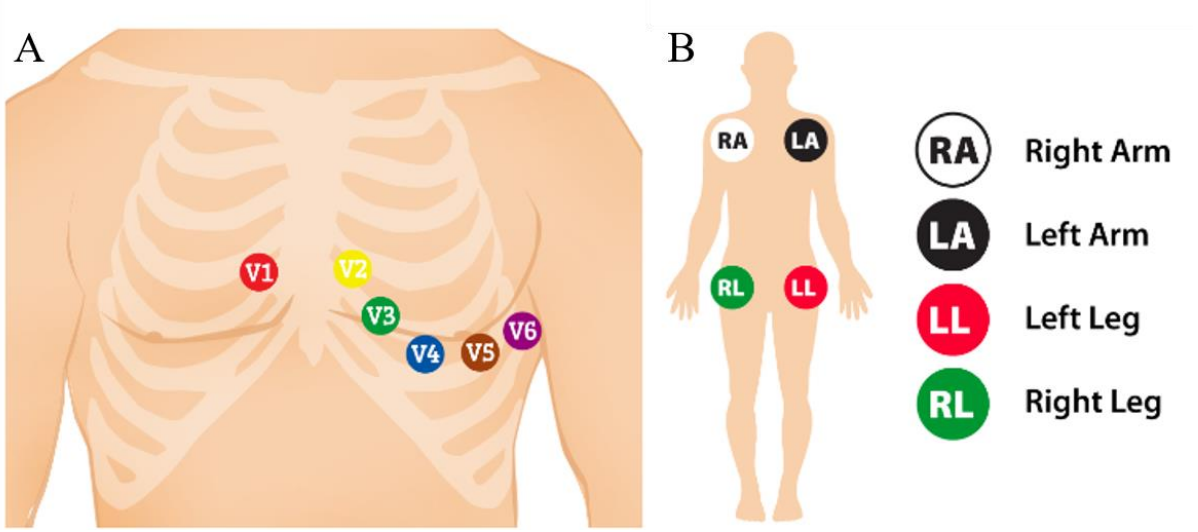

Figure 18: Electrode placement of precordial (**A**) and extremity (**B**) leads performed as a part of the clinical consultation and assessment of cardiorespiratory fitness for participants of Study 2. Adapted from Cables and Sensors (2019).

Briefly, skin was prepared via light abrasion to ensure optimal signal strength. Extremity leads were placed on the upper left and right arms, and on the lower left and right sections of the oblique abdominal area in order to minimise noise artefact during exercise (Figure 18B).

Precordial leads V1-V2 were placed in the fourth intercostal space on right and left side of the sternum, V4 was placed in the fifth intercostal space, parallel to the mid-clavicular line, with V3 placed directly between leads V2-V4. Lead V5 was placed in parallel to the anterior axillary line at the same horizontal level as V4, with V6 placed in parallel to the mid auxiliary line at the same horizontal level as V4-V5 (Figure 18A). Participants resting ECG was observed at rest in a supine position for 10 minutes and synchronised to an online metabolic analyser (MedGraphics, Ultima Series, UK). Participants were then seated on a semi-recumbent cycle ergometer and completed an incremental exercise to volitional exhaustion (section 3.8). All observations were made in the presence of a medically qualified clinician and any irregularities or contraindications to exercise noted and reported to the participants GP.

3.7. Haematology

3.7.1. Cannulation and blood sampling

Venous blood samples were obtained at rest from all participants and in response to maximal exercise in Study 2. A tourniquet (Prameta, Germany) was fitted around the arm and constriction was applied 7-10 cm above the desired puncture site. Prominent antecubital veins were assessed for cannulation and selected based on the most prominent vessel. An 18 or 20 gauge cannula (Becton Dickenson, Oxford, UK) was selected based on the size of the selected vessel and used to gain access. Following entry into the vessel, which was confirmed via blood flashback, the needle was removed and a 3-way tap (Connecta plus 3, Becton Dickenson, Oxford, UK) was fitted, enabling repeated blood withdrawals. The cannula was then secured in place using transpore tape (3M, Bracknell, UK) suitable for skin application. Medical standard saline (Baxter, Norfolk, UK) was flushed through the cannula to ensure line patency between sample collections. All cannulations were monitored and logged for auditing purposes. During blood sampling, a luer adaptor and vacutainer holder (Becton and Dickenson, Oxford, UK) was connected to the 3-way tap and subsequently opened. A 6 mL vacutainer was collected and discarded to avoid sample dilution from the saline flush. A 10 mL potassium-ethylenediamine tetraacetic acid (K-EDTA, Becton and Dickenson, Oxford, UK) vacutainer was used for collection of blood plasma. Following removal of the luer adaptor and vacutainer holder, a 1 mL syringe (Becton and Dickenson, Oxford, UK) was used to collect whole blood. Immediately after blood collection, vacutainers were centrifuged at 600*g* at 4°C for 10 minutes (Hettich Totanta 460R, Hettich Tuttlingen, Germany). Plasma samples were separated and decanted into sterile 2 mL cryovials (Cryovial, Simport, Quebec, Canada) prior to immediate

flash-freezing using liquid nitrogen (-196°C). Plasma samples underwent analysis within 24 hours of collection.

3.7.2. Haemoglobin (Hb)

Haemoglobin concentration was determined using an analyser (HemoCue, 201, Angelholm, Sweden) and microcuvettes (HemoCue, 201, Angelholm, Sweden). The haemoglobin analyser served as a measurement apparatus and was factory calibrated against the haemiglobincyanide (HiCN) method, which is considered as the international reference method for determination of blood haemoglobin concentration (HemoCue, 2008). The microcuvette served as a pipette, reaction vessel and measuring cuvette contained with dry reagent that required no dilution method. Using a 1 mL syringe, whole blood was collected and approximately 0.1 mL was decanted into a microcuvette. The microcuvette was filled in a continuous process to ensure no air bubbles entered. Excess blood on the outside of the microcuvette was wiped away ensuring that blood was not removed from inside the cuvette. The mirocuvette was placed into the tray of the haemoglobin analyser and slider closed for analysis. Haemoglobin concentration was calculated using the analyser whereby the dry reagent contained within the microcuvette was activated. Briefly, sodium deoxycholate haemolyses erythrocytes to release haemoglobin. The haemoglobin was converted by sodium nitrite to methaemoglobin and sodium azide produced azidemethaemoglobin which was sampled at wavelengths of 570nm and 880nm (HemoCue, 2008). To account for varying sample turbidity, analysis was performed in duplicate and the average recorded. Intra- and inter-assay coefficients of variation (CVs) were both <5%.

3.7.3. Haematocrit

Haematocrit concentration was determined by decanting blood into microcapillary tubes (Na-heparinised, Hawksley & Sons, Sussex, UK), which were centrifuged for 3 minutes at 600*g* and placed onto a haematocrit analyser (Hawksley & Sons, Sussex, UK). Analysis was performed in duplicate and the average recorded. Intra- and inter assay CVs were both <5%.

3.7.4. Free radicals

3.7.4.1. Electron paramagnetic resonance (EPR) spectroscopy

EPR spectroscopy is the only direct method to detect free radicals that play a role in the metabolic and chemical changes within biological systems (Buettner and Jurkiewicz, 1993). Traditionally, radicals have short lifetimes (10^{-3}-10^{-9}s) because of their high reactivity which undergo reactions between 10^{-6}-10^{-9} $M^{-1}s^{-1}$. Consequently, free radicals are notoriously difficult

to detect and require spin-trapping methods to prolong their lifetime (Buettner and Jurkiewicz, 1993). With the development of such spin-trapping methods, a wealth of knowledge regarding free radical interactions with biochemical processes has been established (Janzen, 1990). Unlike most free radicals, ascorbate free radical ($A^{\bullet-}$) has a relatively long lifetime and is detectable at room temperature using EPR spectroscopy, which is derived from the one electron oxidation of the endogenous water-soluble antioxidant, ascorbate anion (Yamazaki et al., 1960, Buettner and Jurkiewicz, 1993). Given the prolonged lifetime of $A^{\bullet-}$, spin-trapping is not required and offers an effective measurement of global free radical flux. Furthermore, the low reduction potential of ascorbate anion ensures that reactions are possible with most oxidising radicals to form $A^{\bullet-}$ (Buettner and Jurkiewicz, 1993). Therefore, $A^{\bullet-}$ offers a feasible method for direct detection of global free radical formation in human blood (Bailey et al., 2009).

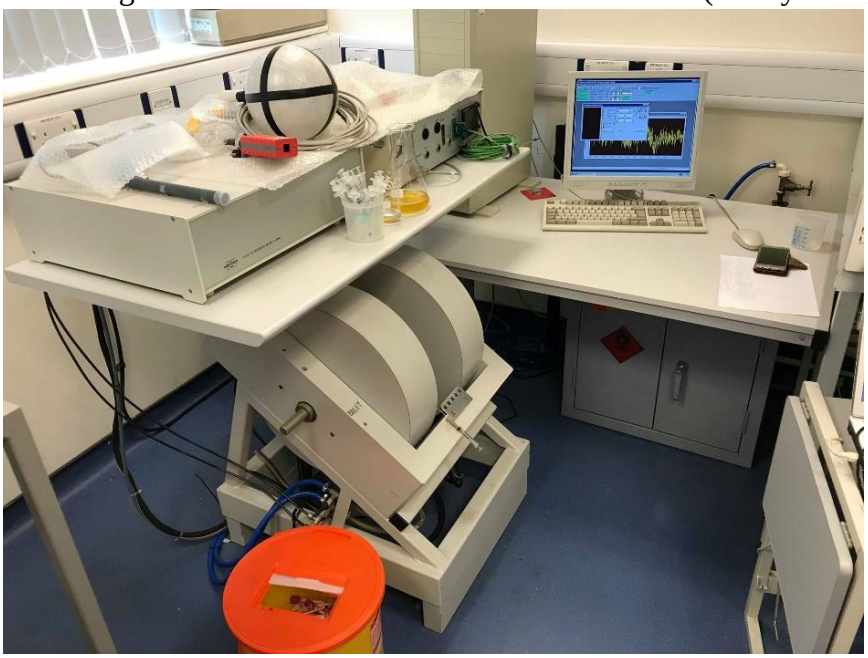

Figure 19: EPR spectrometer at the Neurovascular Research Laboratory.

Exactly 1 mL of plasma was thawed at 37°C in a water bath (GD120, Grant, Royston, GB) for five-minutes and injected into a high-sensitivity multiple-bore sample cell (AquaX, Bruker Instruments Inc, Billerica, USA) housed within a TM_{110} cavity and analysed by X-band EPR spectroscopy operating at 100 kHz modulation frequency, 0.65 Gauss modulation amplitude, 10 mW microwave power, 2 x 10^5 receiver gain and 41 ms time constant for 10 incremental scans (Figure 19). Spectra were subsequently filtered identically using WINEPR (Version 2.11) and the double integral of each doublet calculated using Origin software (Figure 20, Origin pro 8.6, Northampton, USA). Analysis was performed in duplicate and the average recorded. Intra- and inter-assay CVs were both <5%.

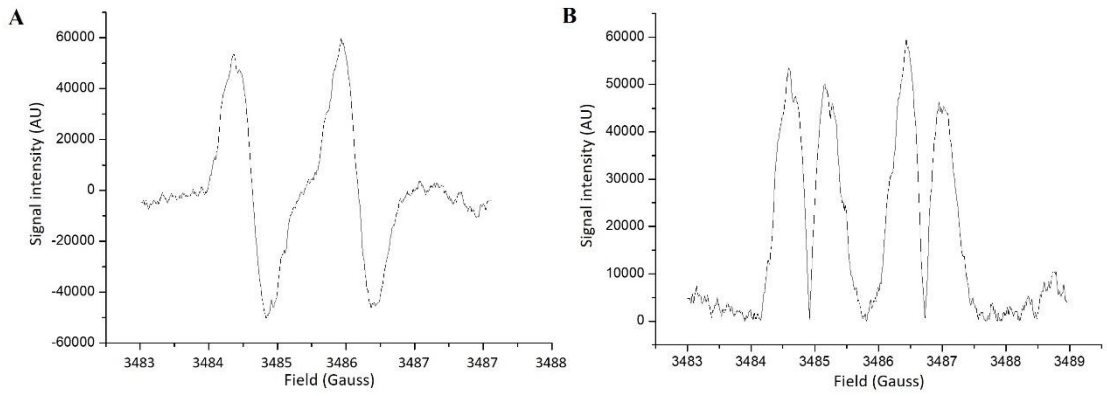

Figure 20: Ascorbate radical doublet (**A**) before and (**B**) after undergoing processing using Origin peak software. Values presented as raw sample of one participant.

3.7.5. Ozone based chemiluminescence (OBC)

Nitric oxide is a key anti-athrogenic molecule associated with a variety of signalling pathways (Pinder et al., 2008), including the maintenance of vascular function (Tousoulis et al., 2012). NO is a highly reactive free radical species which is notoriously difficult and complex to measure (Pinder et al., 2008). Subsequently, the measurement of NO metabolites are often performed, which are the reaction products of NO and include NO^-_3, NO^-_2 and protein bound NO. Two NO metabolites which have often undergone analysis are NO^-_2 and S-nitrosothiols (RSNO, Bailey et al., 2017). This is since NO derived from endothelial nitric oxide synthase (eNOS) at rest and during exercise promotes not only vasodilation, but oxidation in plasma to produce NO^-_2 and RSNO. Such metabolites are stable and are cable of transducing NO bioactivity 'long distance' within the human microcirculation (Jia et al., 1996, Bailey et al., 2017).

To measure these NO metabolites, OBC offers an accurate and sensitive method for the detection and measurement of NO. However, direct measurement may only be achieved by cleaving the metabolite of interest or reducing it back to NO (Pinder et al., 2008). To achieve such states, the use of chemical cleavage reagents or photolysis are required.

3.7.5.1. Principles of OBC

Ozone based chemiluminescence is a form of measurement whereby the luminescent nature of specific chemical reactions can be exploited to quantify NO (Pinder et al., 2008). To measure NO, it must be either cleaved from a parent compound or reduced back to a form a radical. Chemical reagents allow for this to be achieved as NO can be transported in an inert gas stream

within a constant flow rate of nitrogen (N_2, Pinder et al., 2008). During sample processing, N_2 flows through a purge vessel where the cleaving reagent is housed, before reaching a sodium hydroxide (NaOH) trap. The NaOH trap serves to ensure measurement quality by ensuring that potential contaminants are not converted to NO. Thereafter, a solvent filter is placed along the line before gases reach the nitric oxide analyser (Figure 21B, NOA, Model 280i, NOA®, Sievers, Boulder, CO, USA), which serves to protect the analyser from hot acid vapour damage (Pinder et al., 2008). Subsequently, the gas is transported to the NOA that is capable of generating ozone (O_3) within a reaction cell. When NO enters the NOA, it reacts with O_3 to form nitrogen dioxide (NO_2) and oxygen (Pinder et al., 2008). NO_2 is produced in an electrically excited state (NO_2*) which is unstable and releases excess energy as photons when returning to their normal state. The photons are focused through a low pass-filter lens operating at < 900 nm into a photomultiplier tube that subsequently amplifies the signal to produce a reading in the form of millivolts (Pinder et al., 2008). Therefore the following reactions take place (Pinder et al., 2008):

$$NO + O_3 \rightarrow NO_2 + O_2 + h\nu \rightarrow PMT \rightarrow Signal\ (mV)$$

3.7.5.2. Technique

Ozone based chemiluminescence was used to determine the concentration of NO_2^- and RSNO derived from plasma (Pinder et al., 2008, Pelletier et al., 2006) using an acidified tri-iodide solution. Techniques used for the preparation and measurement of chemicals, reagents and samples followed the methods of Bailey et al. (2017, Figure 21A) with all reagent solutions prepared fresh on a daily basis . To prepare the solution, 70 mL of acetic acid (Sigma-Aldrich Ltd., Gillingham, Dorset, UK), 650mg of iodine crystals (Sigma-Aldrich Ltd., Gillingham, Dorset, UK) and 1g of potassium (Sigma-Aldrich Ltd., Gillingham, Dorset, UK) was added and dissolved into 20 mL of HPLC-grade water. Thereafter, 5 mL of tri-iodide reagent was decanted into a purge vessel (GPE Scientific, Leighton, UK) and 30 µL of antifoam solution (204, Sigma-Aldrich Ltd., Gillingham, Dorset, UK) before placing the lock cap onto the top of the purge vessel. Plasma samples (200μL) were thawed in a water bath (GD120, Grant, Royston, GB) at 37°C for five minutes before being injected into the tri-iodide reagent. Reagent was replaced for each sample to eliminate the risk of sample contamination. After processing samples, a peak analysis package (Origin Pro 8.6, Northhampton, USA) was used to calculate the area under the curve (mV/s) for each peak (Figure 21C). Sample concentrations for combined NO_2^- and RSNO were calculated by converting standard curves of known concentrations of a sodium nitrite ($NaNO_2$, Fisher Scientific, Loughborough, UK) stock

solution. Standard curves were composed of a baseline 200 μL injection of HPLC water, followed by dilutions of 62.5, 125, 250, 500, 1000nmol $NaNO_2$ which was derived from a stock solution of 10mM. Intra- and inter-assay CVs were both <5%.

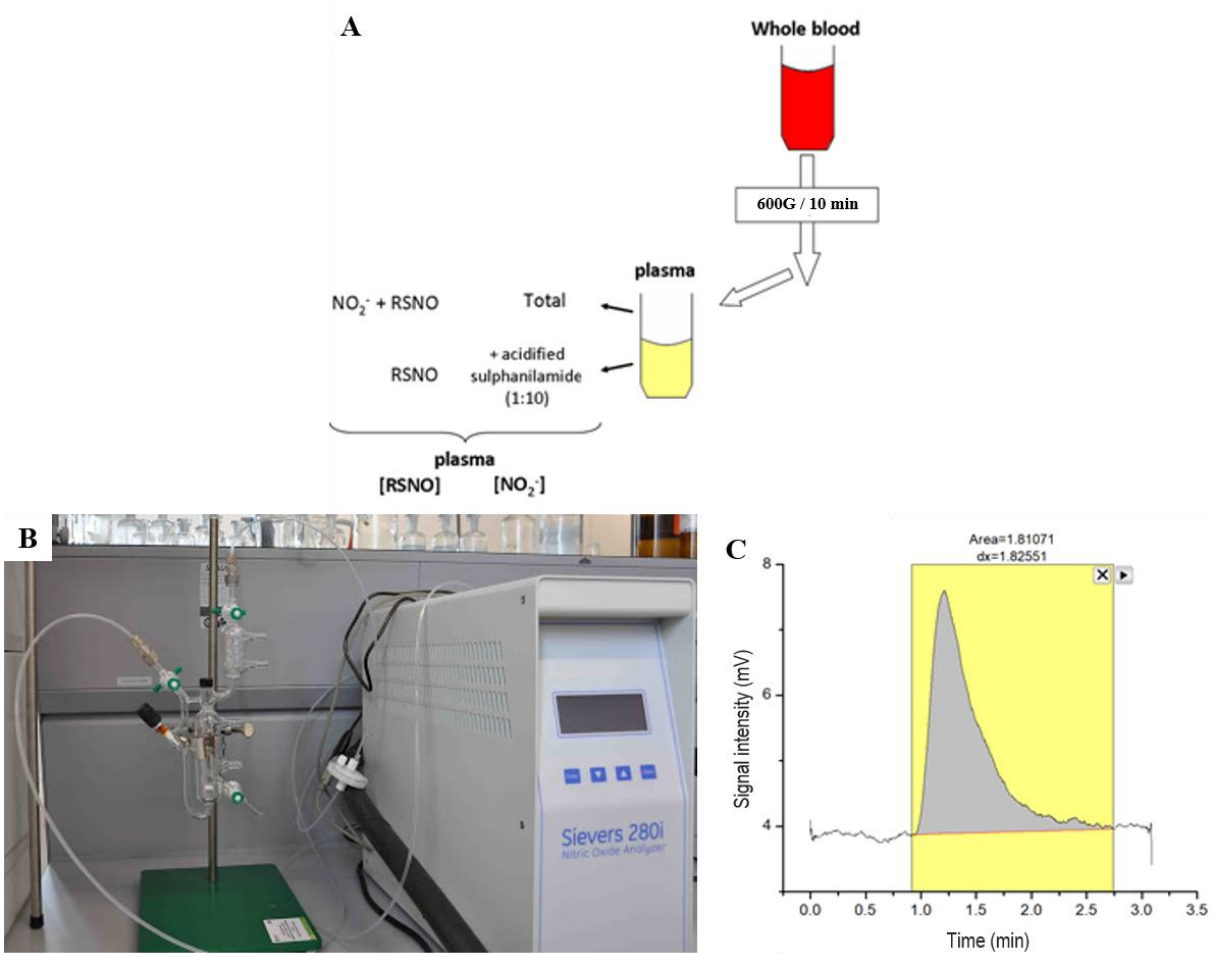

Figure 21: Simplified diagram of sample preparation prior to analysis of nitrite (NO_2^-) and S-nitrosothiols (RNSO, **A**) using Sievers 280i nitric oxide analyser (**B**) and integration of output curve using Origin peak software (**C**). Values presented as raw sample of one participant.

3.7.5.3. Validity of OBC

According to Pinder et al. (2008) there is consensus that OBC offers the most sensitive and robust method for quantifying NO metabolites. This is supported by Pelletier et al. (2006) and various studies have utilised OBC for the detection of NO metabolites (Rassaf et al., 2007) using the same NOA utilised in the current thesis (Gomes et al., 2008, Sandbakk et al., 2015, Bailey et al., 2017). While no studies were identified which specifically address the quality control and repeatability of OBC for analysis of biological samples, the fact that OBC is commonly used as a measurement of such metabolites in the published literature (often with

clinical relevance or outcome) stand virtue to the robust and reliable nature of the method (Pinder et al., 2008).

3.8. Exercise stress test (Study 2 - Retired [aged] players study only)

An incremental exercise test to volitional exhaustion was utilised during Study 2 in order to rule out contraindications to exercise, assess the cerebrovascular response to exercise and establish cardiorespiratory fitness.

3.8.1. Measurement technique

Participants were seated onto an electronically braked semi-recumbent cycle ergometer (Lode Corival, Cranlea & Company, UK, Figure 22). The participant's feet were placed into the pedals and secured using straps. Seat position was adjusted to ensure partial knee flexion (165 – 180°) during maximal pedal stroke length (Ruby and Hull, 1993). A mouthpiece and nose clip was fitted to the participant for the collection of respiratory data via breath-by-breath gas analysis using an online metabolic analyser (MedGraphics, Ultima Series, UK). Prior calibration of the analyser was achieved via methods recommended by the manufacturer. Briefly, a 3-litre syringe was attached to a pneumotach and used to mimic five inspiratory and expiratory flow patterns from the known syringe volume. Thereafter, an automated two-point calibration was performed using gases recommended by the manufacturer (17% O_2/5% CO_2 for the CO_2 analyser and 100% for the O_2 analyser), enabling volume to be calculated via integration of flow pattern and time. Following a two-minute rest period, participants were instructed to pedal at 70 revolutions per minute (RPM) and completed a two-minute warm-up period under no ergometer load. Ergometer workload was calculated via established criteria ensuring automated gradual increases to exercise intensity based from age x weight predicted algorithm until volitional exhaustion (Wasserman, 2012). Throughout the exercise assessment, the ventilatory equivalent of oxygen ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) consumption were determined including end-tidal carbon dioxide (PET_{CO2}), respiratory exchange ratio (RER) and HR was recorded. Maximal performance was confirmed if three of the following criteria was met; participant $\dot{V}O_2$ remained unchanged under increasing ergometer load, cadence fell below 60 RPM, RER in excess of 1.15 arbitrary units (AU), HR within 10% of age predicted maximum or a rating of perceived exertion of 20 points (Davies et al., 2016). Participants completed a two-minute cool down under no ergometer load and a further 10 minutes of supine recovery on a bed.

Figure 22: Incremental exercise assessment using semi-recumbent cycle ergometer.

3.8. Haemodynamic function

3.8.1. Transcranial Doppler ultrasonography

Aaslid et al. (1982) first assessed the intracranial arteries using Doppler Ultrasound. TCD is a non-invasive measure that allows continuous measurement of various cerebral arteries through the transtemporal window at high resolution (Willie et al., 2011). TCD has become a widely used technique for cerebral haemodynamic function in both clinical and research settings due to the high repeatability of the technique (Adams et al., 1997, Bailey et al., 2013a). TCD can assess the internal anterior, middle and posterior cerebral arteries, although the MCA is most commonly assessed since ~80% of all cerebral blood flow is delivered via this vessel (Willie et al., 2011).

As TCD is an ultrasound measure, it does not measure blood flow. Rather, the Doppler shift principle is used to identify flow velocities. This is because the diameter of the insonated vessel is often unknown. However, the MCA was believed to be less susceptible to diameter changes than the arterioles deep within the cerebrovasculature incurred via vasoactive stimuli including CO_2 (Serrador et al., 2000a). As such, the velocity of blood through the MCA is proportional to the 4^{th} power of the vessel radius and the relationship of CBF and blood velocity is considered proportional (Giller et al., 1993b, Nichols et al., 2005).

3.8.1.1. Principles

TCD utilises the Doppler shift principle (Nichols et al., 2005). The ultrasound probe known as a transducer is both a transmitter and receiver. When assessing the vessel of interest (e.g. MCA), the transducer emits a pulsed ultrasonic Doppler beam at 2 MHz which reflects from the red blood cells (RBCs) travelling through the vessel and back to the transducer. The RBC velocity is determined via the difference in frequency being transmitted and received by the transducer.

The Doppler frequency shift calculation is determined by the frequency being received (f_r), the frequency being transmitted (f_t), velocity of red blood cells (V), angle of the ultrasound beam in relation to the direction of blood flow ($\cos\theta$) and speed of sound within blood (C, 1540 $m.s^1$).

$$\text{Doppler Shift} = f_r - f_t = \frac{2 \times V \times ft \times cos\theta}{C}$$

Further, a factor of two is required since the Doppler effect occurs twice, once during transmission and once when receiving the signal (Nichols 2005). As the Doppler is three-dimensional (time, frequency and signal intensity), it is processed via spectral analysis before

being able to view the signal as two-dimensional waveforms, known as the spectral envelope. Once complete, the waveforms are displayed by motion-mode sonography on the monitor with the *y* axis indicating blood blow velocity while the *x* axis indicates time. The waveforms are displayed on a colour coded graph with blue representing weak and red representing strong signal intensity (Figure 23).

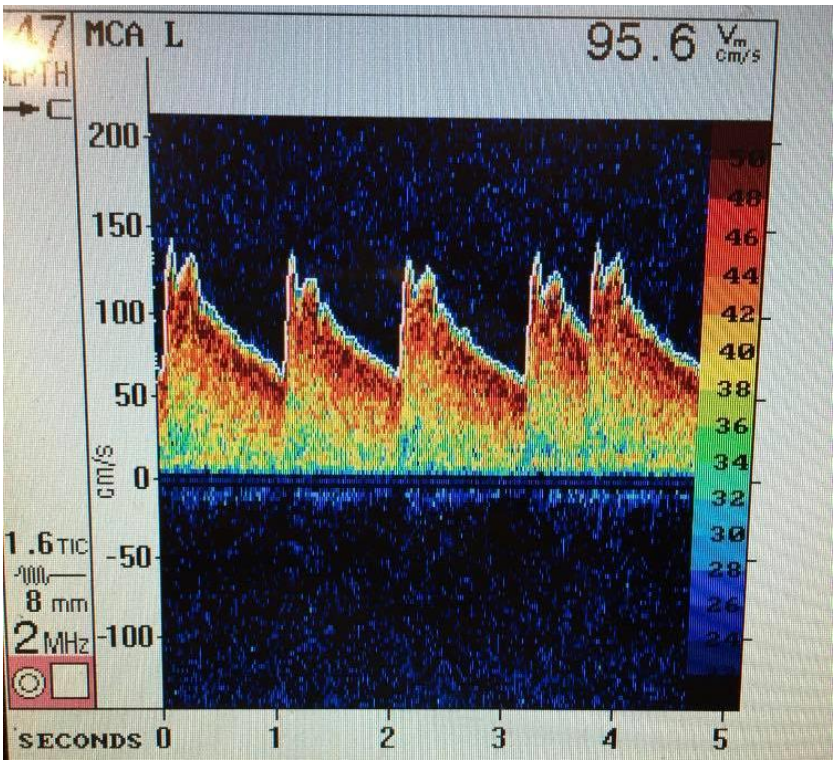

Figure 23: Transcranial Doppler ultrasound waveforms indicative of strong middle cerebral artery signal. Values presented as raw sample of one participant.

3.8.1.2. Measurement technique

All participants were seated in an upright position and instrumented using an adjustable plastic headset (Spencer Technologies, Nicolet Instruments, USA). Thereafter, the transducer (PMD 100, Spencer Technologies, Washington, USA) was attached to the headband for optimal insonation over the transtemporal window and prevention of movement artefact (Appenzeller et al., 2006).

For both studies, TCD was used to assess the right or left MCAv via search methods described by Aaslid et al. (1982). The left MCA was only assessed when insonation of the right MCA was not possible. When locating a signal, a water-based ultrasound gel (Aquasonic 100, Parker, USA) was applied between the skin and transducer to aid conduction. The transtemporal window is located on the temporal bone, which is positioned above the zygomatic arch (Figure 24A). The transducer was positioned differently in this area for each participant due to natural

anatomical variation in the MCA location. To obtain a signal, the transducer was positioned over the area until faint noise was apparent, indicative of an MCA signal. Once achieved, an insonation depth (44-73 mm) was selected, before altering the angle of the transducer for optimal signal intensity (Figure 24B). For repeatability, each measurement parameter was noted for future reference.

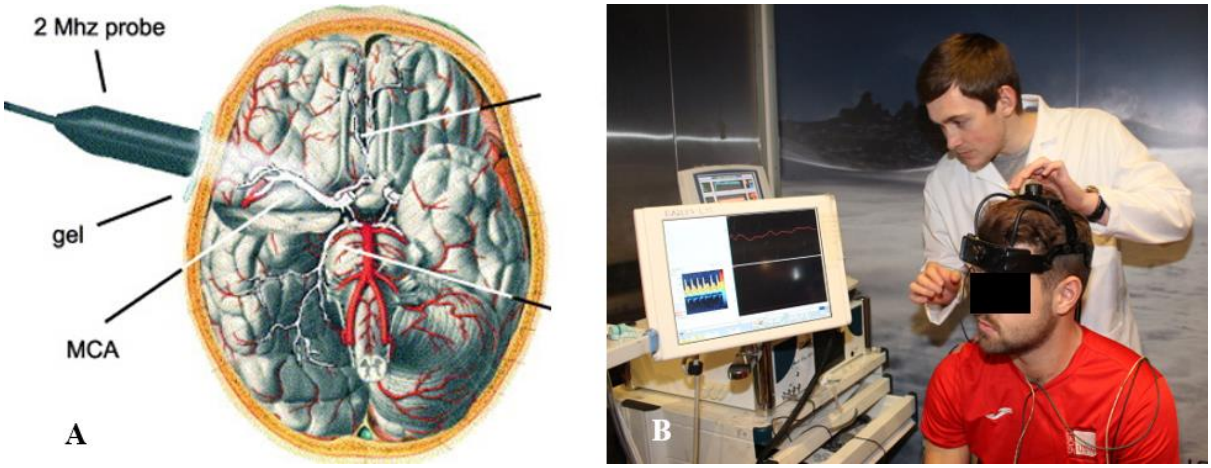

Figure 24: Location of transtemporal window and probe placement (**A**). Insonation of the middle cerebral artery (**B**); MCA, middle cerebral artery. Values presented as raw sample of one participant.

3.8.1.3. Validity

There are various limitations reported throughout the literature for using TCD as an assessment of CBF. However, there is supporting evidence its application. One of the most reported limitations of TCD is the inability to measure the diameter of the insonated vessel. Rather, TCD is solely able to identify flow velocities in sections of the larger intracranial vessels and fails to account for vessel diameter (Sloan et al., 2004). While this may be true, the continuous measurement of MCAv using TCD is valid since changes to flow velocity are relative. It has been speculated that no definitive relationship between MCAv and absolute CBF exists. Several studies have attempted to investigate this relationship and reported inconsistent results. Nuttall et al. (1996) demonstrated poor correlation between the Kety-Schmidt technique of CBF measurement and TCD monitoring of the MCAv. Bishop et al. (1986) concluded that a moderate correlation existed between resting MCAv and absolute CBF using xenon infusion and clearance, however reported a strong correlation between blood flow responses during hypercapnia when expressed as a reactivity index. When comparing MRI, magnetic resonance angiography and TCD, Razumovsky et al. (1999) supported that TCD was a good indicator of CBF.

At the forefront of speculation regarding the validity of TCD is the vasoreactivity of the MCA. Historically it was believed that the MCA was not influenced by alterations in arterial BP or $PaCO_2$. Rather, it was speculated that the arterioles deep within the cerebrovasculature were susceptible to changing $PaCO_2$ rather than the larger intracranial vessels (Serrador et al., 2000a). Serrador et al. (2000a) reported that MCA diameter remained unchanged in response to a reduction of 40 mmHg in arterial BP using lower body negative pressure. It was also reported that MCA diameter remained unaltered in response to hypercapnia or hypocapnia challenges (Serrador et al., 2000a). Similar observations were made by Giller et al. (1993a), Valdueza et al. (1997) and Ter Minassian et al. (1998) who noted that the MCA was not susceptible to vasoactive stimuli despite changes to $PaCO_2$ ranging from 20-60 mmHg. More recently, Coverdale et al. (2014) and Verbree et al. (2014) challenged the vasoactivity of the MCA. They concluded that the MCA was susceptible to mild vasoactive stimuli and contradicted earlier findings. Indeed, alterations of +9 mmHg and -15 mmHg in PET_{CO2} were sufficient to detect changes in the cross-sectional area of the MCA in response to hyper/hypocapnia using conventional MRI methods (Coverdale et al., 2014). Therefore, caution must be applied when assessing CBF via the MCA in response to vasoactive stimuli as dilation of the MCA would underestimate blood flow velocity and the converse true for constriction of the MCA.

3.8.1.4. Reliability

While the true extent to which TCD can be used to accurately assess global CBF is speculative, good reliability of TCD has reported. Demolis et al. (1993) first reported good intra-observer repeatability ($r = 0.902$), between-observer repeatability ($r = 0.908$) and long-term repeatability ($r = 0.951$) using TCD. More recently, the reliability of TCD remains apparent (Kaczynski et al., 2018). Diurnal blood pressure changes have been observed which may lower MCAv throughout the day, thus TCD should be performed at the same time of day where possible (Demolis et al., 1993). Furthermore, repeated trials may cause slight alterations in MCAv due to differences in placement of the transducer from one laboratory visit to the next (Serrador et al., 2000b).

TCD is a valid measure of CBF with good reliability provided several considerations are made. Firstly, PET_{CO2} should be monitored and maintained at predefined concentrations at rest, as well as in response to hyper/hypocapnia to prevent and control for differences in MCA dilation or constriction. Due to the continuous measurement capabilities of TCD, MCAv can be reported as absolute values or relative changes. Time of assessment and placement of the probe

is also important in order to maintain high reliability. While it is impossible to rule out that the anatomical placement of the transducer was changed between visits in the present thesis, investigators performed measurements at the same time of day with participants, noted the individual position of the transducer over the transtemporal window, and obtained a photograph for maximum repeatability.

3.8.2. Near Infrared Spectroscopy (NIRS)

In 1977, brain tissue oxygenation in animals was first quantified non-invasively by NIRS and later utilised in humans from 1985 (Jobsis, 1977, Ferrari et al., 1985). With developments and technological advances, NIRS has been used to quantify muscle oxidative metabolism and the activation of the cerebral cortex in humans (Boushel and Piantadosi, 2000, Hoshi, 2003). NIRS has been a popular and widely used tool for quantifying brain tissue oxygenation since measurements are easily performed, non-invasive and repeatable (Ferrari et al., 2004). To investigate cortical oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (THb) concentration, NIRS was utilised in Studies 1 and 2 using commercially available equipment (Oxymon Mk III Artinis Medical Systems, BV, The Netherlands).

3.8.2.1. Principles

NIRS utilises near infrared light capable of penetrating skin, subcutaneous fat, bone and muscle which is absorbed or scattered by tissue. This is due to several light attenuating factors including: 1) varying concentration of chromophores dependant on oxygen absorption including Hb, myoglobin and cytochrome oxidase; 2) the absorption of light from fixed concentration chromophores including skin melanine; and 3) light scattering following penetration of tissue (Delpy and Cope, 1997). Subsequently, changes in tissue oxygenation may be calculated since each tissue has a distinctive absorbance spectrum, characterised by the differences in emitted and detected light (Wahr et al., 1996). The equipment used to observe changes in O_2Hb and HHb utilise two optodes that are placed directly onto the surface of the skin. One optode serves as a source by emitting continuous wave (CW) light which is directed and distributed through the different tissue layers. The light is scattered and subsequently absorbed via small vessels (<1mm diameter) including the capillaries, arterioles and venules, while the second optode (detection optode) measures the returning CW light intensity. Maximal brain sensitivity is observed at a depth of 1.5 cm below the surface of the skin, therefore source-detection optode separations of 3-5 cm ensure that CW light extend above and below the region of optimal sensitivity, resembling the shape of a banana (Figure 25).

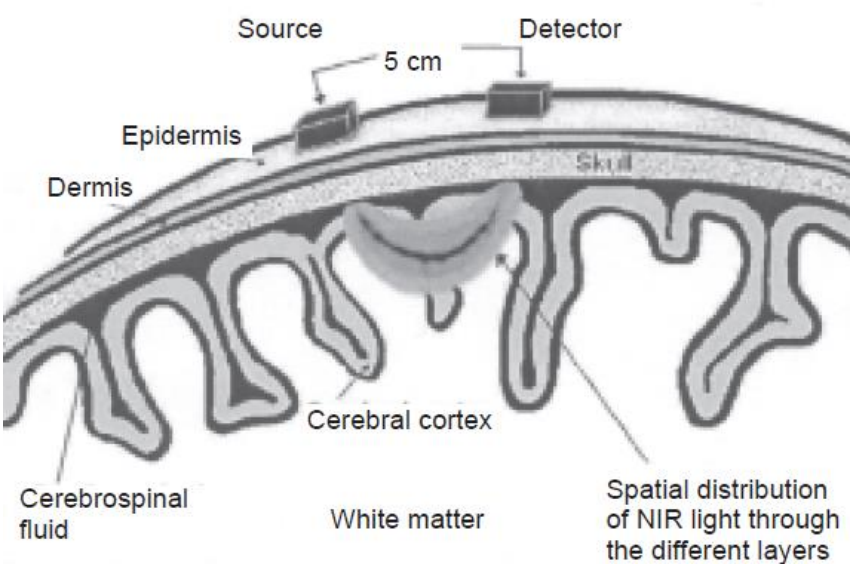

Figure 25: Continuous wave near infrared light travelling through the head for calculation of cortical oxyhaemoglobin, deoxyhaemoglobin, and total haemoglobin. Adapted from (Ferrari et al., 2004).

O_2Hb and HHb are calculated as a result of the CW detection light received with addition to a modified Lambert-Beer law (Delpy and Cope, 1997) that incorporates a differential path length factor (DPF) calculated as:

$$\text{DPF} \times (\text{source} - \text{decector separation})$$

It is important to consider that although NIRS is not effective for quantifying the precise concentration of cortical O_2Hb, HHb and THb, it is correlated strongly with other cerebral haemodynamic measurements including TCD and cerebrovascular reactivity challenges designed to alter PET_{CO2} (Smielewski et al., 1995). Furthermore, the reproducibility of NIRS has been confirmed when undertaking orthostatic challenges to observe cerebral oxygenation (Mehagnoul-Schipper et al., 2001).

3.8.2.2. Measurement technique

Participants were seated in an upright and relaxed position. An alcohol swab was used to sterilise the participant's forehead and dried using a paper towel. Double sided adhesive tape (Double sided stick disks, 3M, Bracknell, UK) was applied to the side of the optodes, ensuring that tape remained clear of the optode body. Participants were seated in an upright and relaxed position. The device was then placed onto the participants forehead with the source optode placed in the centre of the forehead and directly in line with the nasal bridge. The detection optode was placed distally to the centre of the forehead, thus placed over the cerebral cortex of

the frontal brain lobe (Figure 26A). Once secured, a TCD headset was placed over the optodes to minimise noise artefact and loss of CW light (Figure 26B). All data were captured onto a laptop at 50 Hz in response to all cerebral haemodynamic challenges and saved for retrospective analysis.

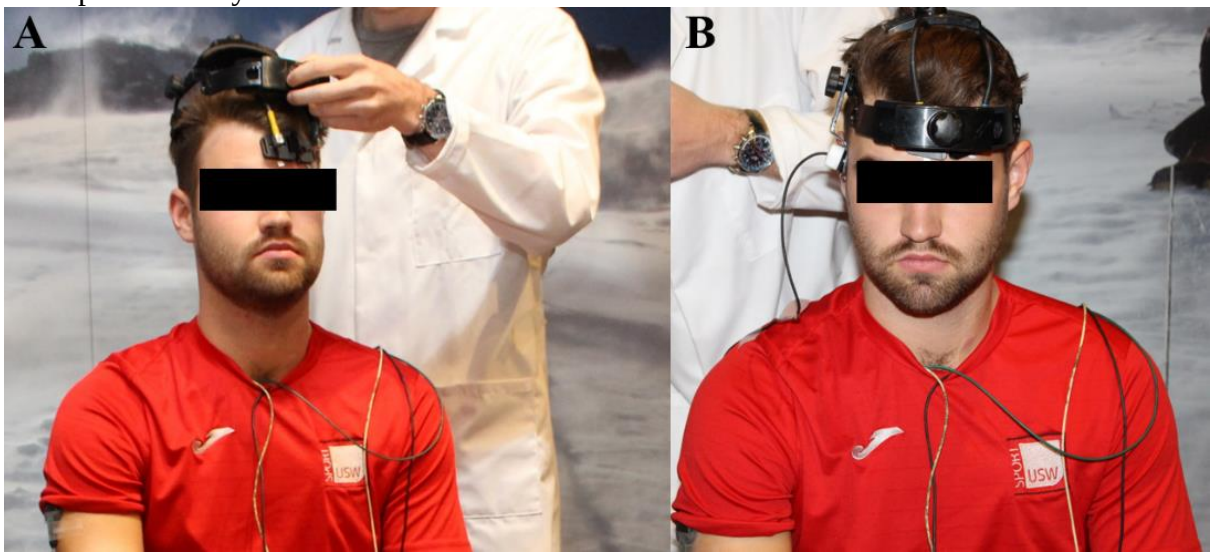

Figure 26: Placement of NIRS optodes (**A**) and headset (**B**).

3.8.2.3. Validity

While there are limitations to NIRS (Murkin and Arango, 2009), it is generally accepted as a validated measurement for assessment of tissue oxygenation. NIRS has been shown to correlate with comparable cerebral haemodynamic measurements, including TCD (Smielewski et al., 1995, Kirkpatrick et al., 1998), electroencephalography (Rigamonti et al., 2005) and positron emission tomography (Rostrup et al., 2002) at rest and in response to alterations in PET_{CO2}. However others have questioned the validity and reliability of NIRS (Kahn and Anyanwu, 2018), thus highlighting the importance of measurement techniques. Cortical O_2Hb, HHb and THb can be recorded continuously and is presented using relative changes. This rectifies the limitation that NIRS is incapable of quantifying exact cortical oxygenation at baseline and in response to vasodilatory challenges. Given that NIRS optodes are placed on the surface of the skin, Miyazawa et al. (2013) observed that thermoregulatory changes may influence captured data, particularly in response to exercise. Precautionary methods may be implemented to limit the influence of thermoregulatory changes by ensuring room temperature is kept constant for all participants, however a degree of caution should be applied when interpreting exercise data.

3.8.2.4. Reliability

The reliability of NIRS has been long established. Mehagnoul-Schipper et al. (2001) confirmed day-to-day reproducibility of NIRS in elderly cohorts during orthostatic challenges.

Furthermore, in clinical settings, Samra et al. (2000) and Hirofumi et al. (2003) confirmed high sensitivity (80%) and specificity (82%) using NIRS when observing frontal cortex oxygenation of patients undergoing surgery, which was able to detect cut off points for intraoperative brain ischemia. NIRS was used in Studies 1 and 2 and presented as relative changes in response to hyper/hypocapnia. In order to limit the thermoregulatory differences, room temperature was maintained (21°C) for all participants of the studies.

3.8.3. Finger photoplethysmography

Throughout all cerebral haemodynamic challenges, finger photoplethysmography (Finapress and Portapress, TPD Biomedical Instrumentation, The Netherlands, Figure 27A) was used to observe beat-by-beat arterial blood pressure. Finger photoplethysmography was developed by Wesseling (1990) and underpinned by methods utilised for the volume-clamp by Penaz (1973). Finger photoplethysmography is widely applied in both research and clinical settings due to the simple operation and non-invasive nature of the assessment (Allen, 2007).

3.8.3.1. Principles

The Finapress and Portapress utilises an enclosed servo-controlled pressure system, to which an adjustable finger cuff can be attached and pressure regulated via an infrared photoplethysmograph. Arterial BP is indirectly measured by simultaneous changes to cuff pressure that are applied with fluctuating arterial pressure (Bogert and van Lieshout, 2005). Throughout a measurement, the finger cuff applies automatic pressure adjustments in response to changes in artery diameter during the cardiac cycle (Bogert and van Lieshout, 2005), thus ensuring that the artery diameter remains constant and complies to the volume clamp method (Penaz, 1973). Subsequently, the arterial wall can be described as unloaded since the pressure of the arterial wall is zero, given that arterial pressure is equal to the pressure of the external surface (Boehmer, 1987). The unloaded phase is maintained by continuous pressure adjustments to the finger cuff, which is achieved via the pressure servo evaluating the small variations in vessel pressure and the unloaded set-point. Therefore, any fluctuations away from the unloaded set-point are counterbalanced by adjustments to finger cuff pressure, ensuring equilibrium of the vessel diameter (Imholz et al., 1998). In turn, any changes to intra-arterial pressure cause cuff pressure to simultaneously change and provide an indirect measurement of BP (Bogert and van Lieshout, 2005). Continuous waveforms are presented live onto a monitor with supplementary display of HR, systolic BP and diastolic BP with an effective range of 20-260 mmHg.

3.8.3.2. Measurement technique

Participant anthropometrics (age, gender, stature, mass) were uploaded into the Finapres and height calibration was performed by attaching height sensors together and resetting the distance function. The height sensors are connected via tubing where fluid pressure from inside the tube is subtracted from finger pressure (Bogert and van Lieshout, 2005). Thereafter, the pressure-servo box was placed loosely onto the back of the wrist, ensuring that the strap did not occlude flow to the hand. The finger cuff was placed around the middle phalynx of the right middle finger and secured via velcro strapping integrated onto the external side of the finger cuff (Figure 27B). To account for the pressure gradient of blood flow from the brachial artery to the finger, a height correction sensor was applied to the finger cuff and onto the proximal side of the arm, in line with the 4^{th} intercostal and the heart.

Despite the ability of the system to maintain the accuracy of intra-arterial pressure during pressure fluctuations incurred via postural changes, participants were instructed to sit in an upright position on a chair with the arm freely hung to the side, thus limiting noise artefact and fluctuations to blood pressure during measurement. Participants held a heated bag to improve measure reproducibility. Data were recorded live onto Labchart (section 3.8.7) with systolic and diastolic pressures reported, including automatic measurement of mean arterial pressure (MAP) calculated as: MAP (mmHg) = 1/3 systolic BP + 2/3 Diastolic BP

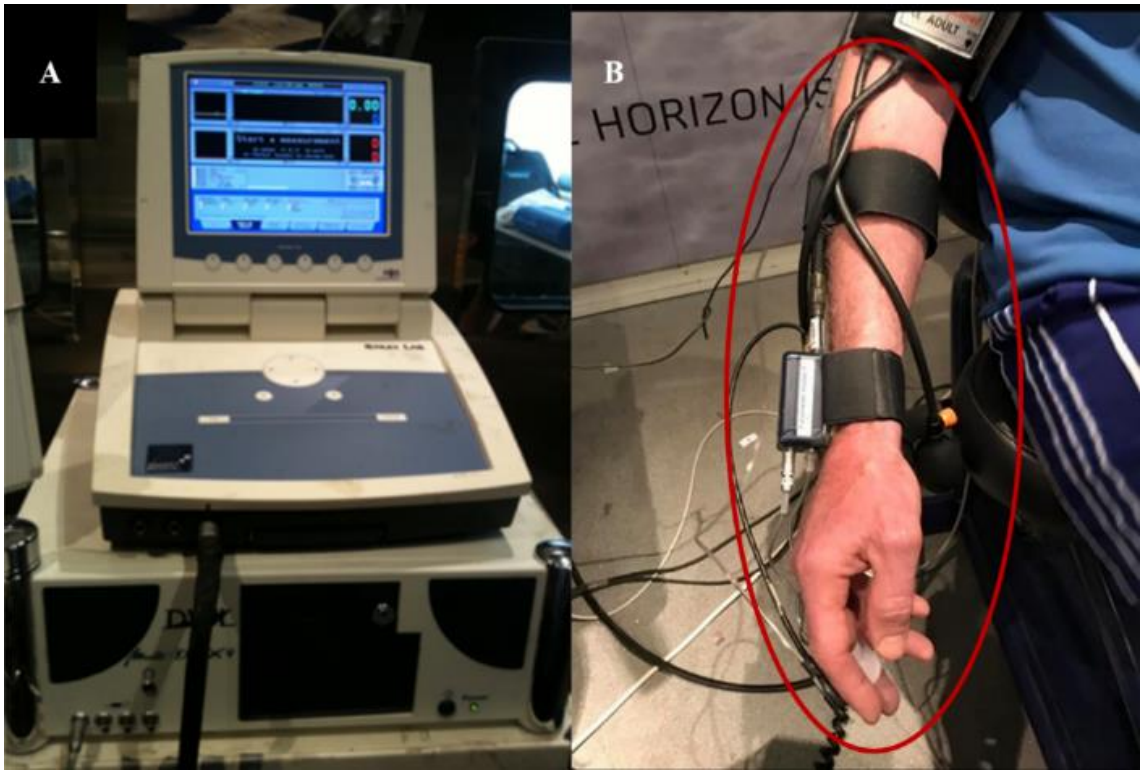

Figure 27: Finapress used to determine beat-by-beat arterial blood pressure (**A**), placement of finger cuff and pressure servo box on a participant (**B**).

3.8.3.3.Validity

Finger photoplethysmography is considered a validated measurement for obtaining arterial BP and has been used for both clinical practice and investigative research with good repeatability (Imholz et al., 1998). Nonetheless, limitations of finger photoplethysmography have been observed (Gomides et al., 2010). Parati et al. (1989) demonstrated the validity of finger photoplethysmography by comparing arterial BP measurements obtained via the Finapress with intra-arterial pressure obtained via radial artery catheterisation. Comparisons were performed under resting conditions and in response to several interventions designed to cause fluctuations in BP including hand-grip test, cold pressor test, diving test, Valsalva manoeuvre, intravenous injections of vasoactive stimulators, lower body negative pressure and passive leg raising (Parati et al., 1989). During a 30-minute resting period, the average pressure differences obtained from both measurement techniques was trivial revealing that systolic and diastolic pressures were within 7 mmHg and 5 mmHg respectively. Similarly, beat-by-beat pressure was comparable throughout all challenges using both Finapress and arterial catheterisation which allowed Parati et al. (1989) to conclude that finger photoplethysmography was an accurate and non-invasive measurement of continuous arterial BP monitoring. Further evidence supporting finger photoplethysmography was provided by Imholz et al. (1998) who conducted a review

of 43 studies which utilised both young and aged populations. It was concluded that finger photoplethysmography was indeed a sufficient method to obtain continuous measurements of arterial BP (Imholz et al., 1998).

Finger photoplethysmography cannot obtain arterial BP accurately during exercise. Nijboer et al. (1988) first reported this by comparing an upper arm sphygmomanometer method and finger photoplethysmography to identify that blood pressure could not be monitored accurately after surpassing 40% of $\dot{V}O_{2Max}$. Similar observations were noted by Gomides et al. (2010) who compared intra-arterial and finger photoplethysmography measurements during resistance training to reveal that BP was overestimated using the latter technique.

3.8.3.4. Reliability

The day-to-day and long term reliability of finger photoplethysmography has been established and discussed by Imholz et al. (1998) and further supported by Allen (2007). However, finger photoplethysmography has limitations during exercise. As a result, manual arm sphygmomanometers were used to assess arterial BP during the incremental exercise test in Study 2, while finger photoplethysmography was used for all other measurements obtained in Study 1 and Study 2.

3.8.4. CVR_{CO2}

The cerebral vasculature is sensitive to alterations in the $PaCO_2$ for the maintenance of CBF regulation (Ainslie and Duffin, 2009). Hypercapnia refers to a condition where $PaCO_2$ is elevated causing vasodilation of the cerebral arterioles, which subsequently increases CBF. Hypocapnia causes vasoconstriction in the cerebral arterioles and reduces CBF as a result of lowered $PaCO_2$ (Kety and Schmidt, 1948, Wasserman and Patterson, 1961). Therefore, the CBF responses to changing concentrations of CO_2 are attributed to stabilised breathing and are referred to as CVR_{CO2}. At present, CVR_{CO2} is used as an index to assess the vasodilatory potential of the cerebrovascular bed to changes in $PaCO_2$ (Ainslie and Duffin, 2009).

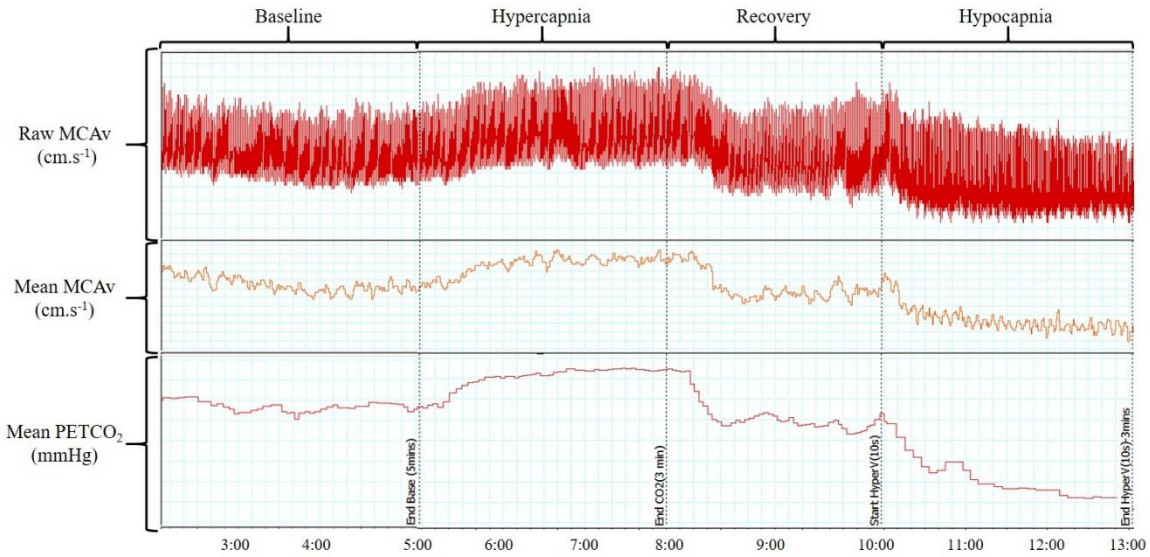

Figure 28: Middle cerebral artery velocity (MCAv) and end tidal carbon dioxide (PET_{CO2}) at baseline and in response to hypercapnia and hypocapnia. Values presented as raw sample of one participant.

As outlined in the literature review, decreased CBF after concussion may be alleviated days after injury, however prolonged cerebrovascular impairments may also be apparent. Participants underwent CVR_{CO2} assessments during TCD of the MCA. Participants were seated at rest in an upright position and a mouthpiece fitted. CVR_{CO2} in response to hypercapnia ($CVR_{CO2HYPER}$) was measured by attaching a Douglas bag (Cranlea and Co, Bourneville, UK) fitted to a 2-way valve and falconia tubing (Cranlea and Co, Bourneville, UK) onto the mouthpiece. Following five minutes of breathing ambient room air, the 2-way valve was opened, allowing participants to breathe a 5% CO_2 – 21% O_2 gas mixture (Medical specification; British Oxygen Company, UK) for 3-minutes. A 2-minute recovery was undertaken after hypercapnia to ensure PET_{CO2} had returned to baseline. CVR_{CO2} in response to hypocapnia ($CVRCO_{2HYPO}$) was determined via instructed hyperventilation for 3-minutes. Participants inhaled/exhaled at 15 breaths per minute using verbal cues. For both assessments, PET_{CO2} was monitored and maintained from baseline by controlling the depth of participant breathing.

Data were collected live using Labchart and retrospectively analysed for CVR_{CO2} in response to hypercapnia and hypocapnia (Figure 28). The final 30 seconds of each challenge was used for analysis when steady state had been achieved. $CVR_{CO2HYPER}$ and $CVR_{CO2HYPO}$ was calculated as:

$$CVR_{CO2}\,(\%.mmHg^{-1}) =$$

$$\frac{100\ \times \text{MCAv (final)} - \text{MCAv (baseline)}}{\text{MCAv (baseline)}}\ /\ \text{PETCO2 (final)} - \text{PETCO2 (baseline)}$$

To determine the combined ability of the cerebrovasculature to respond to changes in PET_{CO2}, $CVR_{CO2RANGE}$ was calculated as the sum of vasodilation and vasoconstriction observed during hypercapnia and hypocapnia (Bailey et al., 2013b):

$$CVRCO_{2RANGE}\ (\%.mmHg) = CVR_{CO2HYPER} + CVR_{CO2HYPO}$$

3.8.5. 3-lead ECG

During all visits, a 3-lead ECG (Powerlab, ADInstruments, Colorado Springs, CO, USA) was used to record HR throughout cerebral haemodynamic measurements. Skin was prepared with a skin abrasive pad and sterilised using an alcohol swab. Electrodes were placed in accordance with standard procedures and lead misplacement ruled out using Einthoven's triangle (Einthoven, 1901). R-R intervals were used to determine HR (Malik et al., 2002).

3.8.6. Capnography

Expired gases were monitored throughout all cerebral haemodynamic challenges using capnography (ML 206, ADInstruments, Oxford, UK). A two-point calibration was conducted using 100% nitrogen and 17% O_2 – 5% CO_2 gases (British Oxygen Company, Guilford, UK) prior to each experimental visit. Expired gases were sampled through a line attached to a mouthpiece and 1000 $L.min^{-1}$ flow head (ADInstruments, Oxford, UK) at 100 $mL.min^{-1}$. The fraction of expired O_2 and CO_2 was logged onto Labchart software. PET_{CO2} was derived from the conversion of expired CO_2 via Daltons law (Dalton, 1802) and presented on Labchart. PET_{CO2} was calculated as:

$$PETCO_2 = (\frac{FETCO2(\%)}{100}) \times (Barometric\ pressure - 47)$$

FET_{CO2} is the fraction of CO_2 measured via capnography, with the partial pressure of water vapour measured at 47 mmHg at body temperature. Barometric pressure was recorded for each experimental visit using a mercury Fortin barometer (Cranlea, Bourneville, UK).

3.8.7. Data logging

All haemodynamic data were recorded continuously using an analogue-to-digital converter (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO, USA) and presented on a

laptop (Figure 29). To account for the delay (-1.07s) in signal output of MAP with TCD, data were 'time aligned' automatically using Labchart software and stored for analysis. Further parameters were calculated and outlined below (Bailey et al., 2013b, Bailey et al., 2019):
Mean MCAv (cm/s) = 1/3 Systolic MCAv (cm/s) + 2/3 Diastolic MCAv (cm/s).

Cerebrovascular resistance (CVRi) = MAP/MCAv.

Cerebrovascular conductance (CVCi) = MCAv/MAP.

Pulsatility index (PI) = (Systolic MCAv – Diastolic MCAv)/Mean MCAv.

Cerebral oxygen delivery (CDO_2) = MCAv x (1.39 x Hb x arterial oxygen saturation [SaO_2]/100) [excluding O_2 dissolved in plasma, negligible (0.03 mmHg at a PaO_2 of 100 mmHg)].

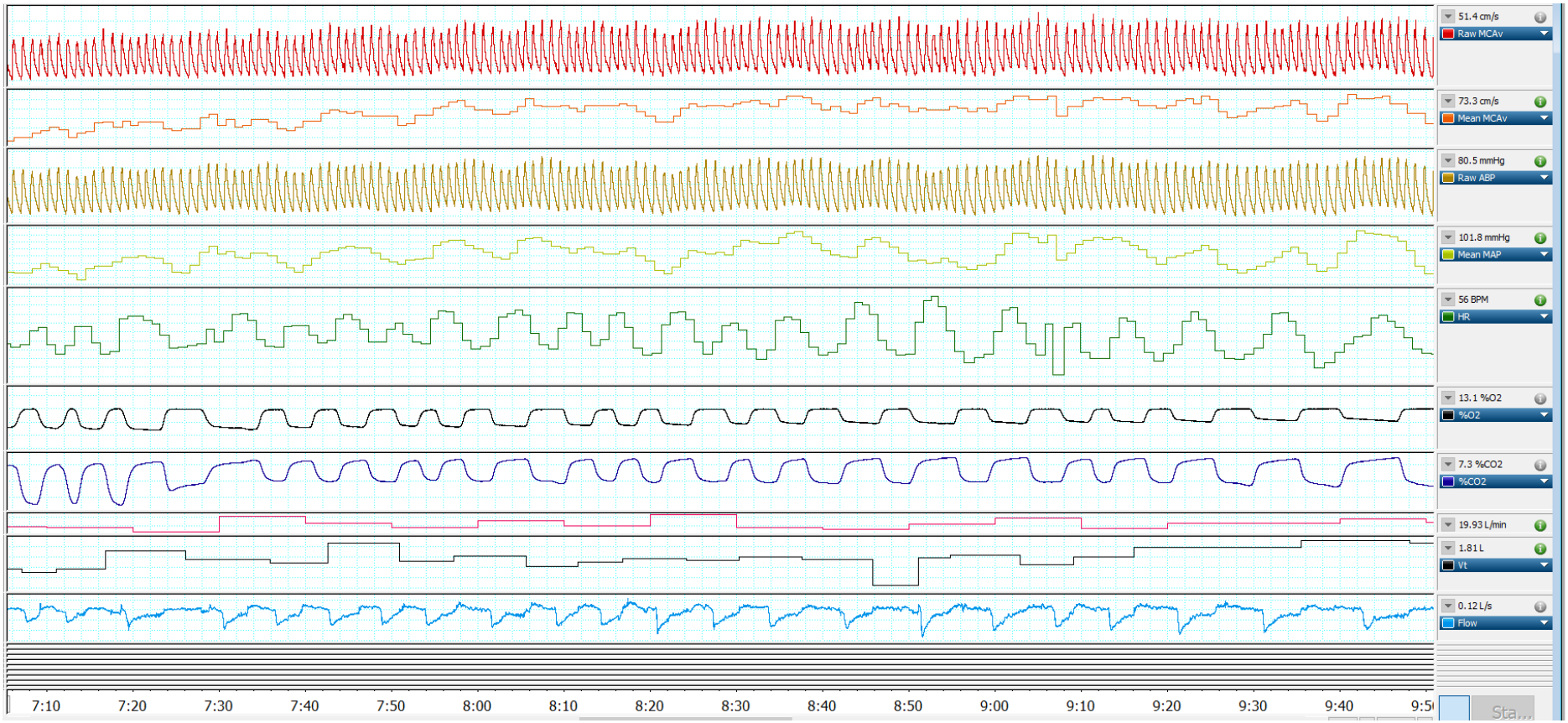

Figure 29: Cerebral haemodynamic and cardiovascular data recording on Labchart version 8. MCAv, middle cerebral artery velocity; ABP, arterial blood pressure; MAP, mean arterial blood pressure; O_2, oxygen, CO_2, carbon dioxide; Vt, ventilation. Values presented as raw sample of one participant.

3.9. Cognition

3.9.1. Cognitive testing battery

Cognition was scrutinised via cognitive assessments which considered the criteria for the symptomatic phase prior to the onset of neurodegeneration as recommended by the National Institute for Ageing and the Alzheimer's Association (Albert et al., 2011). Each of the tests were completed in a separate quiet room to enable participants to concentrate and prevent distraction from other environmental factors.

3.9.2. Memory

Rey Auditory Verbal Learning Test

Developed by Rey (1941) and translated into English for administration in 1983 (Lezak, 1983), the Rey Auditory Verbal Learning Test A (RAVLT-A) is used to assess immediate memory and learning while the Rey Auditory Verbal Learning Test B (RAVLT-B) determines susceptibility to interference. Administration of the RAVLT-A is completed by reading a set of 15 nouns aloud to the participant (List A), who must then recall as many of the words as possible. The list is read to the participant five times who must consistently repeat as many of the nouns as possible during each trial. Following the fifth trial, an interference set of 15 nouns (List B) are read aloud to the participant, who must repeat as many nouns as possible. Immediately after recalling List B, the participant was required to repeat as many nouns as possible from List A with no cue. The RAVLT-A1:A5 was scored as the sum of nouns recalled from trials A1 to A5 (A1+A2+A3+A4+A5), while the RAVLT-B was scored as the sum of words correctly identified from list B1. The RAVLT-A6 was scored as the sum of words correctly identified from list A6 and the RAVLT-A6-A5 calculated by subtracting the A5 trial score from the A6 trial score. For both RAVLT-A and RAVLT-B a higher score was indicative of better cognitive performance.

Digit Span Test (DST): Repetition of Digits Forwards (RDF) and Repetition of Digits Backwards (RDB)

The DST was used to assess short-term and working memory. It was incorporated within the Wechsler intelligence and memory assessment battery (Wechsler, 1981, Lezak, 2004). During the RDF, a string of numbers was read aloud to the participant, who was required to repeat the list back in the correct order. During the RDB, participants were required to repeat a new set of numbers in backward order. During the testing protocol, the list of numbers was continually

increased by one number every two trials until an error was made or the test was completed. Scoring of the RDF and RDB was calculated by adding the number of successfully completed trails. A total DST score was calculated as:

Repetition of Digits Total (RDT) = RDF + RDB

Higher scores achieved during the DST, RDB and RDB was indicative of greater performance.

3.9.3. Concentration

Trail Making Tests

The TMT-A and TMT-B assess executive function, attention and working memory (Lezak, 2004). The TMT-A is comprised of 25 randomly distributed numbers that are circled and displayed on the sheet. The participant was required to draw a line which joined each of the numbers in ascending order until complete. Similarly, the TMT-B required the participant to draw a line in numerical-alpha order until the trial was complete. Participants were instructed to begin on the indicated start point, not to withdraw their pen from the sheet, and to complete the tests as quickly as possible. Practice samples for both TMT-A and TMT-B were completed prior to formal assessment. The completion time for each formal assessment was noted upon completion with quicker times in both components indicative of greater performance.

Digit Symbol Substitution Test

The DSST was conducted to assess working memory, attention and visuo-motor coordination and is a recognised as a validated assessment by the Wechsler Battery (Lezak, 2004, Wechsler, 1945). A sheet was provided to the participant which contained a coding key. Within the key, the numbers 1-10 had a corresponding symbol below them. A table of randomly generated numbers was then provided with blank boxes below each number. Participants were required to fill as many boxes as possible with the correct symbol within 90 seconds. The participant was required to fill 7 blank boxes as a familiarisation task before attempting the test. Higher scores (i.e. more boxes correctly filled) achieved within the 90 second time limit were indicative of greater performance (Wechsler, 1981).

3.9.4. Executive function

Grooved Pegboard Dexterity Test: Dominant (GPD) and Non-dominant (GPND) Hands

The Grooved Pegboard test was used to assess executive function and fine-motor coordination (Lezak et al., 2012). Participants filled a board of 25 identical slots that were positioned differently with metal pegs. Pegs were placed in a tray at the bottom of the board before the test. Each peg had a spine which had to be positioned correctly to fit into each slot. The participant manoeuvred the pegs into each of the 25 slots, beginning at the top and working from left to right as quickly as possible. This test was administered using the dominant and non-dominant hands on separate trials. The time to completion during both trials was noted with quicker times indicative of greater performance.

3.9.5. Mild cognitive impairment

Montreal Cognitive Assessment (MoCA)

The MoCA is used to screen for MCI, but is also used as a common dementia screening tool (Nasreddine et al., 2005). The MoCA is considered a more sensitive measure of MCI in comparison to other screening tools such as the Mini Mental State Examination, due to its ability to detect more subtle alterations in cognition. In order to ensure validity, the test was administered with the MoCA script. Various cognitive domains were assessed throughout the MoCA including; executive function, visuo-constructional skills, memory, attention, concentration, language, conceptual thinking and orientation.

Visuospatial coordination and executive function was assessed via three tests, which included alterative trail making, similar to the TMT-B previously mentioned. Participants were asked to draw a three-dimensional cube and draw a clock with a specified time. Memory was assessed via two assessments. Firstly, participants were asked to name three animals pictured on the assessment. Secondly, participants were required to repeat five words immediately and after five minutes of hearing the list. Attention was assessed via three challenges. Participants were asked to repeat a string of numbers similar to the DST in a forwards and backwards order, followed by a challenge where participants tapped their hand on the table each time the letter ‘A’ was read from a list of randomly selected letters. Participants were then asked to complete a subtraction challenge by continually subtracting 7 from a specified value (e.g. 100) five times. Language and fluency was assessed via pronunciation of two separate sentences and a challenge where participants named as many words beginning with a specific letter from the

alphabet (e.g. ‘F’) in one minute. Abstraction was assessed via a familiarity task. Orientation was assessed by requesting the participant to recall the date, month, year, day, place and the nearest city to where the assessment was conducted. The scores from each section was totalled with a score of ≥ 26 deemed as normal.

3.9.6. Sports concussion assessment

SCAT5

Following the consensus summary and agreement statement of the first international conference of concussion in sport, Vienna 2001 (Aubry et al., 2002), the SCAT was introduced as a combined grading measurement of concussion and recovery within sport. The SCAT was modified in 2008 (McCrory et al., 2009), 2012 (McCrory et al., 2013) and 2017 to the current iteration referred to as SCAT5 (McCrory et al., 2017). The SCAT5 is a standardised tool for the evaluation of concussive symptoms. The SCAT5 consists of an on-field and off-field assessment for evaluation recovery of concussion. The on-field assessment consists of a five-step evaluation immediately after suspected concussion, while a six step off-field assessment can be used to note athlete characteristics at baseline or throughout the recovery period after concussion.

Participants of both studies completed a baseline SCAT5 in order to evaluate the presence of concussion symptomology and to assess neurologic function (McCrory et al., 2017). The SCAT5 off-field assessment was completed at baseline during pre-season and post-season in Study 1 and at baseline for Study 2. Participants were assessed individually by a trained physician in a separate quiet room using the instructions provided.

Participants first completed the athlete background questions by responding verbally to the investigator. The SCAT5 symptom evaluation checklist was then handed to the participant to read aloud and complete. Once completed, the participant calculated the number of symptoms and their symptom severity score before handing the SCAT5 back to the investigator.

A cognitive screening was completed using the Standardised Assessment of Concussion (McCrea, 2001), which included questions relating to orientation where the participant responded verbally. Upon completion of the orientation questions, a list of ten words was read out loud to the participant. The investigator chose one of the three standardised lists (G-I) and participants were instructed to repeat back as many words as possible in any order. The investigator repeated the list twice providing identical instructions before each trial.

Participants then completed a digits backwards assessment. The investigator chose a list (A-F) and read a list of numbers to the participant. Each time the participant correctly repeated the list of numbers in reverse order, the list would increase in length until the participant failed to answer correctly. To conclude the cognitive screening, the participant was asked to name the months of the year in reverse order. At each of the stages, the investigator recorded the score.

A neurological screening was conducted by the investigator. Firstly, the investigator noted whether the participant was able to read aloud. Secondly, the investigator assessed cervical spine movement. Thirdly, the participant was asked to look side-to-side and up-and-down and asked whether they experienced double vision. Coordination was assessed using the finger-to-nose test. Tandem gait was assessed on a three-meter tape of 38 mm diameter in both directions. The Modified Balance Error Scoring System was used assessed balance in double-leg, single-leg and tandem stances (Guskiewicz, 2003).

Delayed recall was assessed at least ten minutes after the participant first heard the list of words from the cognitive screening. Participants repeated as many of the words as possible with the total number of words correctly repeated noted by the investigator and scored.

The investigator totalled the scores from each domain to complete the summary checklist. As all participants attended the laboratory without concussion, no formal concussion diagnosis was attempted.

3.10. Notational analysis (Study 1 - Professional [young] players only)

Notational analysis is the study of movement patterns, strategy and tactics in sports whereby consistent and reliable measurements of critical performance events are quantified (Bartlett, 2001). Notational analysis has been applied across all forms of professional sport to facilitate future sporting performance by providing visual, audible and statistical feedback (Hughes and Franks, 2004). More recently, notational analysis can facilitate the observation of injury during competition with several epidemiological studies reporting injury incidence sustained throughout play (James, 2006, Prien et al., 2018).

Throughout the 2017-2018 season, high speed cameras were used to record all games played by the team. Data were saved on a laptop for retrospective analysis via the team analyst and logged onto a specified coding window using commercially available software (Hudl Sports Code, London, UK). All key match events were recorded and logged onto the coding window for each individual player. All injuries were reported and a video file saved for assistance with

medical follow-up (if required). In addition to the 'in-game' SCAT5 and Head Injury Assessment (HIA) protocol, suspected concussive events identified during notational analysis were coded accordingly, allowing for retrospective screening and diagnosis via the team clinician. Data were processed and transferred to the research team for interpretation following each game of the season. Data were collated and analysed to observe head injuries, including concussion incidence and match events. Concussion incidence was calculated as number of concussions per 1000 match hours (Rafferty et al., 2018):

$$\text{Injury incidence (per 1000 match hours)} = \left(\frac{\text{Number of injuries}}{\text{Match exposure (h)}}\right) * 1000$$

Due to the varying positional demands of rugby union players, incidence rates were investigated between the forwards and backs to identify differences between members of the team (Quarrie et al., 2013). Forwards were identified as props, hookers, locks (second row), flankers and number eights, with backs consisting of half-backs (fly-half and scrum-half), centres, wingers and full backs.

3.11. Statistical analysis

All statistical analysis was performed using commercially available software (SPSS 25.0, International Business Machines, Chigaco, USA) with the exception of prospective power calculations (section 3.3.1). For all analysis, data distribution was confirmed using repeated Shapiro Wilks *W* tests. Following confirmation of data normality, various statistical analyses were performed in accordance to the suitability of the analysis required. Statistical analysis performed for Study 1 and Study 2 are outlined in sections 4.2.7 and 5.2.6. Data is presented as mean ± SD with significance set at $P < 0.05$ for all two-tailed results.

Chapter 4

Research study 1 - Contact events in rugby union and the link to reduced cognition: evidence for impaired redox-regulation of cerebrovascular function.

4.1. Introduction

Following the advent of professionalism in rugby union, elite-level players may be exposed to over 11,000 contact events per-season comprised of tackles, collisions, mauls, rucks and scrums (Fuller et al., 2007a). Tackles are the most common contact-match event in rugby union and cause 52% of all injuries (Cross et al., 2017, England Rugby, 2019).

While the propensity of match-events and injury risk are generally understood, the relationship between player position and the physiological implications of contact remain to be established. Physical contact and head trauma promotes an elevation in systemic OXNOS (Vagnozzi et al., 2007, Giza and Hovda, 2014, Poole et al., 2014, Khatri et al., 2018) defined by a free radical-mediated reduction in vascular nitric oxide bioavailability, that has been associated with cerebral hypoperfusion and impaired cerebrovascular reactivity (CVR, Meier et al., 2015, Bailey et al., 2019). Given that the brain's almost exclusive reliance on a continuous and adequate flux of O_2 and glucose, suppressed cerebral haemodynamic function can further impair cognition (Bailey et al., 2013a, Churchill et al., 2017a). Moreover, Breedlove et al. (2012) and Abbas et al. (2015) have demonstrated that contact events correlate with disruption of functional brain networks, while Stewart et al. (2016) and Lee et al. (2019) have speculated that neurodegeneration is an under-recognised consequence of rugby union. Therefore, it is surprising that molecular profiling and corresponding changes in cerebrovascular function have not previously been performed among elite-level players.

Thus, for the first time, the present study sought to determine the molecular, cerebrovascular and cognitive signatures of professional rugby union players over a single season, stratified by frequency of contact events, playing position and concussion risk. We hypothesised that compared to controls, current rugby union players would present with elevated OXNOS, suppressed cerebrovascular function and a decline in cognition with subtle alterations in these metrics detected over one season.

4.2. Methodology

4.2.1. Ethical approval

The study was approved by the University of South Wales ethics committee (reference number: 0617LSETOE0). Verbal and written consent was obtained from all participants and anonymity ensured via a random coding generator. All procedures were carried out in accordance with the most (7th) recent amendment of the Declaration of Helsinki of the World Medical Association (2013), with the exception that it was not registered in a publicly accessible database prior to recruitment.

4.2.2. Participants

Twenty-one professional rugby union players aged 25 ± 4 years with 3 ± 2 previous concussions incurred over 16 ± 4 years were compared with 17 sex, age-, physical activity- and education-matched controls, with no participation in contact sports or concussion history (Table 1). Each player was contracted to a full rugby union season with their team to attain follow-up data. Forwards were identified as props, hookers, locks (second row), flankers and number eights, while backs consisted of half-backs (fly-half and scrum-half), centres, wingers and full backs.

4.2.3. Study design

Participants were initially contacted via telephone and completed an eligibility questionnaire for inclusion in the study (Section 3.2). Once included, a longitudinal study design was implemented with measurements obtained at three time-points; pre-season, in-season and post-season (Figure 30). Controls attended the Neurovascular Research Laboratory at the University of South Wales and players attended the Vale Training Pavilion before (pre-season) and after (post-season) the 2017-18 rugby union season (measurements outlined below). Ahead of the pre-/post-season assessments, participants refrained from physical activity, caffeine and alcohol, followed a low nitrate/nitrite diet and had completed a 12 hour overnight fast prior to experimentation (Wang et al., 1997, Bailey et al., 2017). Notational analysis was conducted to log all match events sustained throughout the season. Control participants were asked to continue with normal daily living during the ‘in-season’ phase of the research study.

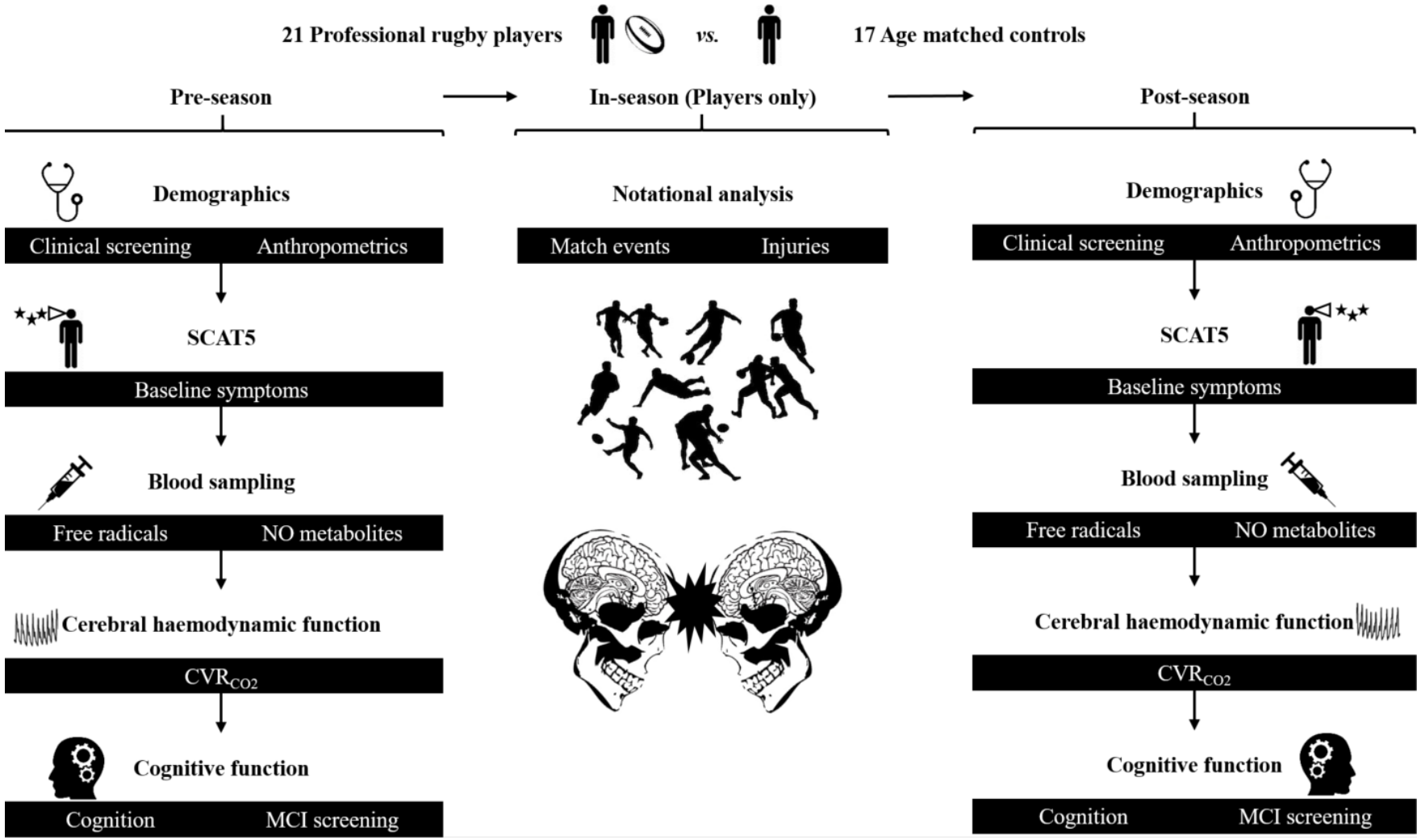

Figure 30: Experimental design; SCAT 5, Sports Concussion Assessment Tool 5th edition; NO, nitric oxide; CVR_{CO2}, cerebrovascular reactivity to changes in end tidal carbon dioxide; MCI, mild cognitive impairment.

4.2.4. Experimental procedures: Pre-season

Anthropometrics

Stature was measured using a stadiometer. Bioelectrical impedance analysis obtained body mass, BMI and body composition (section 3.5).

Concussion history

Players: Concussion history was determined via medical records from the team doctor. Concussion history preceding available medical records was determined via self-recall and cross-checked using the SCAT5 at baseline (section 3.9.6, Davis et al., 2017). Players confirmed an average of 3 ± 2 previous concussions incurred over an average playing career of 16 ± 4 years. Players confirmed 14 ± 12 months since their most recent concussion. The average time between concussions was 17 ± 7 months and return-to-play granted following 17 ± 19 days.

Controls: Concussion history was determined via self-recall and cross-checked using the SCAT5 at baseline.

Blood sampling

A cannula was placed into an antecubital vein of the forearm (section 3.7.1). Blood samples (40 mL) were collected in vacutainers, centrifuged at 600 *g* (4°C) for 10 minutes and the plasma supernatant snap-frozen in liquid nitrogen. Samples were thawed at 37°C for five minutes before batch analysis.

Free radicals

Plasma ascorbate free radical ($A^{\bullet-}$) was measured as a biomarker of global free radical flux (Bailey et al., 2009) and analysed by X-band EPR spectroscopy (section 3.7.4). Spectra were filtered identically using WINEPR (Version 2.11) and the double integral of each doublet calculated using Origin software. Intra- and inter-assay CVs were both <5%.

Total bioactive NO

A 200 μl sample was injected into tri-iodide reagent to determine total bioactive NO (NO_2^- + RSNO), via ozone based chemiluminescence (section 3.7.5, Bailey et al., 2017). All calculations were determined using Origin software. Intra- and inter-assay CVs were both <5%.

Haemoglobin and haematocrit

Whole blood from a 1 mL syringe was decanted into a microcuvette and placed into an analyser to determine haemoglobin concentration (section 3.7.2). A small volume was also decanted into microcapillary tubes, centrifuged for five minutes at 600*g* and placed onto a haematocrit analyser (section 3.7.3). Analysis was performed in duplicate and the average recorded. Intra- and inter assay CVs were both <5%.

Cerebral haemodynamic function

Middle cerebral artery blood flow velocity was determined after 5 minutes from a seated position using TCD ultrasonography (section 3.8.1). CVR_{CO2} was assessed in response to inspiratory hyper/hypocapnia and used to calculate $CVR_{CO2RANGE}$ (section 3.8.4).

Near-infrared spectroscopy (NIRS)

NIRS was used to determine cortical O_2Hb, HHb and tHb throughout cerebral haemodynamic challenges (section 3.8.2). Given that absolute changes in cO_2Hb, HHb and tHb cannot be obtained by NIRS alone due to the unknown pathway length of the photons (Murkin and Arango, 2009), relative changes were calculated and expressed as a percentage change from baseline for analysis.

Cardiovascular function

Beat-by-beat arterial blood pressure was determined by finger photoplethysmography (Imholz et al., 1998). A Finometer was placed on the middle finger which determined MAP and cardiac output (Q) using an automated model flow method throughout (section 3.8.3, Wesseling et al., 1993). Heart rate was determined via a 3-lead ECG.

Data logging/analysis

All cerebral haemodynamic data were recorded continuously at 1 kHz using an analogue-to-digital converter (section 3.8.7). Throughout all cerebral haemodynamic challenges, CVRi, TPR, CVCi, PI and CDO_2 were calculated (section 3.8.7).

Cognition

The MoCA was used as clinical screening tool for MCI (Nasreddine et al., 2005). This was complimented by a battery of validated psychometric tests that considered the criteria for the symptomatic phase prior to the onset of neurodegeneration as recommended by the National Institute for Ageing and the Alzheimer's Association (Albert et al., 2011). The RAVLT-A and

RAVLT-B were used to determine parameters of learning and memory (Lezak, 1983, Rey, 1941). Working memory was assessed via the RBD (Koppitz, 1977) and TMT-B (Tombaugh, 2004). The TMT-A (Tombaugh, 2004), RDF (Koppitz, 1977) and DSST (Salthouse, 1992) were used to measure attention and information processing. Visuospatial coordination and executive function were assessed via the GPD and GPND (Trites, 1977). Higher scores in the RAVLT-A, RAVLT-B, RDB, RDF, DSST and MoCA, or lower scores in the TMT-A, TMT-B, GPD and GPND indicated superior performance (Marley et al., 2017).

4.2.5. Experimental procedures: In-season

Notational analysis

Notational analysis was performed for 31 games played during the season. All match events throughout the season were logged via notational analysis (section 3.10). The frequency of match events was calculated per-game/season. The total number of games and exposure for each player was determined through video analysis.

Concussion incidence and propensity

Concussion/head injury incidence was calculated as the mean number of injuries per 1000 match hours and injury propensity calculated per 1000 match events (Rafferty et al., 2018).

4.2.6. Experimental procedures: Post-season

All experimental procedures were repeated after completion of the rugby season.

4.2.7. Statistical analysis

Datasets were analysed using commercially available software (SPSS V 25.0). Following confirmation of distribution normality (Shapiro Wilks *W* tests), two-way [Group: controls *vs.* players × Timepoint: pre- *vs.* post-season repeated measures analyses of variance (RM-ANOVA) were employed to assess demographics, SCAT5 performance, molecular profile, CVR, changes in cortical oxygenation and cognition to determine within- and between-group differences across the playing season. Changes in cerebrovascular function were performed using a three-way [Group: controls *vs.* players × Timepoint: pre- *vs.* post-season × Condition: normocapnia *vs.* hyper/hypocapnia)] RM-ANOVA. Where interaction effects were identified, *post hoc* analyses were performed using Bonferonni corrected pairwise comparisons (paired (within) or independent (between) sample *t*-tests. Differences in match events between forwards and backs were also determined using independent samples t-tests. Concussion incidence and match event injury propensity between-groups were calculated using rate ratios

(RR) and percentile bootstrapped to confirm 95% confidence intervals (CI's). Injury incidence/propensity were deemed different if the 95% CI's for the RR did not overlap with unity (Rafferty *et al.*, 2019). Data are displayed as mean ± SD with statistical significance for all two-tailed tests established at P <0.050.

4.3. Results

Demographics

Baseline measures: Both groups were matched for age (P = 0.210), physical activity (P = 0.202), and education (P = 0.057, Table 1). By design players reported an average of 16 ± 4 years participation in rugby union and reported 3 ± 2 previous concussions across their lifetime compared to the non-contact sport, non-concussed controls (P < 0.001). Players were taller (P = 0.001) and heavier (P < 0.001) compared to the control group during both pre- and post-season.

Seasonal changes: Players were leaner during post-season, relative to the pre-season (P < 0.001), while controls gained body fat (P < 0.001). Players completed more resistance training relative to the control group (P = 0.006). Players presented with lower haemoglobin concentration compared to controls during post-season (P < 0.001; Table 1).

SCAT5

Players had higher SCAT5 concentration scores compared to the control group during pre-season (P = 0.007; Table 2), with no differences observed post-season (P = 0.734). Delayed recall was impaired in rugby players (P = 0.032) and lower balance scores were observed in controls across the season (P < 0.001).

Molecular function

Elevated oxidative stress indicated via increased $A^{\bullet-}$ concentration was apparent in players when compared with controls across the season (P < 0.001; Figure 31A). Players had lower bioactive NO relative to the control group (P < 0.001, Figure 31B). Total bioactive NO decreased in both groups across the season (P < 0.001).

Table 1: Demographics

Group	**Controls (*n*=17)**			**Players (*n*=21)**			***P* value**		
Characteristic	**Pre-season**	**Post-season**	**Δ**	**Pre-season**	**Post-season**	**Δ**	**Group**	**Time Point**	**Interaction**
Clinical									
Age (years)	28 ± 8	29 ± 8*****	1 ± 1	25 ± 4	26 ± 4*****	1 ± 0	0.210	0.000	0.026
Mean arterial pressure (mmHg)	86 ± 22	90 ± 12		86 ± 15	78 ± 18	-7 ± 24	0.076	0.006	0.115
Haemoglobin (g/dL)	15.2 ± 1.2	15.7 ± 1.8*	0.5 ± 1.9	15.5 ± 0.8	13.9 ± 0.9*	-1.6 ± 6.9†	0.023	0.029	0.000
Haematocrit (%)	46.1 ± 4.4	46.0 ± 2.3	0.0 ± 4.2	46.4 ± 1.7	45.6 ± 3.5	-0.8 ± 3.6	0.959	0.544	0.574
Anthropometrics									
Stature (cm)	177 ± 6	177 ± 6	0 ± 1	185 ± 7	185 ± 7	0 ± 1	0.001	0.293	0.074
Mass (kg)	73 ± 9	73 ± 8	0 ± 1	104 ± 14	103 ± 4	-1 ± 3	0.000	0.432	0.130
BMI (kg/m^2)	23 ± 3	23 ± 3	0 ± 1	31 ± 4	30 ± 4	1 ± 1	0.000	0.430	0.191
Body fat (%)	11 ± 4	12 ± 5	1 ± 3	20 ± 5†	17 ± 5*†	-3 ± 2†	0.000	0.018	0.000
Education									
Total education (years)	16 ± 2	16 ± 2	0 ± 1	14 ± 2	15 ± 2	0 ± 1	0.057	0.067	0.780
Contact/activity									
Concussions (n)	0 ± 0	0 ± 0	0 ± 0	3 ± 2	3 ± 2	0 ± 1	0.000	0.394	0.394
Rugby career (years)	0 ± 0	0 ± 0	0 ± 0	16 ± 4	17 ± 4	1 ± 0†	0.000	0.658	0.658
Physical activity (min/week)	353 ± 169	397 ± 254	44 ± 184	437 ± 158	323 ± 157	14 ± 219	0.202	0.386	0.657
Aerobic activity (min/week)	259 ± 149	305 ± 209	46 ± 170	283 ± 147	323 ± 157	40 ± 191	0.653	0.156	0.992
Resistance activity (min/week)	94 ± 73	92 ± 67	-2 ± 51	154 ± 42	129 ± 52	-26 ± 61	0.006	0.148	0.205

Values presented as mean ± SD; BMI; body mass index; †, $P < 0.05$ *vs.* control; *, $P < 0.05$ within groups.

Table 2: Sports Concussion Assessment

Group	Controls (*n*=17)			Players (*n*=21)			*P* value		
Characteristic	**Pre-season**	**Post-season**	**Δ**	**Pre-season**	**Post-season**	**Δ**	**Group**	**Time Point**	**Interaction**
Total symptoms	1 ± 1	2 ± 4*	-1 ± 3	1 ± 2	1 ± 2	1 ± 1	0.485	0.328	0.027
Severity score	1 ± 1	3 ± 7	-2 ± 7	2 ± 2	1 ± 2	1 ± 2	0.382	0.022	0.096
Orientation	5 ± 0	5 ± 0	0 ± 0	5 ± 0	5 ± 0	0 ± 0	1.000	1.000	0.167
Memory (immediate recall)	24 ± 3	23 ± 2	1 ± 3	21 ± 2	22 ± 4	-1 ± 4	0.094	0.600	0.122
Concentration	3 ± 1	4 ± 1	0 ± 1	4 ± 1†	4 ±1	0 ± 1	0.070	1.000	0.027
Balance errors	6 ± 3	6 ± 2	1 ± 4	2 ± 2	2 ± 2	0 ± 3	0.000	0.467	0.677
Memory (delayed recall)	8 ± 2	8 ± 1	0 ± 1	7 ± 1	6 ± 2	1 ± 2	0.032	0.425	0.143

Values presented as mean ± SD; *, P <0.05 within groups; †, P <0.05 *vs.* controls.

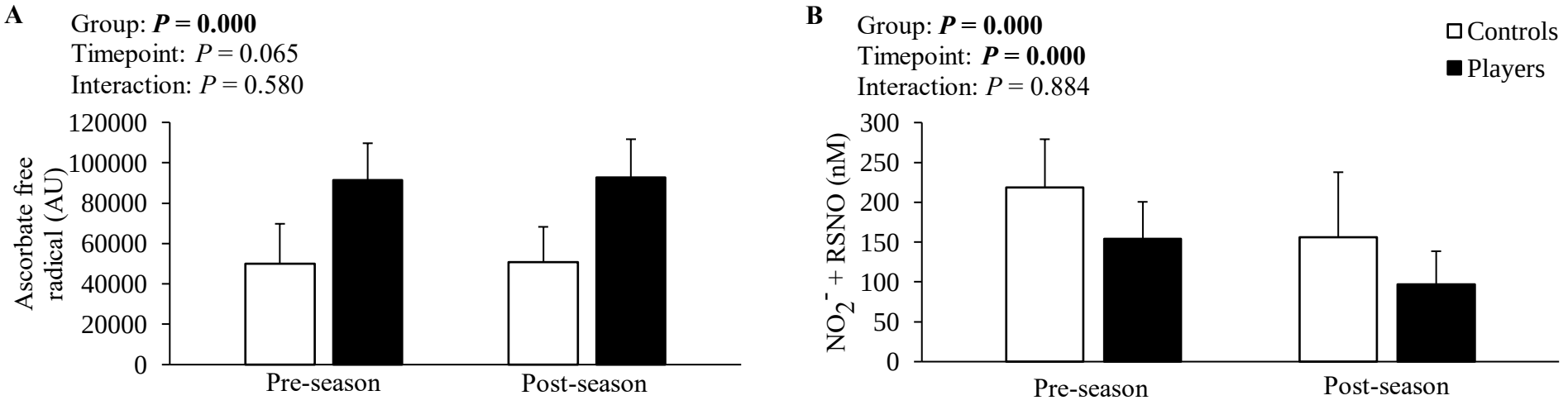

Figure 31: Ascorbate free radical (**A**) and cumulative bioactive nitric oxide concentration (**B**) during pre-season and post-season; NO_2^- nitrite; RSNO; S-nitrosothiols. Values presented as mean ± SD; controls (n = 17); players (n = 21).

Cerebrovascular reactivity

CDO_2 was lower in players at rest (P = 0.010), however no further differences in resting cerebral haemodynamic function were apparent between groups (Table 3-4). Cerebral perfusion in response to hypercapnia was lower in players compared to controls during post-season (P = 0.032; Table 3). Players confirmed a reduction in normocapnic CDO_2 (P = 0.006, Figure 32A) and hypocapnic CDO_2 (P = 0.007, Figure 32B) across the season compared to controls. No other relative differences were detected across the season for any cerebrovascular or cardiopulmonary variables (P > 0.05).

CVR_{CO2}

Players' $CVR_{CO2HYPER}$ and $CVR_{CO2HYPO}$ reduced from pre- to post-season (P < 0.001 and P = 0.003, Figure 33A). Players had lower $CVRCO_{2RANGE}$ during pre-season compared to the controls (P = 0.034), while no differences were apparent during post-season (P = 0.975; Figure 33 B). $CVR_{CO2RANGE}$ reduced in players from pre-to-post-season (P = 0.008), which was also observed in the control group (P < 0.001).

Cortical oxygenation

No changes in O_2Hb (P = 0.920 and P = 0.130), HHb (P = 0.671 and P = 0.724) or tHb (P = 0.959 and P = 0.128) across the season were detected during either cerebrovascular challenge (Figure 34).

Table 3: Cerebrovascular and cardiovascular responses to hypercapnia.

Condition	**Controls (*n*=17)**				**Players (*n*=21)**			
Group	**Baseline**		**Hypercapnia**		**Baseline**		**Hypercapnia**	
Time point	**Pre-season**	**Post-season**	**Pre-season**	**Post-season**	**Pre-season**	**Post-season**	**Pre-season**	**Post-season**
Cerebrovascular								
MCAv (cm/s)	60 ± 11	64 ± 11	82 ± 13*	78 ± 14*	55 ± 12	57 ± 11	73 ± 12*	72 ± 15*†
Condition: *P* = 0.000, Group: *P* = 0.057, Time: *P* = 0.652, Interaction: *P* = 0.030								
SMCAv (cm/s)	96 ± 17	102 ± 18	122 ± 17	120 ± 23	90 ± 19	91 ± 17	110 ± 21	109 ± 22
Condition: *P* = 0.000, Group: *P* = 0.088, Time: *P* = 0.699, Interaction: *P* = 0.095								
DMCAv (cm/s)	40 ± 8	44 ± 8	58 ± 10*	55 ± 10*	38 ± 9	39 ± 8	53 ± 10*	52 ± 11*
Condition: *P* = 0.000, Group: *P* = 0.134, Time: *P* = 0.788, Interaction: *P* = 0.029								
CVRi (mmHg/cm/s)	1.43 ± 0.28	1.44 ± 0.32	1.13 ± 0.31	1.22 ± 0.29	1.64 ± 0.45	1.42 ± 0.40	1.25 ± 0.34	1.23 ± 0.34
Condition: *P* = 0.000, Group: *P* = 0.329, Time: *P* = 0.580, Interaction: *P* = 0.319								
CVCi (cm/s/mmHg)	0.73 ± 0.20	0.73 ± 0.15	1.07 ± 0.85	0.86 ± 0.19	0.67 ± 0.28	0.76 ± 0.22	0.87 ± 0.31	0.87 ± 0.21
Condition: *P* = 0.000, Group: *P* = 0.488, Time: *P* = 0.577, Interaction: *P* = 0.446								
PI (AU)	0.94 ± 0.12	0.91 ± 0.13	0.79 ± 0.09	0.83 ± 0.10	0.96 ± 0.12	0.92 ± 0.12	0.81 ± 0.14	0.80 ± 0.12
Condition: *P* = 0.000, Group: *P* = 0.978, Time: *P* = 0.655, Interaction: *P* = 0.156								
CDO_2 (mL/cm/s)	1,225 ± 241	1,353 ± 246	1,679 ± 286	1,645 ± 325	1,149 ± 242	1,070 ± 207	1,514 ± 299	1,354 ± 271
Condition: *P* = 0.000, Group: *P* = 0.010, Time: *P* = 0.255, Interaction: *P* = 0.167								
Cardiopulmonary								
Heart Rate (bpm)	66 ± 13	66 ± 22	67 ± 11	67 ± 21	59 ± 7	59 ± 10	60 ± 7	60 ± 8
Condition: *P* = 0.196, Group: *P* = 0.048, Time: *P* = 0.958, Interaction: *P* =0.982								
MAP (mmHg)	86 ± 22	90 ± 12	91 ± 22	92 ± 15	86 ± 15	78 ± 17	87 ± 15	85 ± 15
Condition: *P* = 0.006, Group: *P* = 0.154, Time: *P* = 0.820, Interaction: *P* = 0.096								
TPR (mmHg/L/min)	13.50 ± 4.87	14.36 ± 2.99	13.62 ± 4.02	14.34 ± 3.46	14.48 ± 2.97	13.81 ± 3.43	14.30 ± 3.11	14.44 ± 2.91
Condition: *P* = 0.638, Group: *P* = 0.721, Time: *P* = 0.693, Interaction: *P* = 0.304								
PET_{CO2} (mmHg)	42 ± 7	47 ± 3	52 ± 5	56 ± 5	42 ± 8	43 ± 4	53 ± 4	55 ± 3
Condition: *P* = 0.000, Group: *P* = 0.347, Time: *P* = 0.003 Interaction: *P* = 0.219								

Values presented as mean ± SD; MCAv, middle cerebral artery velocity; SMCAv, systolic middle cerebral artery velocity; DMCAv, diastolic middle cerebral artery velocity; CVRi, cerebrovascular resistance index; CVCi, cerebrovascular conductance index; PI, pulsatility index; CDO_2, cerebral oxygen delivery; MAP, mean arterial pressure; TPR, total peripheral resistance, PET_{CO2}, end-tidal carbon dioxide; *, *P* <0.05 within groups; †, *P* <0.05 *vs.* controls.

Table 4: Cerebrovascular and cardiovascular responses to hypocapnia.

Condition	**Controls (*n*=17)**				**Players (*n*=21)**			
Group	**Baseline**		**Hypocapnia**		**Baseline**		**Hypocapnia**	
Time point	**Pre-season**	**Post-season**	**Pre-season**	**Post-season**	**Pre-season**	**Post-season**	**Pre-season**	**Post-season**
Cerebrovascular								
MCAv (cm/s)	59 ± 11	60 ± 11	36 ± 6	40 ± 9	54 ± 10	56 ± 11	38 ± 6	40 ± 7
Condition: *P* = 0.000, Group: *P* = 0.480, Time: *P* = 0.106, Interaction: *P* = 0.481								
SMCAv (cm/s)	95 ± 16	98 ± 19	72 ± 12	78 ± 15	91 ± 16	90 ± 16	72 ± 11	74 ± 13
Condition: *P* = 0.000, Group: *P* = 0.364, Time: *P* = 0.196, Interaction: *P* = 0.843								
DMCAv (cm/s)	39 ± 8	40 ± 8	21 ± 4	24 ± 7	37 ± 7	37 ± 8	24 ± 5	25 ± 6
Condition: *P* = 0.000, Group: *P* = 0.802, Time: *P* = 0.134, Interaction: *P* = 0.636								
CVRi (mmHg/cm/s)	1.54 ± 0.47	1.60 ± 0.38	2.20 ± 0.80	2.24 ± 0.57	1.62 ± 0.41	1.57 ± 0.40	2.07 ± 0.56	1.98 ± 0.56
Condition: *P* = 0.000, Group: *P* = 0.520, Time: *P* = 0.937, Interaction: *P* = 0.865								
CVCi (cm/s/mmHg)	0.80 ± 0.67	0.66 ± 0.15	0.59 ± 0.51	0.47 ± 0.31	0.68 ± 0.27	0.67 ± 0.15	0.53 ± 0.19	0.55
Condition: *P* = 0.000, Group: *P* = 0.752, Time: *P* = 0.317, Interaction: *P* = 0.885								
PI (AU)	0.97 ± 0.18	0.97 ± 0.14	1.41 ± 0.20	1.38 ± 0.28	1.01 ± 0.16	0.96 ± 0.15	1.30 ± 0.21	1.23 ± 0.22
Condition: *P* = 0.000, Group: *P* = 0.271, Time: *P* = 0.174, Interaction: *P* = 0.784								
CDO_2 (mL/cm/s)	1,207 ± 233	1,258 ± 248	745 ± 126	829 ± 157	1,138 ± 223	1,045 ± 205	795 ± 126	750 ± 122
Condition: *P* = 0.000, Group: *P* = 0.121, Time: *P* = 0.968, Interaction: *P* = 0.713								
Cardiopulmonary								
Heart Rate (bpm)	66 ± 11	69 ± 21	74 ± 11	76 ± 20	61 ± 8	60 ± 8	68 ± 9	63 ± 8
Condition: *P* = 0.000, Group: *P* = 0.013, Time: *P* = 0.820, Interaction: *P* = 0.386								
MAP (mmHg)	88 ± 23	92 ± 14	77 ± 23	85 ± 15	84 ± 14	85 ± 15	76 ± 15	77 ± 17
Condition: *P* = 0.000, Group: *P* = 0.221, Time: *P* = 0.323, Interaction: *P* = 0.411								
TPR (mmHg/L/min)	13.38 ± 4.22	3.36	9.57 ± 3.16	11.21 ± 2.61	13.54 ± 2.37	14.23 ± 2.59	11.04 ± 2.40	12.38 ± 3.24
Condition: *P* = 0.000, Group: *P* = 0.390, Time: *P* = 0.036, Interaction: *P* = 0.942								
PET_{CO2} (mmHg)	41 ± 5	45 ± 3	28 ± 6*	29 ± 5*	40 ± 8	43 ± 4	29 ± 7*	31 ± 4*
Condition: *P* = 0.000, Group: *P* = 0.907, Time: *P* = 0.007, Interaction: *P* = 0.044								

Values presented as mean ± SD; MCAv, middle cerebral artery velocity; SMCAv, systolic middle cerebral artery velocity; DMCAv, diastolic middle cerebral artery velocity; CVRi, cerebrovascular resistance index; CVCi, cerebrovascular conductance index; PI, pulsatility index; CDO_2, cerebral oxygen delivery; MAP, mean arterial pressure; TPR, total peripheral resistance, PET_{CO2}, end-tidal carbon dioxide; *, *P* <0.05 within groups.

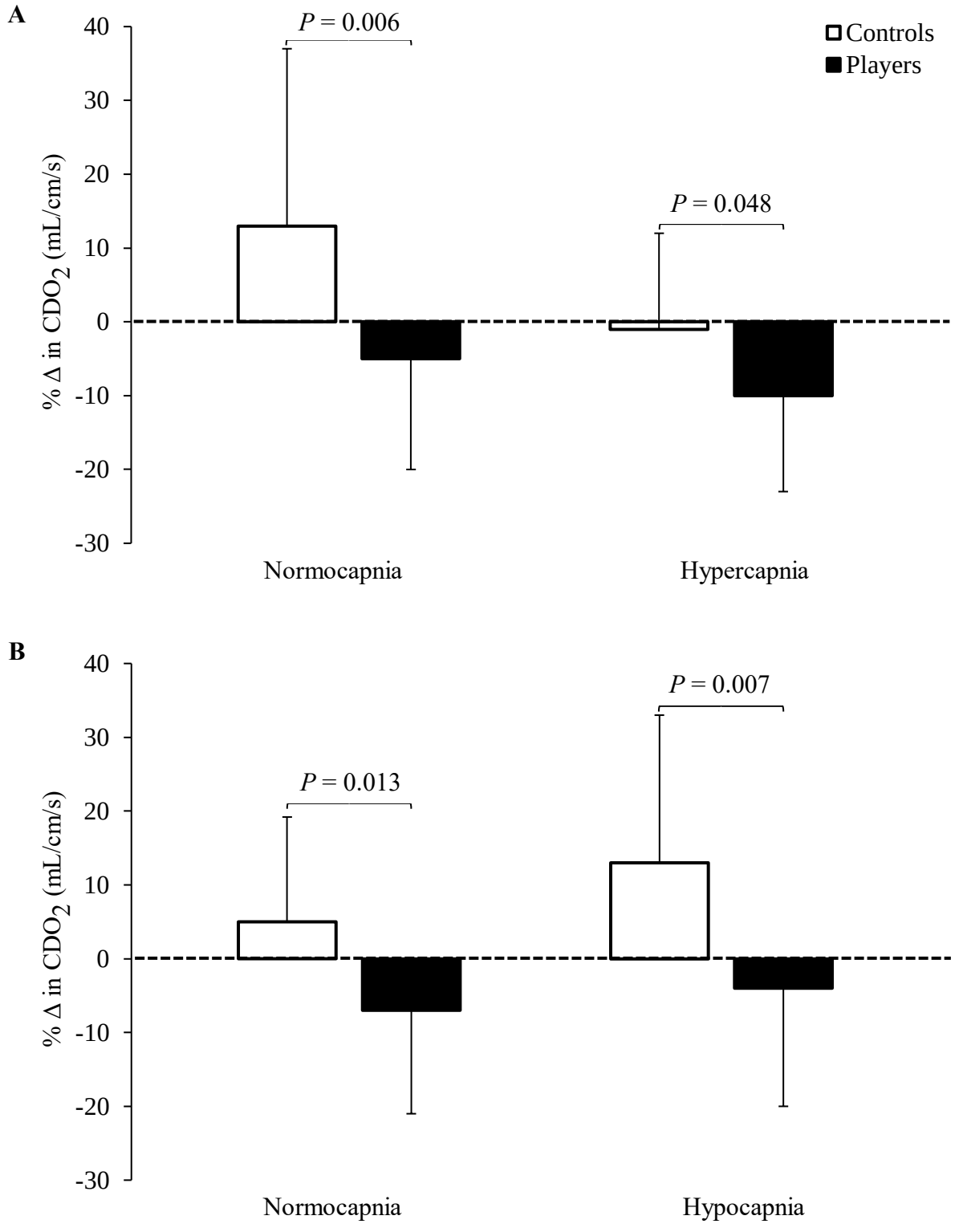

Figure 32: Relative changes in cerebral oxygen delivery (CDO_2) across the season in normocapnia and in response to hypercapnia (**A**) and hypocapnia (**B**). Values presented as mean ± SD; controls (n = 17); players (n = 21).

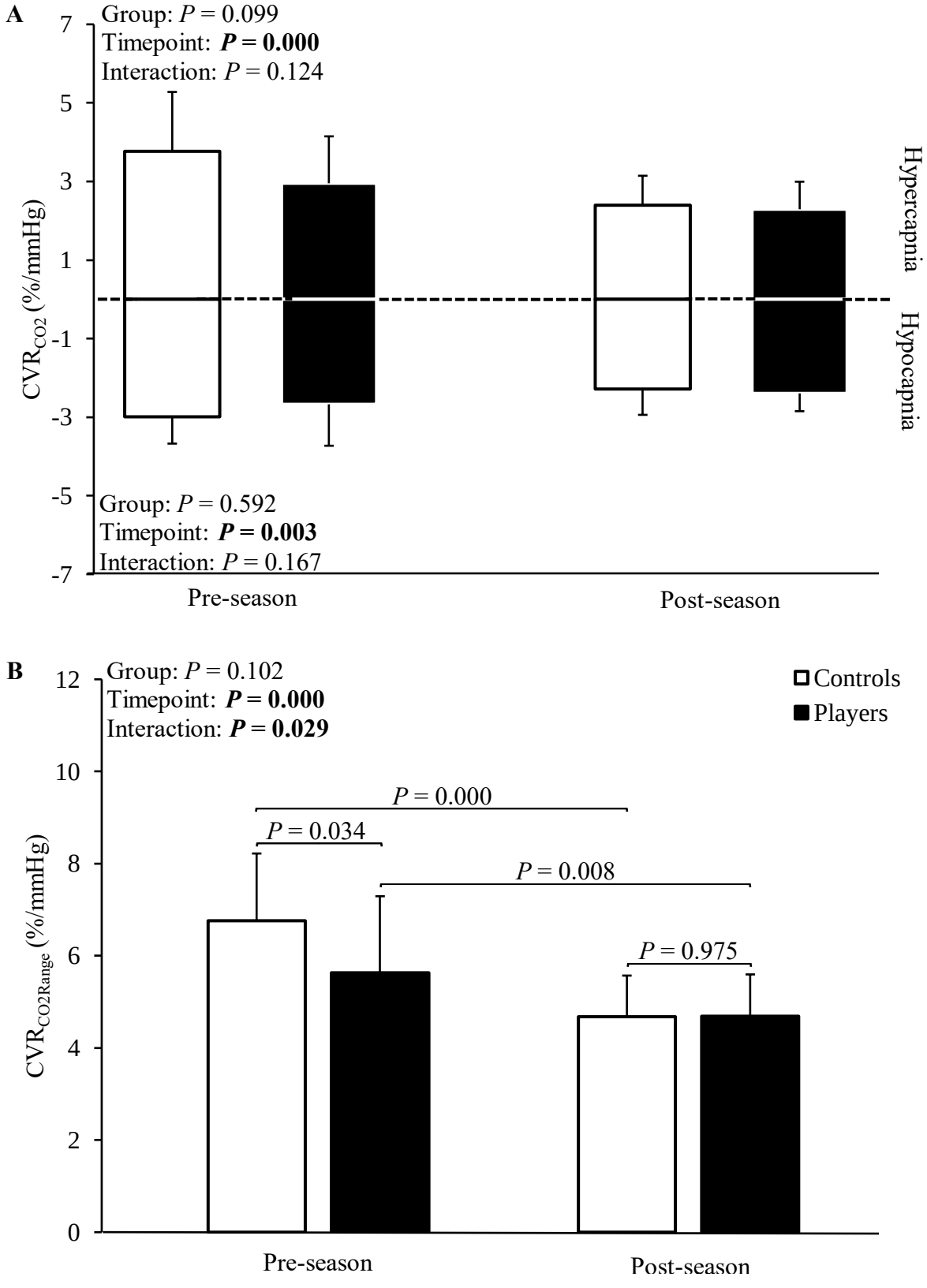

Figure 33: Cerebrovascular reactivity to carbon dioxide (CVR_{CO2}) across the season in response to hypercapnia and hypocapnia (**A**) and corresponding vasomotor range ($CVR_{CO2RANGE}$, **B**). Values presented as mean ± SD; controls (*n* = 17); players (*n* = 21).

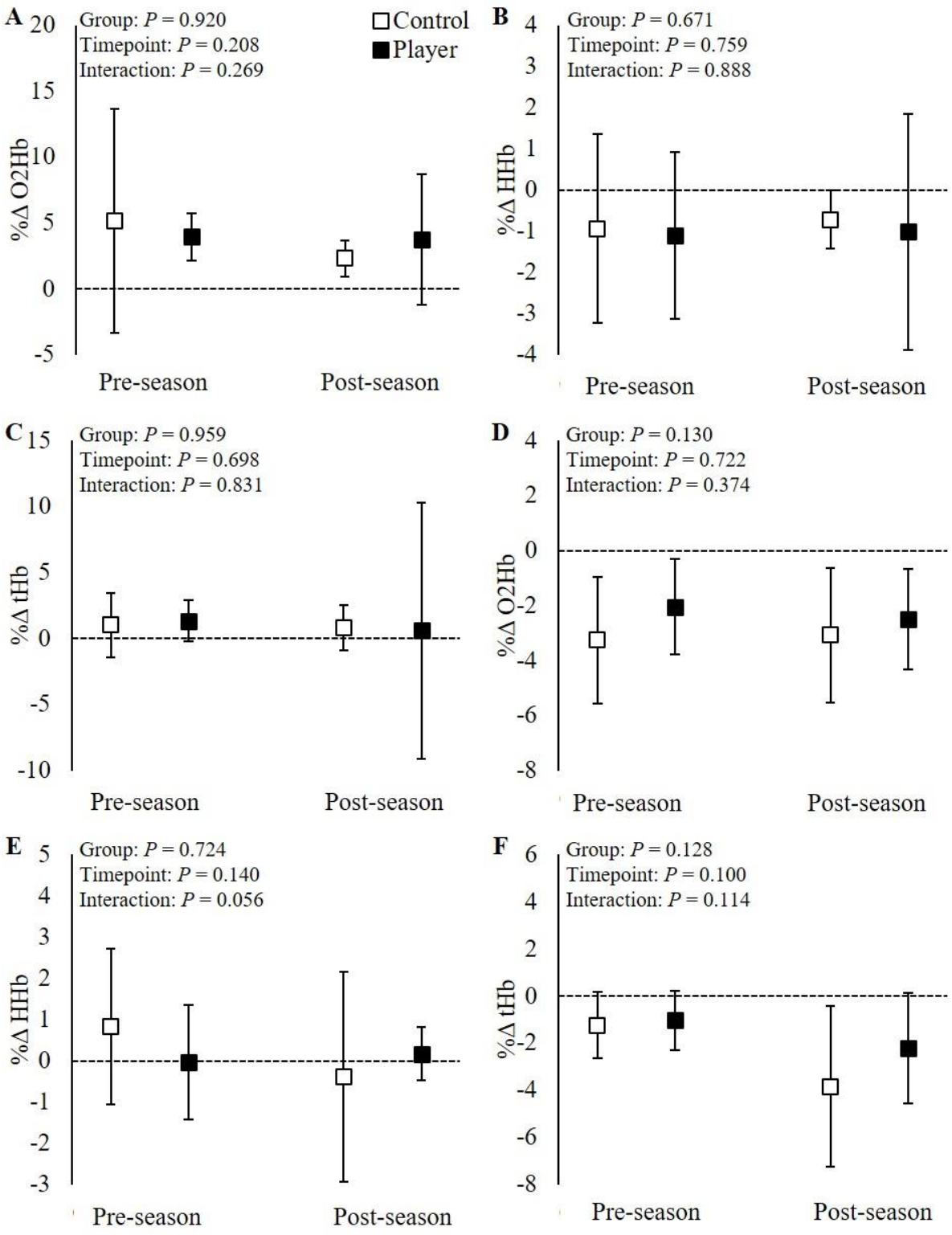

Figure 34: Relative changes in cortical oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb) and total haemoglobin (tHb) from rest to hypercapnia **(A-C)** and from rest to hypocapnia **(D-F)** during pre/post-season. Values presented as mean ± SD; controls (*n* = 17); players (*n* = 21).

Cognition

Baseline measures: While no MCI was detected using the MoCA ($P = 0.095$), players exhibited depressed immediate memory (RAVLT-B1, $P < 0.001$) and delayed recall (RAVLT-A6, $P < 0.001$) during pre-season. No differences were evident during post-season between-groups (Table 5). Players had impaired fine motor coordination compared to controls as indicated via slower completion times of the GPD and GPND compared to controls ($P < 0.001$). Across the season, RAVLT-B scores improved in players and reduced in controls ($P = 0.006$), while RAVLT-A6 scores improved in rugby players and controls ($P < 0.001$, Table 5).

Table 5: Cognition

Group	Controls (*n*=17)			Players (*n*=21)					
Characteristic	**Pre-season**	**Post-season**	**Δ**	**Pre-season**	**Post-season**	**Δ**	**Group**	**Time point**	**Interaction**
Global cognition									
Montreal Cognitive Assessment (score)	28 ± 2	28 ± 1	0 ± 2	28 ± 2	26 ± 2	-2 ± 2	0.068	0.095	0.099
Visuomotor coordination									
Grooved Pegboard Dominant Hand (s)	59 ± 7	58 ± 7	-1 ± 6	71 ± 10	66 ± 8	-5 ± 8	0.012	0.000	0.141
Grooved Pegboard Non-Dominant Hand (s)	66 ± 9	63 ± 8	-2 ± 8	80 ± 16	77 ± 14	-3 ± 14	0.231	0.000	0.876
Learning and memory									
Rey Auditory Verbal Learning Test A1:A5 (*n*)	53 ± 9	58 ± 11	5 ± 9	49 ± 8	53 ± 6	4 ± 7	0.002	0.102	0.630
Rey Auditory Verbal Learning Test B1 (*n*)	7 ± 2	6 ± 2	-1 ± 2	5 ± 1†	6 ± 2	1 ± 2†	0.543	0.012	0.014
Rey Auditory Verbal Learning Test A6 (*n*)	11 ± 4	12 ± 3	1 ± 2	6 ± 2†	11 ± 3*	5 ± 3†	0.000	0.000	0.000
Rey Auditory Verbal Learning Test A6-A5 (*n*)	-1 ± 2	-1 ± 2	0 ± 2	-3 ± 2	-2 ± 2	1 ± 3	0.349	0.020	0.601
Working memory									
Trail Making B (s)	54 ± 15	52 ± 11	-3 ± 17	54 ± 16	47 ± 13	-7 ± 14	0.055	0.578	0.406
Repetition of Digits Backward (score)	7 ± 2	7 ± 2	0 ± 3	6 ± 2	8 ± 2	2 ± 2	0.008	0.996	0.112
Attention / Information processing									
Repetition of Digits Forwards (score)	9 ± 3	9 ± 2	0 ± 3	8 ± 3	8 ± 3	0 ± 3	0.380	0.302	0.973
Trail Making A (s)	23 ± 5	22 ± 6	-1 ± 5	28 ± 9	23 ± 7	-5 ± 12	0.065	0.083	0.281
Digit-Symbol Substitution Test (score)	65 ± 8	71 ± 9	6 ± 6	60 ± 8	65 ± 8	5 ± 6	0.000	0.053	0.612
Repetition of Digits Total (score)	15 ± 4	16 ± 3	1 ± 4	14 ± 4	16 ± 4	2 ± 4	0.029	0.518	0.360

Values presented as mean ± SD; †, $P < 0.05$ *vs.* controls; *****, $P < 0.05$ within groups.

In-season analysis

Match events

Players completed an average of 17.9 ± 10.0 hours of match play over the course of the season. Forwards incurred more match events compared to backs, most notably for collisions (*P* = 0.017; Table 6). Per game, forwards were involved in more collisions (*P* = 0.005), tackles (*P* = 0.028) and jackals (*P* = 0.034) compared to backs. No differences in the average number of ball caries were observed between-groups (*P* = 0.725).

Incidence and propensity

Six concussions were incurred during the season (forwards; n = 5, backs; n = 1) corresponding to a concussion IR of 10/1000 player match hours (95% CI, -0.00 – 28.49). Forwards suffered 12.9 concussions/1000 match hours (95% CI, 0.00 – 38.46) compared to 4.2/1000 player match hours among backs (95% CI, 0.00 – 33.44). Concussion propensity resulting from collisions, tackles, jackals and ball carries was not different between-groups (Table 7).

Table 6: Comparison of contact events

Group	**Forwards (*n* = 13)**	**Backs (*n* = 8)**	***P* Value**	**95% CI**
Collisions	186 ± 129	67 ± 64	**0.017**	25.16 – 228.05
Tackles	120 ± 98	71 ± 40	0.126	-18.17 – 135.59
Jackals	48 ± 56	9 ± 10	0.059	-1.78 – 83.14
Ball carries	59 ± 55	63 ± 30	0.932	-42.59 – 46.29
Collisions (per game)	12 ± 6	5 ± 3	**0.005**	2.58 – 12.40
Tackles (per game)	7 ± 4	4 ± 1	**0.028**	0.42 – 6.56
Jackals (per game)	3 ± 3	1 ± 0	**0.034**	0.18 – 4.24
Ball carries (per game)	4 ± 2	4 ± 1	0.725	-2.16 – 1.53

Data presented as mean ± SD.

Table 7: Injury incidence and match event propensity

Group	**Forwards (*n* = 13)**	**Backs (*n* = 8)**	**Rate ratio (95% CI)**
Concussion (*n*/season)	5	1	-
Concussion/1000 Hours	12.9	4.2	3.08 (0.00 – 28.49)
Concussion/1000 Collisions	1.3	7.4	0.18 (-0.00 – 2.46)
Concussion/1000 Tackles	1.7	1.0	1.68 (0.00 – 2.85)
Concussion/1000 Jackals	4.4	37	0.12 (0.00 – 101.52)
Concussion/1000 Ball carries	2.5	1.1	2.28 (0.00 – 5.66)

Data presented as absolute values.

4.4 Discussion

This functionally integrated translational study, combining molecular (blood-borne), cerebral haemodynamic (perfusion and reactivity) and clinical (cognitive) metrics has identified that when compared to controls, professional rugby union players with concussion history were characterised by elevated systemic OXNOS, supressed CVR, and a decline in immediate memory, delayed recall, fine motor coordination and executive function across the competitive season.

Incidence and match events

Our results concur with the most recent RFU Injury Surveillance Project (England Rugby, 2020) as injury propensity was not influenced by grouped playing position or the type of match event. While the reporting of injury incidence remains important and serves as an objective measure of whether concussion prevalence is being managed successfully within the game, it is a blunt tool for measuring the potential for neurological injury in players. This study identified that match event frequency was higher among forwards compared to backs. While no differences in molecular, cerebrovascular and cognitive function were apparent between playing positions, we demonstrated that a player's molecular and cerebral hemodynamic signature changes across the season. Moreover, players were identified with increased OXNOS, decreased cerebrovascular function and cognition compared to controls, highlighting a need for more rigorous routine measures in rugby union.

Metabolic function

Players exhibited elevated systemic OXNOS compared to controls throughout the season. During pre-season, it appeared that cumulative exposure to prior concussion and an average of 16 years participation in rugby gradually inflicted a persistent molecular shift towards elevated OXNOS. Giza and Hovda (2014) described that mitochondrial hypermetabolism following impact increased ROS generation, namely superoxide and results in the scavenging of NO to form peroxynitrite during the subsequent hypometabolic phase. However, this study demonstrates evidence for increased nitrosative stress in the absence of elevated oxidative stress over the course of the competitive season. Players did not exhibit an increase in systemic $A^{\bullet-}$ across the season and given that a reduction in bioactive NO was observed over the season, other mechanisms are likely accountable. Contact can supress eNOS activity, thereby disturbing NO kinetics (Wada et al., 1998) that may be persistent over the course of a player's active career. Moreover, supressed CDO_2 among players supports a reduction in O_2 required

for NOS to produce NO (Cherian et al., 2000). Similarly, the limited oxygen supplies in response to contact reduce the conversion of citrulline into arginine and administration of L-arginine may be an effective intervention to increase NO and CBF following trauma (Cherian et al., 1999).

Cerebral haemodynamic function

The players CVR decreased across the season and were most apparent when compared to the control group. Players presented with no clinical symptoms of hypoperfusion (i.e. altered mental status, Cipolla, 2009), however CDO_2 was lower at rest and in response to the CO_2 challenges between pre- and post-season. Players had decreased $CVR_{CO2RANGE}$ compared to controls, an albeit indirect reflection of impaired cerebrovascular endothelial function (Glodzik et al., 2013). Given that $CVR_{CO2HYPER}$ is partly mediated by NO (Lavi et al., 2003), the suppressed $CVR_{CO2RANGE}$ observed between players and controls could be explained by increased OXNOS, though further research is required.

It is also important to consider the integrity of the vascular beds and regulation of CBF following concussion. Historically, both primary injury resulting from damage induced by impact trauma and secondary degenerative injury have been argued as mechanisms for reduced CBF following concussion (Zhang et al., 2004). In an uninjured state, vasodilatory challenges including hypercapnia induce NO-mediated increases in CBF (Chen et al., 2008, Fathi et al., 2011). However, structural damage via contact and concussion can create asymmetrical vasodilatory responses by redistributing blood in favour of vascular beds with greater vasodilatory reserve (Ellis et al., 2016). For instance, the thalamus elicits the greatest shear stress in computational models of concussion, whereby neuronal damage can lead to apoptosis and decreased perfusion in capillaries, resulting in functionally disrupted neurons nine months following injury (Grossman et al., 2013). Consequently, it is important to consider that players with apparently normal MCAv may be characterised by hypoperfusion of the Circle of Willis and anterior regions of the cerebral vasculature (Ellis et al., 2016).

Cognition

Players had no clinical indication of cognitive impairment via the MoCA. However, impairments were most apparent for immediate memory, delayed recall, fine-motor coordination and executive function, agreeing with previous studies (McMillan et al., 2017). Players presented with suppressed cerebral perfusion and nutrient delivery (i.e. CDO_2). Positive correlations have consistently been observed between CBF and cognition (Scarmeas

et al., 2003). Both globally and regionally (Ghogawala et al., 2013, Churchill et al., 2017b), hypoperfusion of the cerebral vasculature has been associated with impaired cognition and is a risk factor for dementia (Wolters et al., 2017). While not measured in this study, the cerebral delivery of glucose ($CD_{Glucose}$) may have been reduced and suppressed cognition (Giza and Hovda, 2020). Follow up studies should consider measuring $CD_{Glucose}$ and whether the brain is able to compensate in response to trauma by undergoing periods of increased O_2 and $CD_{Glucose}$ extraction.

Players performed worse in assessments of immediate memory (RAVLT-B1) and delayed recall (RAVLT-A6) compared to controls. Meier et al. (2015) observed cognitive impairments in concussed athletes seven days after injury who were characterised by regional impairments in CBF in the dorsal mid-insular cortex and right superior temporal sulcus. Given that blood flow to these regions is subserved by the MCA (Uddin et al., 2017), impaired perfusion and corresponding substrate delivery may result in compromised white matter integrity and neuroglial damage (Basser, 1995, Wu and Cheung, 2010). The insula is connected via white matter tracts to other important brain regions, namely the frontal, temporal and parietal lobes, including the hippocampus and amygdala (Showers and Lauer, 1961). Memory function is largely governed by the hippocampus (Bird and Burgess, 2008). Consequently, a loss of white matter tracts may cause cognitive dysfunction in these brain regions. Grossman et al. (2013) has further observed reduced CBF via arterial spin labelling, fractional anisotropy and mean kurtosis (MK) in the thalamus of mTBI patients. The thalamus and hippocampus are functionally connected, which serves as a hub for relaying information and upholding memory function (Stein et al., 2000).

We further observed slower completion times of the GPD and GPND in players, indicative of impaired fine-motor coordination and executive function. Both the frontal and temporal lobes of the brain are particularly vulnerable to impact forces when tackling in rugby (Cross et al., 2017). Subsequently, if structural damage was inflicted in these brain regions among players of this study, it would have the potential to impair executive function (Wang et al., 2016).

It is important to consider that while cognitive impairments were apparent in players, our results did not correspond to those of Shuttleworth-Edwards and Radloff (2008) who observed impairments in the DSST, TMT-A and TMT-B. Similarly, cognition in players generally improved over the course of the season, implying some degree of habituation. Shuttleworth-Edwards et al. (2014) has demonstrated that formerly concussed athletes elicit blunted learning

effects, offering unconventional diagnostic potential. However, our results do not correspond with these findings. Apparent improvements in cognition can be misinterpreted unless repeat assessments and correction factors are applied (Marley et al., 2017). Due to time constraints, we were unable to control for habituation effects via repeated baseline testing, which may have increased our potential to identify cognitive decline across the season. Moreover, interpretative caution must be exercised when considering the decline in cognition as previous research indicates that a reliable change index for the MoCA is ± 3 points, albeit in an aged sedentary population (Kopecek et al., 2017) in the absence of intense aerobic-strength training known to compound neuroprotection (Bailey et al., 2013b). Indeed, players performance in the RAVLT, Trail Making Tests and DSST aligned with age-defined reference metrics (Salthouse, 1992, Amodio et al., 2002, Vakil et al., 2010), however player GPND scores at pre-season fell outside of expected performance ranges and corresponded with performance of the 40-54 year-old age group (Ruff and Parker, 1993).

Conclusion

Compared to a non-concussed, non-contact control group, professional rugby union players are characterised by elevated OXNOS, suppressed cerebrovascular reactivity and cognition. Over the course of the season, subtle reductions were observed for some molecular, cerebral hemodynamic and cognitive metrics, although clinical impairments were not observed. The findings of this study imply that persistent impairments are the likely effect of over a decade of contact and prior exposure to concussion in rugby union. To better understand the players physiological phenotype in response to recurrent contact events across a season, future research may consider deploying the measures used in this study to evaluate acute single-match-events at the pitch side, including recovery based on a clearer delineation of the underlying temporal kinetics.

Chapter 5

Research study 2 - Neurovascular implications of life-long exposure to recurrent contact in retired rugby union players; the link to cognition

5.1. Introduction

Following retirement from sport, the chronic consequences of prior-recurrent contact are most evident. Fuller et al. (2007a) identified that rugby players may be exposed to 11,000 contact events per-season, equating to nearly quarter of a million contact events over a 20-year playing career. Retired athletes with three or more concussions are characterised by a fivefold increase in the prevalence of MCI and are susceptible to neurodegeneration, including CTE (Guskiewicz et al., 2005, Gardner et al., 2014a). This is apparent in various sports including American Football, soccer and ice hockey (Gardner et al., 2014a). Rugby union has one of the highest concussion incidence rates of any sport (Gardner et al., 2014b, England Rugby, 2020), but the confirmed diagnosis of CTE among retired rugby players remain relatively sparse. Stewart et al. (2016) has speculated this may be due to the low volume of brain donations provided by retired players, while Lee et al. (2019) noted that mixed neurodegenerative pathology during autopsy may lead to misdiagnosis.

Retired rugby players may be uniquely susceptible to accelerated cognitive impairment. While no consensus has been established, several mechanisms have been proposed. For instance, cerebral hypoperfusion has been consistently associated with MCI and neurodegeneration (Johnson et al., 2005, Wolters et al., 2017) attributable to two potential mechanisms. Firstly, the neurometabolic cascade of concussion promotes mitochondrial dysfunction and elevated OXNOS (Vagnozzi et al., 2007, Tavazzi et al., 2007, Giza and Hovda, 2014), which can compromise cerebral haemodynamic function and CVR (Bailey et al., 2019). Secondly, shear stress inflicted by contact can lead to the loss of neuronal network integrity and promotes atrophy (Grossman et al., 2013). This can cause regional hypoperfusion since functionally impaired neurons no longer demand energy and divert blood flow to non-impaired networks (Ellis et al., 2016). These events may be further compounded by ageing and physical inactivity as natural ageing can reduce CBF by up to a 30% across the lifetime (Ainslie et al., 2008), while decreased cardiorespiratory fitness can reduce cerebral hemodynamic function (Bailey et al., 2013b).

At present, no studies have attempted to understand these mechanisms in retired rugby players. The present study aimed to determine the chronic molecular, cerebrovascular and cognitive signatures of retired rugby players with concussion history. We hypothesised that compared to controls, retired rugby union players would present with elevated OXNOS, suppressed cerebrovascular function and cognition.

5.2. Methodology

5.2.1. Ethical approval

The experimental protocol was approved by the University of South Wales ethics committee (Reference number: 2017TO1102). Verbal and written informed consent was obtained from all participants and anonymity ensured via a random coding generator. All experimental procedures conformed to the standards set by the Declaration of Helsinki (Williams, 2008).

5.2.2. Participants

Forty-four males were recruited into the study and divided into two sub-groups. Twenty-two retired rugby players aged 64 ± 5 years with 3 ± 3 concussions incurred over 22 ± 7 years were compared to 22 age-, CRF- and education-matched controls with no participation in contact sports or concussion history. Forty-one participants were included in the final analysis comprised of 20 retired players and 21 controls. Three participants were excluded from the study due to ill-health (n = 2) and failure to comply with the complete experimental protocol (n = 1). Table 8 outlines participant demographics.

5.2.3. Study design

A cross-sectional observational study was conducted. Following confirmation of interest to participate in the study, participants were contacted via telephone and completed an eligibility questionnaire for screening purposes. If deemed eligible (section 3.2), participants attended the Neurovascular Research Laboratory at the University of South Wales on two occasions (measurements outlined below). Ahead of the study visits, participants refrained from physical activity, caffeine and alcohol, followed a low nitrate/nitrite diet and had completed a 12 hour overnight fast prior to experimentation (Wang et al., 1997, Bailey et al., 2017).

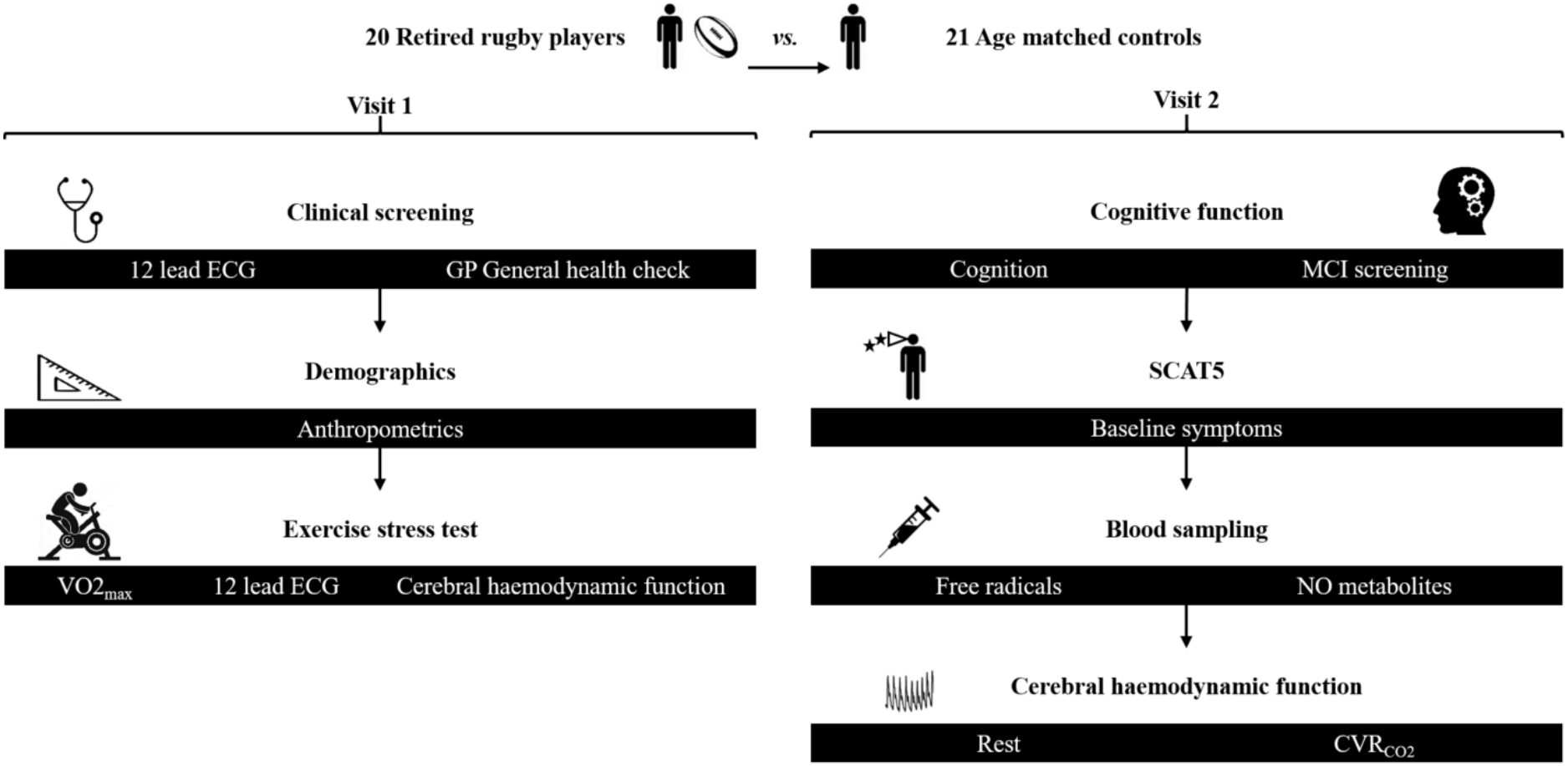

Figure 35: Experimental design; ECG; electrocardiography; GP, general practitioner; $\dot{V}O2_{MAX}$, maximal oxygen consumption; MCI, mild cognitive impairment; SCAT 5, Sports Concussion Assessment Tool 5th edition; NO, nitric oxide; CVR_{CO2}, cerebrovascular reactivity to changes in end tidal carbon dioxide.

5.2.4. Experimental procedures - Visit one

Medical observations

All participants were subject to an extensive clinical examination (section 3.6) via Dr Gareth Jones. The consultation addressed general health status (including identification of any major disease), review of medication, BP, chest auscultation and 12-lead ECG. Participant concussion history was reported via the off-field section of the SCAT5 (section 3.9.6, McCrory et al., 2017). Retired players confirmed an average of 32 ± 12 years since their most recent concussion and returned to play granted following 8 ± 14 days. Controls reported no concussion history.

Anthropometrics

stature was measured using a stadiometer (Seca, Germany). Bioelectrical impedance analysis (Tanita BC-418MA, Japan) obtained body mass, BMI and body composition (section 3.5).

Blood sampling

A cannula was placed in an antecubital vein of the forearm (section 3.7.1). Blood samples were obtained before and immediately after cessation of the exercise stress test (described below). Blood samples were collected in vacutainers, centrifuged at 600 *g* (4°C) for 10 minutes and snap-frozen in liquid nitrogen. Prior to batch analysis, plasma samples were thawed at 37°C for five minutes to determine markers of OXNOS. A 1 mL syringe was used to collect whole blood for analysis of haemoglobin and haematocrit concentration.

Free radicals

Plasma ascorbate free radical was measured as a biomarker of global free radical flux (Bailey et al., 2009) and analysed by X-band EPR spectroscopy (section 3.7.4). Spectra were filtered identically using WINEPR (Version 2.11) and the double integral of each doublet calculated using Origin software. Intra- and inter-assay CVs were both <5%.

Total bioactive NO

A 200 μl sample was injected into tri-iodide reagent to determine total bioactive NO (NO_2^- + RSNO), via ozone based chemiluminescence (section 3.7.5, Bailey et al., 2017). All calculations were determined using Origin software. Intra- and inter-assay CVs were both <5%.

Haemoglobin and haematocrit

Whole blood from a 1 mL syringe was decanted into a microcuvette and placed into an analyser to determine haemoglobin concentration (section 3.7.2). A small volume was also decanted

into microcapillary tubes, centrifuged for five minutes at 600*g* and placed onto a haematocrit analyser (section 3.7.3). Analysis was performed in duplicate and the average recorded. Intra- and inter assay CVs were both <5%.

Electrocardiography

Participants were fitted with a 12-lead ECG (Welch Allyn, Buckinghamshire, UK) to exclude any myocardial abnormalities. Participants remained at rest in a supine position for 10 minutes. Thereafter, a continuous recording of the ECG was performed throughout an exercise stress test (section 3.6).

Cerebral haemodynamic function

Middle cerebral artery velocity during rest and exercise was determined via TCD ultrasonography using a 2 MHz pulsed Doppler ultrasound system (section 3.8.1). Insonation of the M1 segment of the right (or left; when insonation of the right MCA side was unachievable) MCA was conducted at depths of 45-60 mm (Aaslid et al., 1989). The probe was secured over the trans-temporal window using a headband in order to attain optimal insonation and prevent movement artefact. MCAv was collected for five minutes at rest and continuously throughout the exercise stress test.

Exercise stress test

Participants were seated on an electronically braked semi-recumbent cycle ergometer and completed an incremental exercise test to exhaustion (section 3.8). Online breath-by-breath respiratory gas analysis was performed throughout. Heart rate was monitored throughout exercise using the 12 lead ECG. Maximal performance was confirmed in accordance to established criteria (section 3.8.1).

5.2.5. Experimental procedures - Visit two

Cognition

The MoCA was used as clinical screening tool for MCI (Nasreddine et al., 2005). Cognition was assessed further using a battery of tests which considered the criteria for the symptomatic phase prior to the onset of neurodegeneration as recommended by the National Institute for Ageing and the Alzheimer's Association (Albert et al., 2011). The RAVLT-A and RAVLT-B were used to determine parameters of learning and memory (Lezak, 1983, Rey, 1941). Working memory was assessed via the RDB (Koppitz, 1977) and TMT-B (Tombaugh, 2004). The TMT-A (Tombaugh, 2004), RDF (Koppitz, 1977) and the DSST (Salthouse, 1992) was used to

measure attention and information processing. Visuospatial coordination and visuo-constructional skills were assessed via the GPD and GPND (Trites, 1977). Superior performance was indicated by higher scores in the RAVLT-A, RAVLT-B, RDB, RDF, DSST and MoCA, or lower scores in the TMT-A, TMT-B, GPD and GPND (Marley et al., 2017).

Cerebral haemodynamic function

Middle cerebral artery blood flow velocity was determined after 5 minutes from a seated position using TCD ultrasonography (section 3.8.1). CVR_{CO2} was assessed in response to inspiratory hyper/hypocapnia and used to calculate $CVR_{CO2RANGE}$ (section 3.8.4). For all cerebral haemodynamic parameters, absolute changes and percentage changes were calculated and expressed from baseline to hypercapnia or hypocapnia for analysis.

Near-infrared spectroscopy

NIRS was used to determine cortical O_2Hb, HHb and tHb throughout cerebral haemodynamic challenges (section 3.8.2). Given that absolute changes in cO_2Hb, HHb and tHb cannot be obtained by NIRS alone due to the unknown pathway length of the photons (Murkin and Arango, 2009), relative changes were calculated and expressed as a percentage change from baseline for analysis.

Cardiovascular function

Throughout cerebral haemodynamic challenges, beat-by-beat arterial blood pressure was determined by finger photoplethysmography (Imholz et al., 1998). A Finometer was placed on the middle finger which determined MAP and Q using an automated model flow method throughout (section 3.8.3, Wesseling et al., 1993). Heart rate was determined via a 3-lead ECG.

Data logging/analysis

All cerebral haemodynamic data were recorded throughout experimental visits 1 and 2 using an analogue-to-digital converter at 1 KHz (section 3.8.7). Throughout all cerebral haemodynamic challenges, CVRi, TPR, CVCi, PI and CDO_2 was calculated (section 3.8.7).

5.2.6. Statistical analysis

Datasets were analysed using a commercially available statistical package (SPSS 25.0, International Business Machines, Chigaco, USA). Following confirmation of distribution normality via Shapiro Wilk *W* tests, between-group differences were determined via independent samples *t*-tests for participant demographics, molecular profile, cognition, CVR,

SCAT5 performance and changes in cortical oxygenation. A two-way [group (controls *vs.* retired players) × condition (normocapnia *vs.* hyper/hypocapnia)] ANOVA was conducted to determine differences between groups for changes in cerebrovascular function at rest and during hyper/hypocapnia. ANOVA's were performed separately in response to the bi-phasic kinetic of the MCAv during the exercise stress test in order to accurately assess the increase and decrease in perfusion (i.e. vasodilation and vasoconstriction throughout exercise, Figure 40, Ogoh and Ainslie, 2009, Brugniaux et al., 2014). All post-hoc analysis was completed using Bonferroni corrected independent samples *t*-tests and paired samples *t*-tests. Results are displayed as mean ± standard deviation with statistical significance for all two-tailed tests established at $P < 0.05$.

5.3. Results

Demographics

Both groups were matched for age, anthropometrics, physical activity, cardiorespiratory fitness and education level ($P > 0.05$, Table 8 and Table 9). At maximal exercise, both retired players and controls were matched for all respiratory parameters ($P > 0.05$) excluding the ventilatory equivalent of CO_2 ($P = 0.039$, Table 9).

SCAT5

Retired players had prolonged symptoms of concussion (Table 10). Likewise, the severity of symptoms was greater among retired players compared to controls ($P = < 0.001$). No further differences were identified between groups in any remaining metric ($P > 0.05$).

Molecular function

Retired players confirmed lower oxidative stress as indicated via $A^{\bullet-}$ concentration ($P = 0.018$; Figure 36A). Players were also characterised by lower bioactive NO concentration in comparison with controls ($P = 0.049$; Figure 36B).

Table 8: Demographics

	Controls (*n*=21)	Players (*n*=20)	*P* Value
Clinical			
Age (years)	64 ± 7	64 ± 5	0.992
Systolic Blood Pressure (mmHg)	147 ± 15	148 ± 17	0.186
Diastolic Blood Pressure (mmHg)	84 ± 8	87 ± 6	0.424
Mean arterial pressure (mmHg)	105 ± 10	107 ± 9	0.904
Haemoglobin (g/dL)	15.2 ± 1.5	14.6 ± 1.3	0.548
Haematocrit (%)	48 ± 4	47 ± 3	0.274
Anthropometrics			
Stature (cm)	174 ± 7	176 ± 9	0.324
Mass (kg)	84 ± 20	89 ± 14	0.327
BMI (kg/m^2)	28 ± 5	29 ± 4	0.395
Body Fat (%)	24 ± 9	24 ± 6	0.989
Education			
Total education (years)	16 ± 3	17 ± 3	0.303
Contact/activity			
Concussions (*n*)	0 ± 0	3 ± 3†	0.000
Rugby career (years)	0 ± 0	22 ± 7†	0.000
Total physical activity (min/week)	377 ± 293	380 ± 132	0.565
Resistance training (min/week)	58 ± 70	90 ± 68	0.090
Aerobic physical activity (min/week)	320 ± 253	290 ± 95	0.917

Values presented as mean ± SD; BMI, body mass index; †, P <0.050 *vs.* controls.

Table 9: Cardiopulmonary responses to functional diagnostic exercise testing

	Controls (*n*=21)	Players (*n*=20)	*P* Value
$\dot{V}O_{2MAX}$ (mL/kg/min)	27 ± 10	27 ± 6	0.927
$\dot{V}O_{2MAX}$ (L/min)	2.07 ± 0.66	2.36 ± 0.51	0.133
$\dot{V}CO_2$ (L/min)	2.31 ± 0.85	2.68 ± 0.79	0.156
$\dot{V}_E$ (L/min)	68.3 ± 26.1	73.5 ± 21.1	0.489
$\dot{V}_E$ / $\dot{V}O_2$	31.7 ± 6.4	32.9 ± 4.7	0.485
$\dot{V}_E$ / $\dot{V}CO_2$	34.1 ± 2.9	36.4 ± 3.8†	0.039
Respiratory exchange ratio	1.10 ± 0.15	1.10 ± 0.14	0.951
Maximum heart rate (bpm)	126 ± 28	133 ± 16	0.256
Maximum power (Watts)	174 ± 54	193 ± 44	0.220

Values presented as mean ± SD; $\dot{V}O_{2MAX}$, maximal oxygen consumption; $\dot{V}CO_2$, carbon dioxide consumption; $\dot{V}e$, ventilation; $\dot{V}e$ / $\dot{V}O_2$ and $\dot{V}e$ / $\dot{V}CO_2$, ventilatory equivalent of oxygen and carbon dioxide consumption respectively; †, P <0.05 *vs.* controls.

Table 10: Sports Concussion Assessment

Parameter	Controls (*n*=21)	Players (*n*=20)	*P* Value
Total concussion symptoms (*n*)	3 ± 3	7 ± 6†	0.007
Concussion severity score (*n*)	4 ± 7	16 ± 13†	0.000
Orientation (*n*)	5 ± 0	5 ± 0	0.138
Immediate recall (*n*)	17 ± 3	18 ± 4	0.545
Concentration (*n*)	4 ± 1	4 ± 1	0.359
Balance Errors (*n*)	8 ± 5	9 ± 5	0.453
Delayed Recall (*n*)	2 ± 2	3 ± 2	0.382

Values presented as mean ± SD; †, *P* <0.05 *vs.* controls.

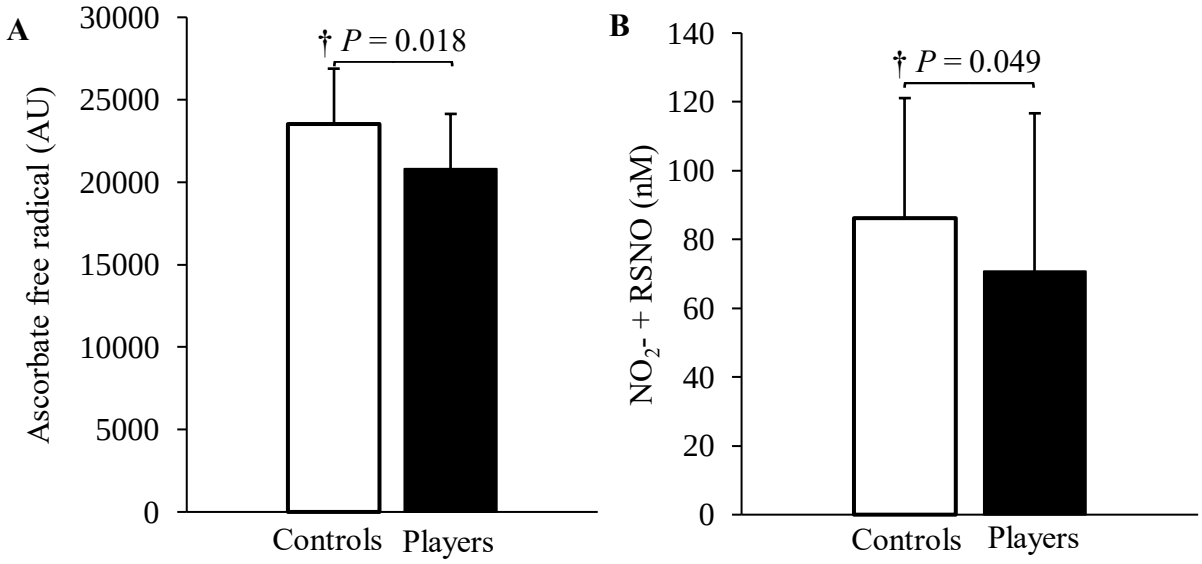

Figure 36: Ascorbate free radical concentration (**A**) and total bioactive nitric oxide concentration at rest (**B**); NO_2^-, nitrite; RSNO, S-nitrosothiols. Values presented as mean ± SD; controls (*n* = 21); players (*n* = 20); †, *P* <0.05 *vs.* control.

Cerebrovascular reactivity

At rest and throughout hypercapnia (Table 11), retired players presented with decreased MCAv (*P* = 0.004), SMCAv (*P* = 0.002), DMCAv (*P* = 0.029) and CDO_2 (*P* = 0.001). Players further presented with higher CVRi (*P* = 0.046), while the change in TPR was lower in comparison to the control group (*P* = 0.028). Throughout hypocapnia (Table 12), no differences in MCAv were apparent between-groups (*P* = 0.080), however retired players had lower SMCAv (*P* = 0.020) and CDO_2 relative to controls (*P* = 0.021). The absolute change in MCAv (*P* = 0.022;

Figure 37A) and CDO_2 (P = 0.013; Figure 37B) in response to hypocapnia was lower in retired players relative to controls. However, respective changes in cortical oxygenation from rest to hypercapnia (Figure 39A) and hypocapnia (Figure 39B) revealed no differences in O_2Hb, HHb and tHb between the retired players and controls (P > 0.05). When accounting for the respective changes in PET_{CO2}, no differences were identified for $CVR_{CO2HYPER}$ (P = 0.917), $CVR_{CO2HYPO}$ (P = 0.072; Figure 38A) or $CVR_{CO2RANGE}$ (P = 0.155; Figure 38B).

Exercise stress test

As exercise intensity increased throughout the exercise stress test, MCAv (Figure 40A) and PET_{CO2} (Figure 40B) gradually increased up to 80% of $\dot{V}O_{2MAX}$. MCAv and PET_{CO2} declined between 80-100% of $\dot{V}O_{2MAX}$ and throughout recovery. No between group differences were identified for MCAv and PET_{CO2} throughout the exercise stress test (P > 0.05, Figure 40).

Table 11: Cerebrovascular and cardiovascular responses to hypercapnia.

Group		**Controls (*n*=21)**			**Players (*n*=20)**			***P* Value**	
Condition	**Baseline**	**Hypercapnia**	**Δ**	**Baseline**	**Hypercapnia**	**Δ**	**Condition**	**Group**	**Interaction**
Cerebrovascular									
MCAv (cm/s)	51 ± 7	69 ± 15	17 ± 12	45 ± 9	57 ± 10	12 ± 7	0.000	0.004	0.076
SMCAv (cm/s)	85 ± 10	109 ± 22	24 ± 16	73 ± 14	87 ± 27	14 ± 22	0.000	0.002	0.104
DMCAv (cm/s)	32 ± 6	44 ± 11	12 ± 7	29 ± 7	37 ± 8	9 ± 5	0.000	0.029	0.107
CVRi (mmHg/cm/s)	1.87 ± 0.38	1.52 ± 0.33	-0.34 ± 0.20	2.18 ± 0.56	1.82 ± 0.64	-0.36 ± 0.32	0.000	0.044	0.879
CVCi (cm/s/mmHg)	0.55 ± 0.12	0.67 ± 0.18	0.12 ± 0.10	0.48 ± 0.16	0.67 ± 0.33	0.18 ± 0.23	0.000	0.560	0.251
PI (AU)	1.05 ± 0.23	0.98 ± 0.20	-0.08 ± 0.13	1.00 ± 0.16	0.97 ± 0.17	-0.04 ± 0.08	0.002	0.631	0.250
CDO_2 (mL/cm/s)	1048 ± 170	1400 ± 283	352 ± 212	878 ± 184	1118 ± 242	243 ± 144	0.000	0.001	0.056
Cardiovascular									
Heart Rate (bpm)	65 ± 16	63 ± 12	-3 ± 9	60 ± 9	60 ± 8	1 ± 3	0.399	0.278	0.100
MAP (mmHg)	92 ± 13	102 ± 17	10 ± 13	94 ± 19	99 ± 23	4 ± 9	0.000	0.905	0.135
TPR (mmHg/L/min)	15.35 ± 4.19	17.12 ± 4.21*	1.77 ± 2.81	16.34 ± 4.15	16.52 ± 4.34	0.19 ± 1.83†	0.012	0.879	0.039
PET_{CO2} (mmHg)	42 ± 3	53 ± 3	11 ± 4	42 ± 4	52 ± 5	10 ± 2	0.000	0.901	0.135

Values presented as mean ± SD; MCAv, middle cerebral artery velocity; SMCAv, systolic middle cerebral artery velocity; DMCAv, diastolic middle cerebral artery velocity; CVRi, cerebrovascular resistance index; CVCi, cerebrovascular conductance index; PI, pulsatility index; CDO_2, cerebral oxygen content; MAP, mean arterial pressure; TPR, total peripheral resistance; PET_{CO2}, end tidal carbon dioxide; †, $P < 0.05$ *vs.* controls; *, $P < 0.05$ within groups.

Table 12: Cerebrovascular and cardiovascular responses to hypocapnia

Group	**Controls (*n*=21)**			**Players (*n*=20)**			***P* Value**		
Condition	**Baseline**	**Hypocapnia**	**%Δ**	**Baseline**	**Hypocapnia**	**%Δ**	**Condition**	**Group**	**Interaction**
Cerebrovascular									
MCAv (cm/s)	49 ± 10	33 ± 7*	-15 ± 6	41 ± 8†	29 ± 7*	-11 ± 5	0.000	0.016	0.022
SMCAv (cm/s)	81 ± 15	63 ± 10*	-18 ± 9	67 ± 12†	55 ± 11†*	-12 ± 8	0.000	0.004	0.022
DMCAv (cm/s)	30 ± 8	19 ± 5	-11 ± 5	25 ± 6	16 ± 6	-9 ± 5	0.000	0.059	0.179
CVRi (mmHg/cm/s)	2.02 ± 0.47	2.69 ± 0.65	0.67 ± 0.37	2.28 ± 0.75	3.03 ± 1.17	0.75 ± 0.84	0.000	0.190	0.707
CVCi (cm/s/mmHg)	0.51 ± 0.13	0.40 ± 0.09	-0.11 ± 0.08	0.49 ± 0.21	0.38 ± 0.14	-0.12 ± 0.22	0.000	0.537	0.882
PI (AU)	1.08 ± 0.25	1.36 ± 0.30	0.28 ± 0.20	1.05 ± 0.17	1.37 ± 0.45	0.32 ± 0.37	0.000	0.854	0.648
CDO_2 (mL/cm/s)	992 ± 197	680 ± 133*	-312 ± 125	797 ± 157†	577 ± 139†*	-222 ± 90†	0.000	0.003	0.009
Cardiovascular									
Heart Rate (bpm)	66 ± 15	69 ± 16	4 ± 7	62 ± 10	68 ± 12	6 ± 6	0.000	0.502	0.262
MAP (mmHg)	94 ± 15	87 ± 14	-8 ± 6	90 ± 24	84 ± 20	-6 ± 20	0.004	0.515	0.717
TPR (mmHg/L/min)	15.33 ± 4.21	13.08 ± 3.65	-2.25 ± 1.52	15.57 ± 3.92	12.33 ± 3.45	-3.24 ± 2.04	0.000	0.829	0.087
PET_{CO2} (mmHg)	41 ± 3	29 ± 5	-12 ± 5	39 ± 6	28 ± 5	-10 ± 8	0.000	0.210	0.451

Values presented as mean ± SD; MCAv, middle cerebral artery velocity; SMCAv, systolic middle cerebral artery velocity; DMCAv, diastolic middle cerebral artery velocity; CVRi, cerebrovascular resistance index; CVCi, cerebrovascular conductance index; PI, pulsatility index; CDO_2, cerebral oxygen content; MAP, mean arterial pressure; TPR, total peripheral resistance; PET_{CO2}, end tidal carbon dioxide; †, P <0.05 *vs.* controls; *, P < 0.05 within groups.

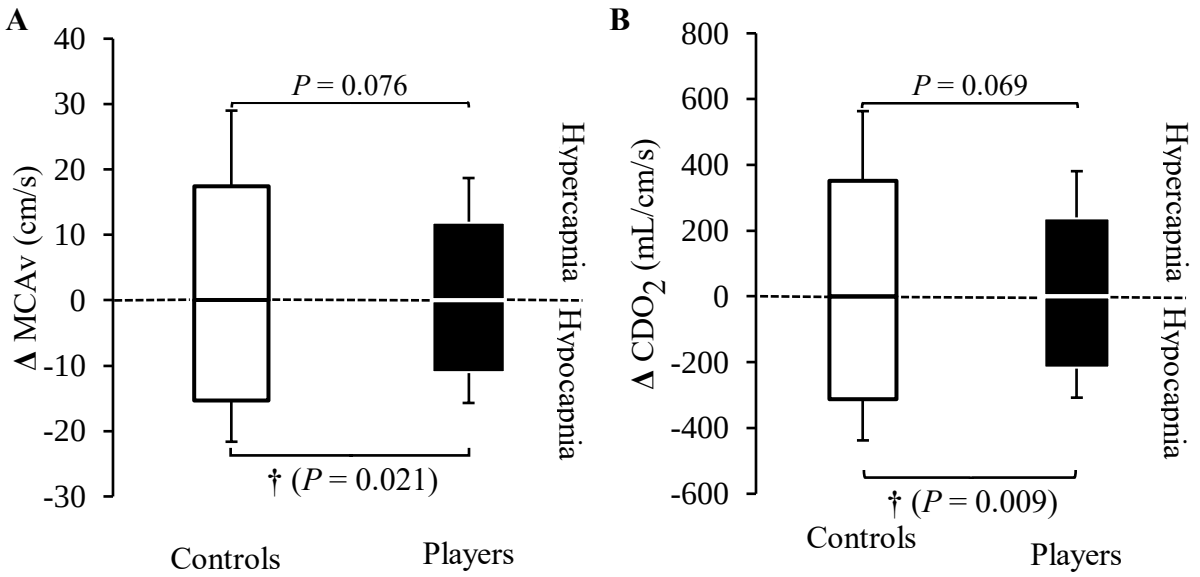

Figure 37: Change in middle cerebral artery velocity **(A)** and cerebral oxygen delivery (**B**) from rest to hypercapnia and hypocapnia respectively. Values presented as mean ± SD; controls (n = 21); players (n = 20); †, $P < 0.05$ *vs.* controls.

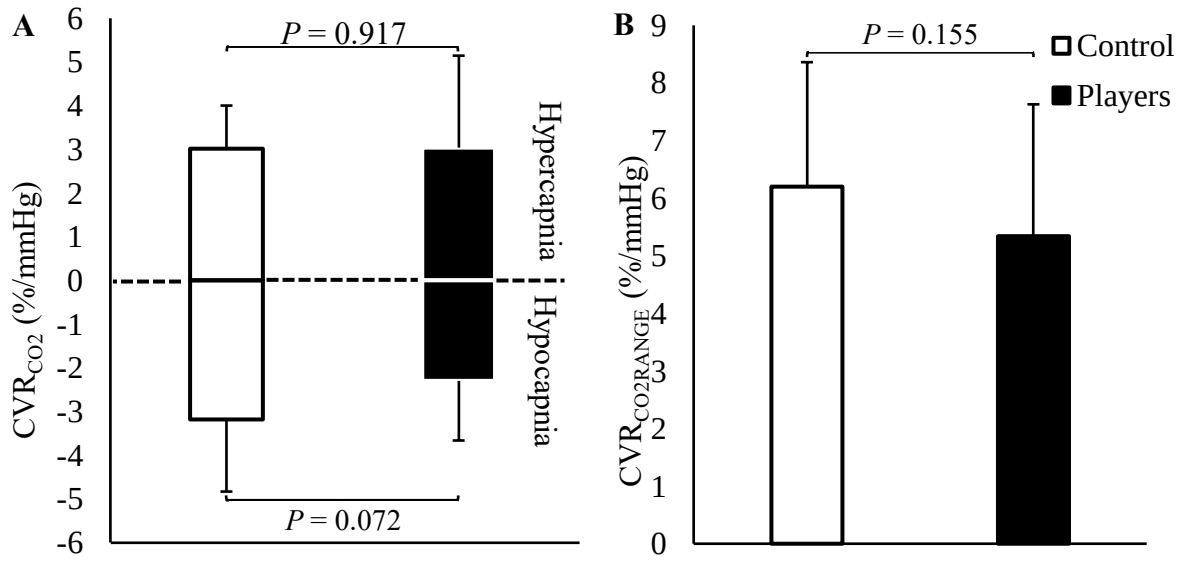

Figure 38: CVR_{CO2} in response to hypercapnia, hypocapnia (**A)** and $CVR_{CO2RANGE}$ (**B**). CVR_{CO2}; Cerebrovascular reactivity to changes in end-tidal carbon dioxide. Values presented as mean ± SD; controls (n = 21); players (n = 20).

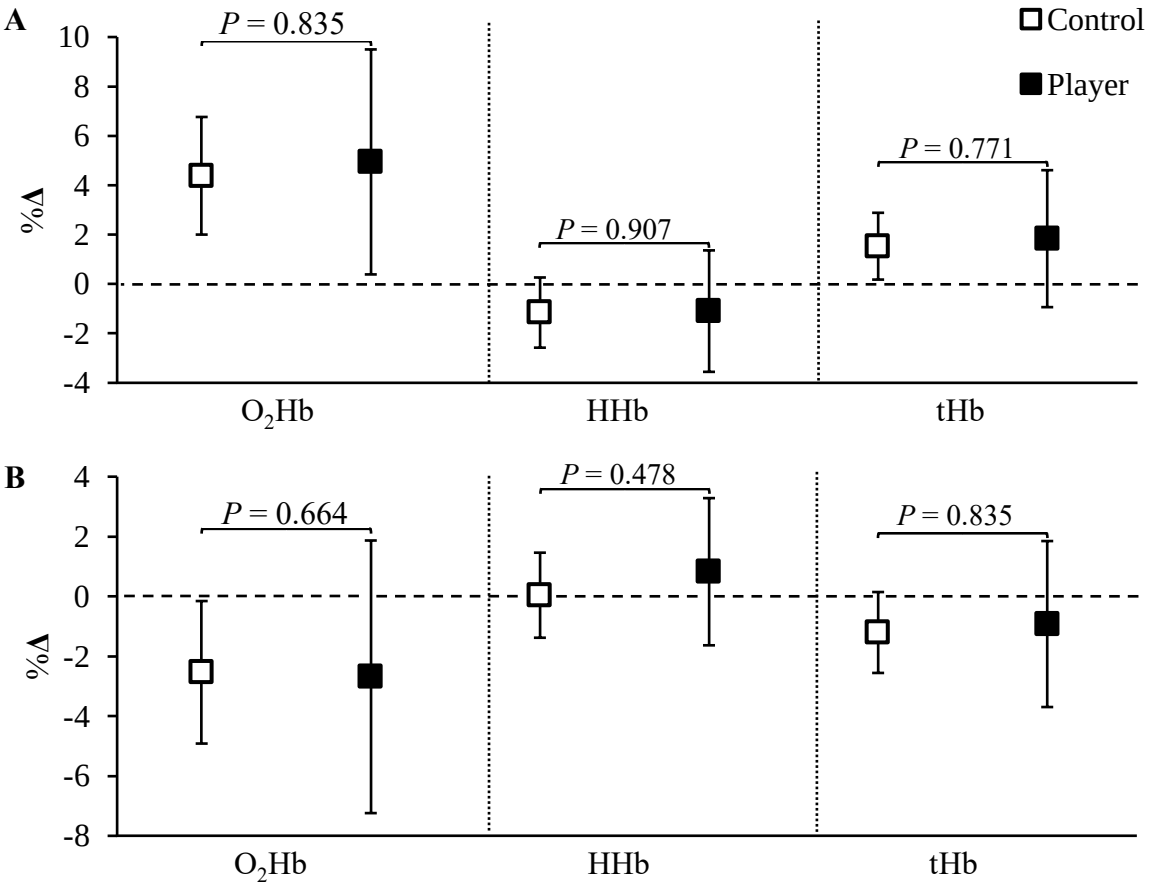

Figure 39: Cortical oxygenation from rest to hypercapnia (**A**) and hypocapnia (**B**) respectively. O_2Hb, oxyhaemoglobin; HHb, deoxyhaemoglobin; tHb, total oxyhaemoglobin. Values presented as mean ± SD; controls (n = 21); players (n = 20).

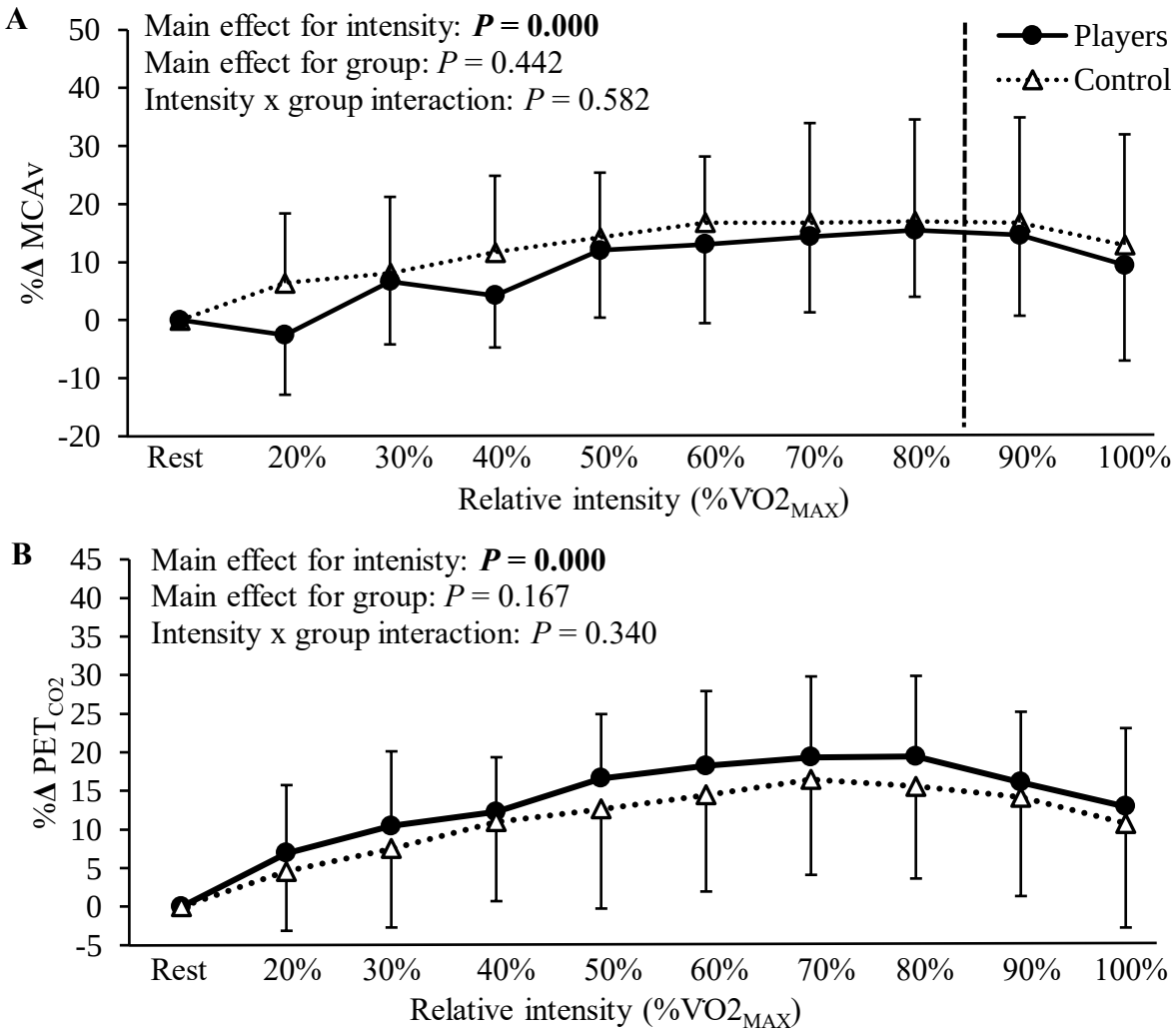

Figure 40: Changes to middle cerebral artery velocity (MCAv, **A**) and end-tidal carbon dioxide (PET_{CO2}, **B**) during relative exercise intensities to maximum oxygen consumption ($\dot{V}O_{2MAX}$) and recovery. Values presented as mean ± SD; controls (*n* = 21); players (*n* = 20).

Cognition

The retired players presented with impaired fine motor coordination using the non-dominant hand and executive function as indicated by slower completion of the GPND trail (*P* = 0.021). Scores indicative of MCI were apparent following completion of the MoCA among retired players in comparison with normal scores of the controls (*P* = 0.020; Table 13). No further between-group differences were apparent in the remaining assessments.

Table 13: Cognition

Assessment	Controls (*n*=21)	Players (*n*=20)	*P* Value
MCI screening			
Montreal Cognitive Assessment (*n*)	26 ± 2	24 ± 3†	0.020
Visuomotor coordination			
Grooved Pegboard Dominant Hand (s)	77 ± 21	78 ± 13	0.230
Grooved Pegboard Non-Dominant Hand (s)	79 ± 15	92 ± 18†	0.021
Learning and memory			
Rey Auditory Verbal Learning Test A1:A5 (*n*)	46 ± 9	43 ± 11	0.583
Rey Auditory Verbal Learning Test B1 (*n*)	6 ± 2	5 ± 1	0.220
Rey Auditory Verbal Learning Test A6 (*n*)	8 ± 3	8 ± 4	0.689
Rey Auditory Verbal Learning Test A6-A5 (*n*)	-3 ± 2	-2 ± 2	0.970
Working memory			
Trail Making B (s)	69 ± 25	71 ± 23	0.696
Repetition of Digits Backward (*n*)	7 ± 3	6 ± 2	0.116
Attention / Information processing			
Repetition of Digits Forward (*n*)	8 ± 3	8 ± 2	0.769
Trail Making Test A (s)	33 ± 11	32 ± 9	0.865
Digit-Symbol Substitution Test (*n*)	51 ± 14	47 ± 9	0.315

Values presented as mean ± SD; †, $P < 0.05$ *vs.* controls.

5.4. Discussion

This study combined molecular (blood-borne), cerebral haemodynamic (blood flow) and clinical (cognitive) metrics to identify that retired rugby union players exposed to recurrent contact over an average playing career of 22 years with three previous concussions were characterised by a systemic reduction in NO bioactivity, as well as reductions in cerebral perfusion, impaired fine-motor coordination using the non-dominant hand and scores indicative of MCI using the MoCA. Contrary to our original hypothesis, the reduction in NO bioactivity was observed alongside a reduction in systemic free radical formation compared to controls that may reflect a functional deficiency in the hormetic benefits of free radicals given their emerging role as signalling transductants. Nonetheless, alterations to the molecular, cerebral haemodynamic and cognitive profiles of formerly concussed retired players are clearly apparent and may serve as useful biomarkers that could help direct player management and care.

Molecular

Following retirement from sport, an apparent uncoupling between OXNOS was apparent since retired players presented with decreased $A^{\bullet-}$ and total NO bioactivity compared to controls. Therefore, lower NO bioactivity in retired players cannot be ascribed to oxidative scavenging as hypothesised. While it is accepted that contact promotes oxidative stress via mitochondrial ROS formation 1-5 days after injury (Vagnozzi et al., 2007, Giza and Hovda, 2014), mitochondrial adaptation to ROS years after recurrent contact has not been explored. Retired players confirmed an average playing career of 22 years, where we anticipated that a lifetime's exposure to recurrent contact may have promoted an oxidative stress ceiling (discussed in Chapter 4). Similarly, others have argued that the mitochondrial theory of ageing would maintain elevated systemic oxidative stress with advancing age, whereby mutations to mitochondrial DNA promote oxidative phosphorylation and overproduction of ROS (Lenaz, 1998, Luo et al., 2020). As a decline in systemic oxidative stress was observed among players, the decline in ascorbate concentration would provide less bioavailable substrate for global oxidation of ROS (Fall et al., 2018). Research is conflicting as to whether ascorbate/vitamin C bioavailability declines throughout ageing (Dickinson et al., 1994, Hampl et al., 2004) and alternative mechanisms may be accountable for the decrease in ascorbate free radical concentration observed in retired players. Such mechanisms include lower ascorbic acid oxidation ratio in players (Lykkesfeldt and Moos, 2005), thereby reducing the plasma concentration of $A^{\bullet-}$. Other potential mechanisms include whether structural damage to

neuronal networks following concussion can promote apoptosis (Ellis et al., 2016). Subsequently, the metabolic demands of the brain are reduced and decrease mitochondrial ROS formation (Giza and Hovda, 2014). Others proffer that mitochondrial hormesis may confer sustained reductions in oxidative stress over time, whereby the initial increase in ROS promote the activation of apoptosis inhibitor survivin, and limit ROS mediated neuronal damage (Zhang et al., 2007, Ristow and Zarse, 2010). To what extent that prolonged reductions in oxidative stress arise as an adaptive mechanism to recurrent contact among retired players with concussion history remain to be established.

Despite unexpectedly lower free radical formation in the retired players, chronic reductions in circulating NO bioactivity prevailed. Several potential explanations can be presented in the form of natural ageing and low consumption of dietary nitrate (Cau et al., 2012). However, given that both groups were prospectively matched for such characteristics, the propagation of other mechanisms are plausible. Following injury, prolonged post-traumatic lactate accumulation occurs (Tsacopoulos and Magistretti, 1996). This unfavourable state can induce acidosis which is capable of promoting neuronal dysfunction, by transporting excess lactate from glia to neurons (Giza and Hovda, 2014, Liu et al., 2017). As a counter mechanism, acidosis promotes the pH dependent reduction of nitrite to NO in a bid to maintain vascular function (Zweier et al., 2010). This generates further implications given that a decrease in bioactive NO reserve can cause endothelial dysfunction (Rassaf et al., 2006).

Cerebral haemodynamic function

Consistent with our hypothesis, retired players were characterised by suppressed cerebral haemodynamic function, in the form of lower perfusion and CDO_2 at rest and in response to hyper/hypocapnia. These observations were independent of differences in CRF given that both groups were matched at baseline for any potential experimental confounders. Therefore, recurrent contact and concussion history further contributed to a decline in resting CBF and concurs with previous findings in retired contact sport athletes (Hart et al., 2013). Likewise, cerebral hypoperfusion and prolonged symptomology have previously been observed (Meier et al., 2015), to which we identified increased symptom severity among the retired players using the SCAT5. While we did not measure the extent of structural damage via neuroimaging, Grossman et al. (2013) has demonstrated that concussion promotes neuronal apoptosis via shear stress. Under these circumstances, nutrient delivery in the vascular bed could diminish locally and globally, given that an uncoupling between the perfused blood vessels and

functionally impaired neurons would occur by distributing blood flow in favour of uncompromised vascular beds (Grossman et al., 2013, Ellis et al., 2016).

Vasoreactivity to CO_2

Despite marked reductions to resting cerebral perfusion, no differences in CVR_{CO2} were identified when comparing retired players and controls. Previously, Bailey et al. (2013a) and Brugniaux et al. (2014) have demonstrated the importance of observing CVR_{CO2} in young and ageing adults to uncover subtle cerebrovascular differences. In response to $CVR_{CO2HYPER}$, no differences were identified between retired players and controls. During $CVR_{CO2HYPO}$, no differences were identified between retired players and controls. However, when absolute changes in MCAv and CDO_2 were observed between groups, clear reductions were identified among retired players.

In addition to NO mediation, Bailey et al. (2013b) has speculated that the CVR_{CO2} response to hypocapnia is governed by unidentified molecular mechanisms. Hypocapnia contributes to respiratory alkalosis by increasing the ratio of bicarbonate to $PaCO_2$ concentration (Battisti-Charbonney et al., 2011). In smooth muscle, potassium channels are inhibited and allow intracellular calcium accumulation, which has been attributed to greater exercise capacity (Bettice et al., 1984). If this process were to occur in the cerebral vasculature, it may be detrimental since the neurometabolic cascade following concussion can create severe ionic imbalances, whereby elevations in intracellular calcium contribute to mitochondrial dysfunction (Giza and Hovda, 2001, Vagnozzi et al., 2007, Giza and Hovda, 2014).

Cognition

Retired players presented with scores indicative of MCI using the MoCA, including reductions in executive function and fine-motor coordination of the non-dominant hand compared to controls. Collectively, prior recurrent contact and concussion history ‘accelerates’ the decline in cognition observed during ‘normal’ ageing with changes independent of inactivity-induced degeneration, given that both groups were matched for CRF. Neurodegenerative diseases are often preceded by MCI, where 10-20% of MCI patients’ transition towards neurodegenerative diagnosis annually in comparison with 1-2% of non-MCI patients (Tierney et al., 1996, Meyer et al., 2002). While structural damage, molecular alterations and suppressed cerebral haemodynamic function are likely contributors, the mechanisms are not fully understood and require further research (McMillan et al., 2017).

Cerebral hypoperfusion and structural damage pose significant risks to the cerebral vasculature, given the brain has high metabolic turnover and is heavily reliant on sustained O_2 and glucose delivery. Cerebral hypoperfusion has been associated with cognitive impairment and increased susceptibility of neurodegeneration (Bailey et al., 2019, Wolters et al., 2017). Indeed, retired players were characterised by diminished global CBF relative to controls, however it is important to consider that regional hypoperfusion may prevail. For instance, brain regions supplied via the MCA have been characterised by hypoperfusion following concussion, namely the dorsal mindinsular cortex, right superior temporal sulcus and regions of the frontal lobe (Meier et al., 2015, Uddin et al., 2017, Churchill et al., 2017a). While concussion incidence between the 'amateur' and professional eras of rugby are largely different (Gardner et al., 2014b, Owens et al., 2019), tackles remain the primary cause of concussion, particularly when involving head-to-head collisions (Cross et al., 2017, Owens et al., 2019). Moreover, these match events increase the risk of damage to both the frontal and temporal lobes given their anatomical position within the skull (Pearce et al., 2018, Tarazi et al., 2018). Neuroimaging studies of concussed athletes have demonstrated structural and functional deficits in these brain regions (Grossman et al., 2013, Meier et al., 2015, Churchill et al., 2020). Thus it is not surprising that we observed MCI, impaired fine-motor coordination and executive dysfunction in retired players, given that these cognitive domains are governed by the dorsolateral prefrontal cortex, anterior cingulate cortex and orbitofrontal cortex of the frontal lobe (Miller and Cohen, 2001).

Conclusion

The age-related decline in cognition and onset of MCI occurs earlier in retired rugby players exposed to recurrent contact and concussion compared to non-concussed controls. Molecular and cerebral haemodynamic impairments were observed among players, which are known risk factors for the development of neurodegenerative diseases. Subsequently, targeted interventions to offset the rate of accelerated cognitive impairment are warranted. Similar observations should be implemented in current players given that molecular and cerebral hemodynamic profiling may serve as useful biomarkers to manage player welfare.

Chapter 6
General discussion

6.1. Overview

Recurrent contact and concussion history can increase the risk of neurodegenerative disease for which there are currently no cures (Gardner et al., 2014a, Nichols et al., 2019). Post-mortem diagnoses of CTE in retired contact sport athletes continue to emerge, but remains relatively uncommon among rugby players despite comparative concussion incidence rates to other collision sports (Gardner et al., 2014a, Stewart et al., 2016). Neurodegeneration is often preceded by cognitive impairment due to recurrent contact, however the complex and likely multifactorial mechanisms remain contested. In a bid to understand the relationship between these mechanisms, this thesis provides novel insights into whether recurrent contact sustained over a career in rugby union accelerates the normal brain ageing response when compared to non-contact control groups.

6.2. Integration of findings and emerging concepts

The summarised findings for Study 1 and 2 are outlined in Figures 41-44 and Figure 46. As hypothesised, current professional rugby union players presented with increased OXNOS, suppressed cerebrovascular function, and cognition compared to controls. Contrary to our original hypothesis, the reduction in NO bioactivity in retired players was accompanied by a decrease as opposed to an increase in systemic free radical formation, that may reflect hormetic adaptation to persistently elevated oxidative stress incurred over decades. Nonetheless, decreased NO bioactivity and cerebral haemodynamic function prevailed in retired players, as well as scores indicative of MCI using the MoCA.

Molecular

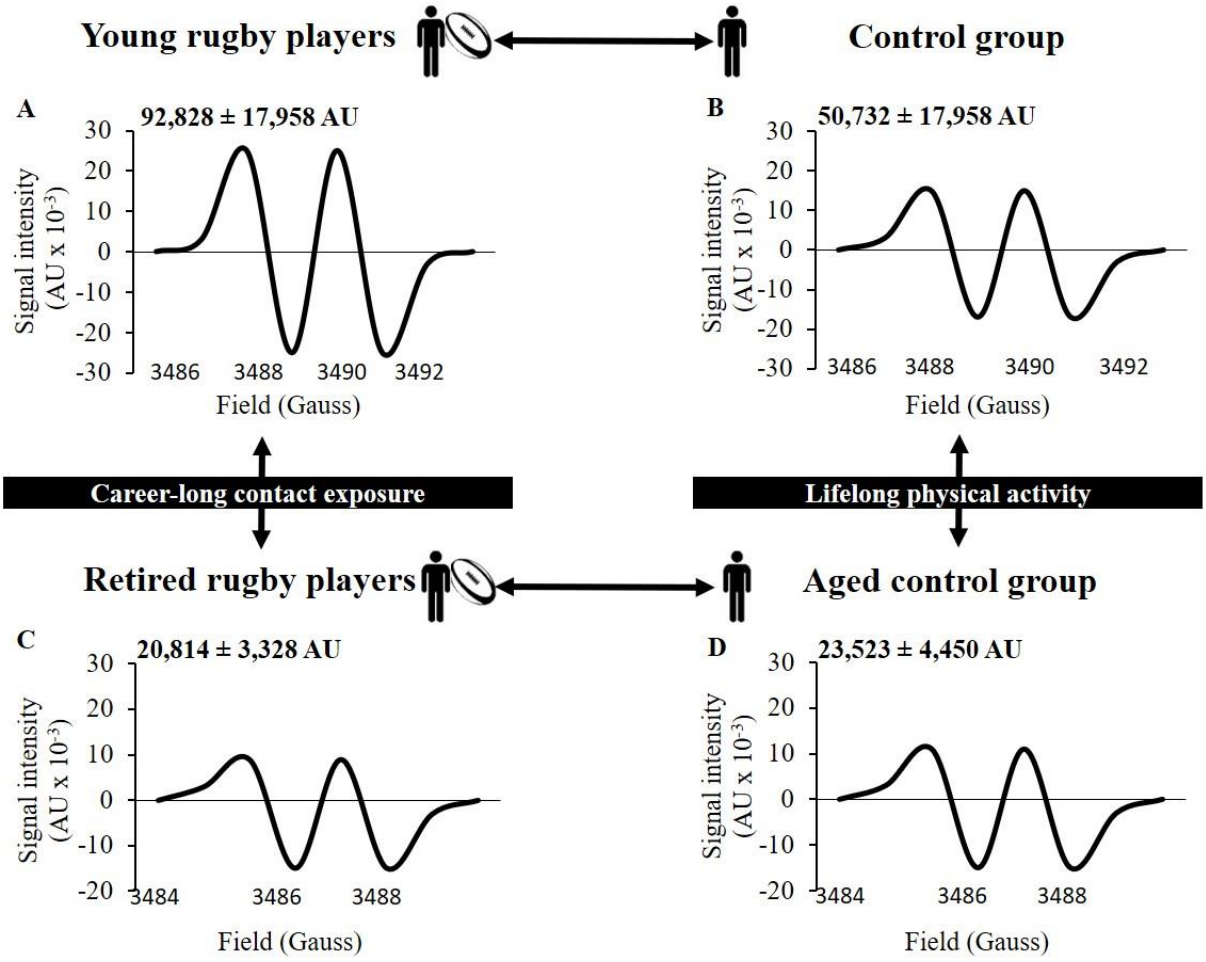

Figure 41: Comparison of typical electron paramagnetic resonance spectra of the ascorbate radical. Values presented as mean ± SD; young rugby players (*n* = 21); young control group (*n* = 17); retired rugby players (*n* = 20); aged control group (*n* = 21).

While it appeared that cumulative exposure to prior recurrent contact through an average of 16 years participation in rugby gradually inflicted a persistent molecular shift towards elevated OXNOS among young players (Figure 41A), post-season observations demonstrated evidence for increased nitrosative stress in the absence of elevated oxidative stress. Over the course of the competitive season, players did not exhibit an increase in systemic $A^{\bullet-}$.Given that a reduction in bioactive NO was observed over the season (Figure 41A), it appears that contact disturbed NO kinetics and Wada et al. (1998) suggests a potential mechanism via the suppression of eNOS activity. Following retirement from rugby, the uncoupling between OXNOS remained apparent. Retired players confirmed lower oxidative stress and total NO bioavailability compared to controls (Figure 41C and Figure 42C). When compared to young players, oxidative stress was 127% lower, while total NO bioavailability was 31% lower in retired players.

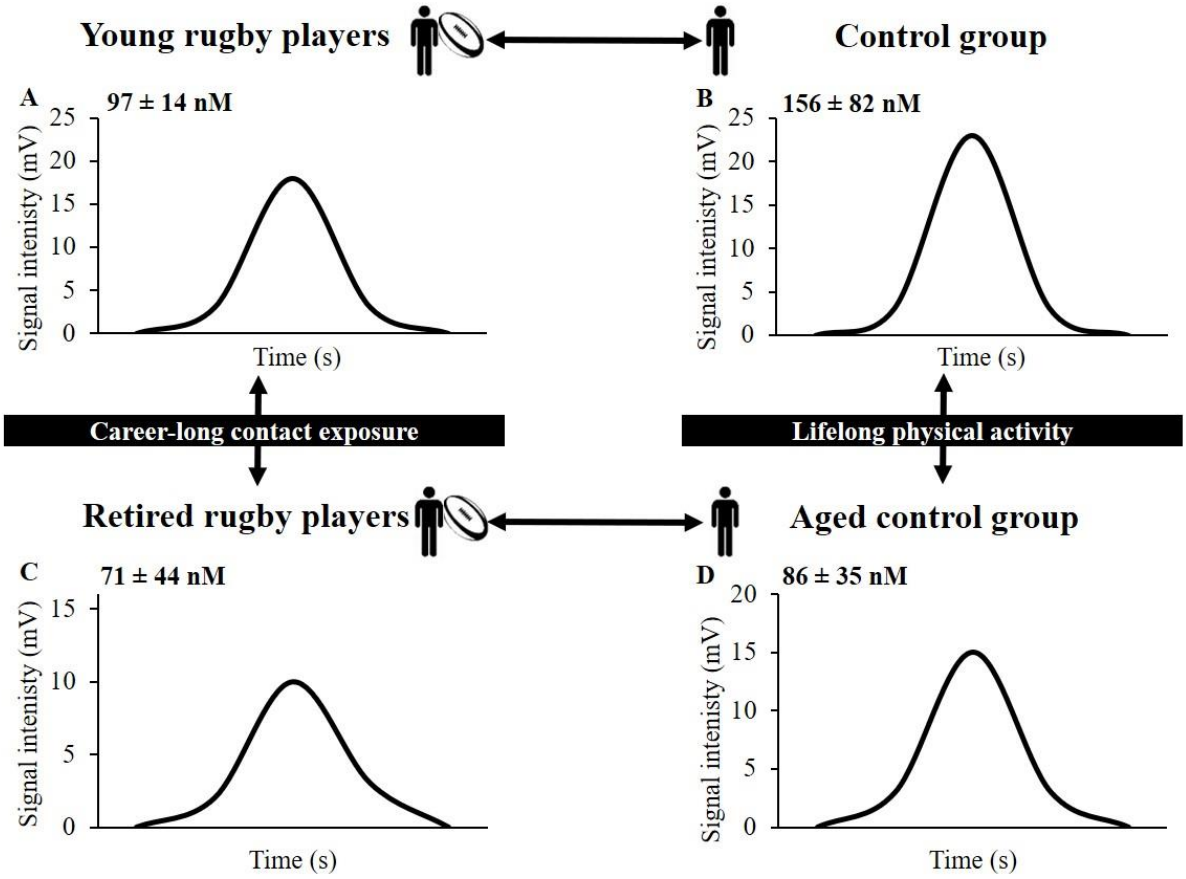

Figure 42: Comparison of typical ozone-based chemiluminescence spectra of bioactive (nitrite + S-nitrosothiols) nitric oxide metabolites. Values presented as mean ± SD; young rugby players (n = 21); young control group (n = 17); retired rugby players (n = 20); aged control group (n = 21).

It was anticipated that retired rugby players would present with elevated oxidative stress compared to controls and indeed, young players. Retired players confirmed an average playing career of 22 years, where a lifetime's exposure to recurrent contact may have promoted an oxidative stress ceiling as observed among young players. Following retirement and with increasing age, it was anticipated that elevated systemic oxidative stress would remain apparent via the mitochondrial theory of ageing, whereby mutations to mitochondrial DNA promote oxidative phosphorylation and overproduction of ROS (Lenaz, 1998, Luo et al., 2020). As a decline in systemic oxidative stress was observed, the decline in ascorbate concentration provided less bioavailable substrate for the global oxidation of ROS (Fall et al., 2018). Research is conflicting as to whether ascorbate/vitamin C bioavailability declines throughout ageing (Dickinson et al., 1994, Hampl et al., 2004) and alternative mechanisms may be accountable for the decrease in ascorbate free radical concentration observed in retired players.

Contact and trauma promote mitochondrial hypermetabolism within the cerebral vasculature, which generate increased ROS known to scavenge NO (Giza and Hovda, 2001, Bailey et al., 2019). This relationship has been coined elevated OXNOS, and has been observed alongside suppressed CVR and cognition (Bailey et al., 2013c, Meier et al., 2015, Churchill et al., 2017b, Bailey et al., 2019). Young players presented with elevated systemic OXNOS during pre-season, thereby conforming to our hypothesis. However, post-season observations revealed an apparent uncoupling of OXNOS, as NO declined across the season in the absence of elevated oxidative stress among young players. Similarities were apparent in the retired players, who presented with a 31% reduction in total bioactive NO compared to young players in the absence of elevated oxidative stress (Figure 41 and Figure 42).

Recurrent contact can disturb NO kinetics through suppression of eNOS activity required for the synthesis of NO (Wada et al., 1998). Therefore, exposure to recurrent contact over a career of rugby union may promote an insurmountable shift towards decreased NO bioavailability. Moreover, nitric oxide is considered a vital metabolite in the regulation of cerebral perfusion (Lavi et al., 2003), whereby reductions in NO can lead to hypoperfusion and suppressed CVR (Bailey et al., 2019). Both young and retired players presented with suppressed CDO_2, while retired players were further characterised by hypoperfusion at rest compared to controls. The reduction in perfusion and therefore O_2 would blunt the energy dependent conversion of citrulline into arginine required for NOS to produce NO (Cherian et al., 2000, Lajtha et al., 2007). Indeed, a 30 g supplement of L-arginine may improve CVR by up to 34% in patients with impaired vasomotor reactivity (Zimmermann and Haberl, 2003). Similar benefits have been observed using a 300 mg/kg infusion of L-arginine following cortical injury in rats, whereby CVR was partially restored through increased substrate availability for eNOS (Golding et al., 2000). While others have shown that incremental supplementation of L-arginine in diabetic patients may impair microvascular and executive function (Beckman et al., 2018), no studies have investigated how L-arginine supplementation may help combat molecular, cerebrovascular and cognitive function in athletes exposed to recurrent contact, thereby granting a potential treatment pathway for players of Studies 1 and 2.

Cerebral haemodynamic function

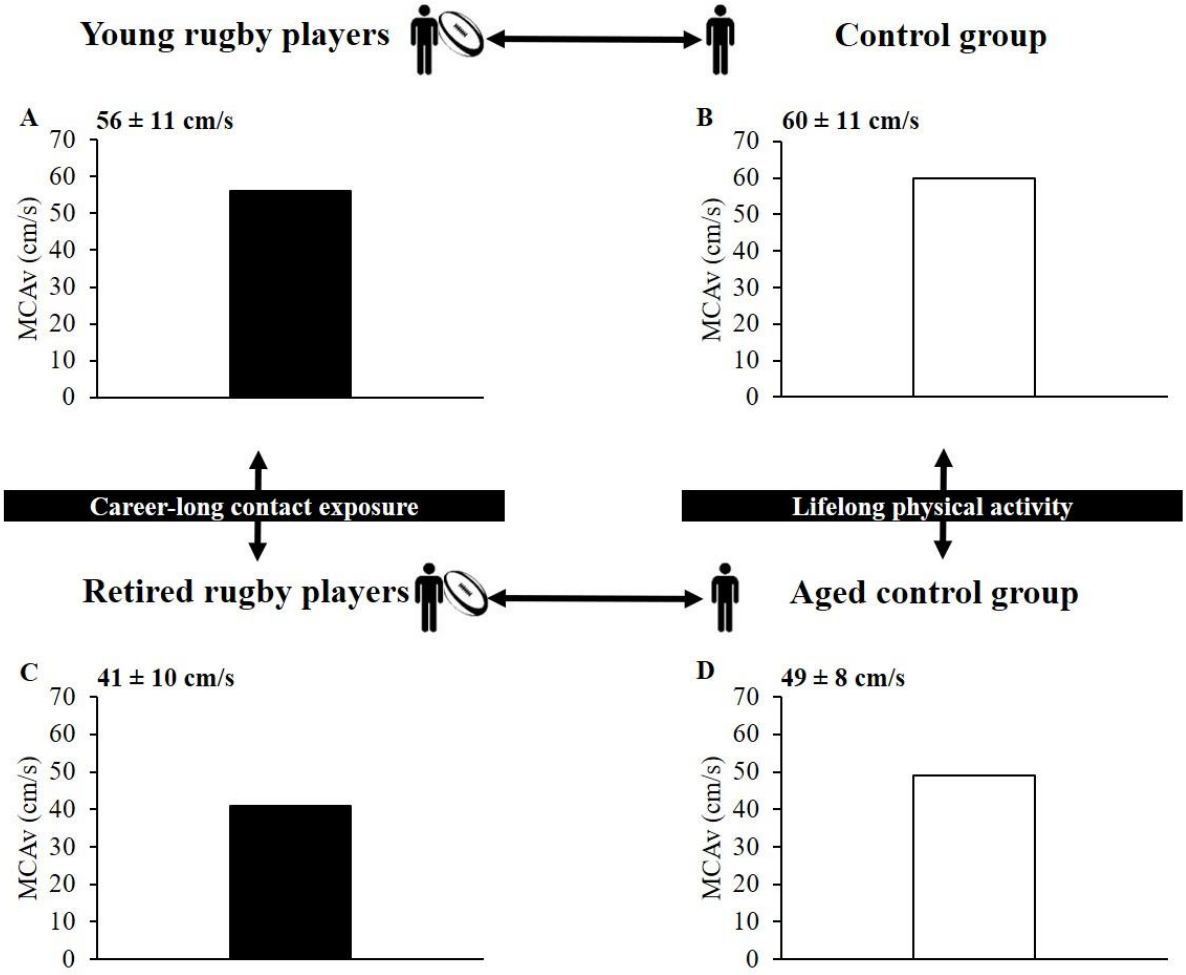

Figure 43: Comparison of resting cerebral perfusion. MCAv; middle cerebral artery blood flow velocity. Values presented as mean ± SD; young rugby players (*n* = 21); young control group (*n* = 17); retired rugby players (*n* = 20); aged control group (*n* = 21).

Both young and retired players presented with supressed cerebral haemodynamic function compared to their respective control groups. Resting CDO_2 decreased by 13% across the season in young players due to a combination of reduced cerebral perfusion, O_2 content, and Hb (Figure 32). Similarly, resting CBF was lower in retired players compared to the control group (Table 12). In an apparently healthy population, the age related decline in resting CBF is estimated at 25-30% using TCD ultrasonography (Ainslie et al., 2008). When comparing the young controls of Study 1 and the aged controls of Study 2, we observed a 26% reduction in CBF (Figure 43B and Figure 43 D). However, when we compared the control group of Study 1 and the retired players of Study 2, CBF had declined by 41% (Figure 43B and Figure 43C. While an accelerated decline in resting cerebral perfusion was apparent in retired players, perfusion remained within normative ranges (34-86 cm/s, Adams et al., 1992), highlighting

that the decline in CBF following exposure to prior recurrent contact does not necessarily bare 'clinical' significance. However, the physical activity status of retired players may have provided a degree of neuroprotection, given that they confirmed an average of 380 minutes of weekly physical activity comprised of both aerobic and resistance training. Bailey et al. (2013b) has observed that males aged ≥ 60 years who completed ≥150 minutes of recreational aerobic activity across the adult lifespan attenuated the age-related decline in MCAv compared to a sedentary control group. Despite the neuroprotective benefits afforded through a physically active lifestyle, retired players were not able to completely 'counter' the cumulative maladaptation to cerebral haemodynamic function incurred through repetitive contact across their career. Moreover, Calverley et al. (2020) noted that physical activity may disassociate the brain's 'biological' age from 'chronological' age by up to as much as a decade. Therefore, it is unknown whether sedentary, retired players not recruited as a part of Study 2 may already exhibit clinical reductions in CBF and CVR, while physically active players may be reliant on 'borrowed time' that partially protects them from further cerebral haemodynamic impairments.

The trajectory for future cerebral haemodynamic impairments among young players must also be considered, given that suppressed CVR was already apparent among players recruited in Study 1 (Figure 44A). Naturally, young players are highly physically active throughout their career, however retirement from professional sport (typically between 25-40 years old) often promotes lifestyle changes comprised of sedentary behaviour, poor diet, alcohol dependency and substance abuse (Guskiewicz et al., 2007a, McKee et al., 2009, Buckley et al., 2019), which correlate with increased risk of neurodegeneration in later-life (Topiwala et al., 2018). As physical activity status may have provided some neuroprotection among retired players of Study 2, current players should receive education programmes promoting the importance of physical activity following retirement from sport. Indeed, high intensity interval training (HIIT) may offer a low risk, yet time efficient insurance policy towards neuroprotection. Both Burley et al. (2016) and Calverley et al. (2020) have highlighted that increased endothelial shear stress during HIIT offers molecular, cerebral haemodynamic and structural benefits through increased expression of NO and trophic factors, which promote endothelial function,

neuroplasticity and motor function that can be tailored to individual needs using a number of recognised training interventions.

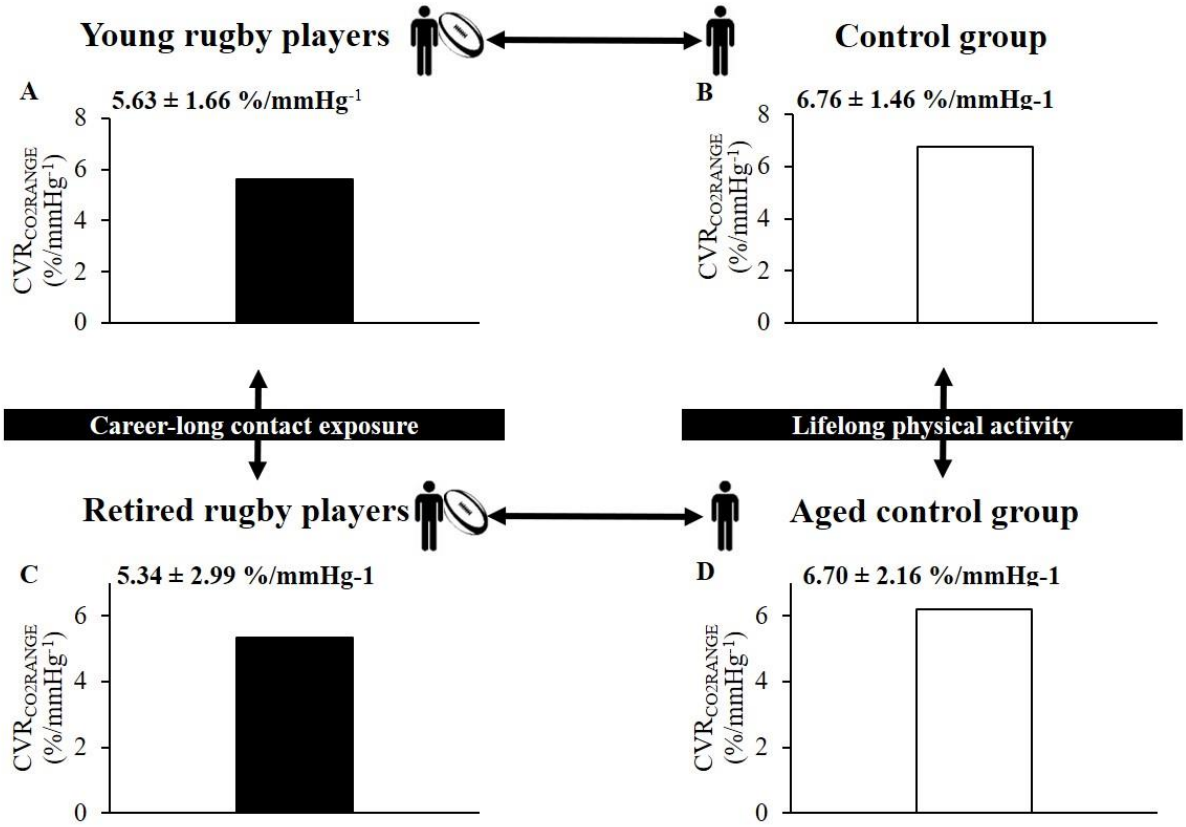

Figure 44: Cerebrovascular reactivity to changes in end-tidal CO_2 via hypercapnia and hypocapnia ($CVR_{CO2RANGE}$). Values presented as mean ± SD; young rugby players (n = 21); young control group (n = 17); retired rugby players (n = 20); aged control group (n = 21).

While cerebral haemodynamic function may appear normal at rest, subtle impairments may be uncovered when the brain is 'challenged' by acute exertional stress. For instance, Brugniaux et al. (2014) observed that MCAv between sedentary and physically active males was not different at rest, but when exposed to incremental exercise, more marked increases in MCAv were apparent when transitioning between low- to moderate exercise intensity in the physically active cohort. As the physically active cohort elicited greater cerebrovascular reactivity, differences in cerebral haemodynamic function would not have been detected had exercise not been used to 'force' the (impaired) signal out of the (biological background) noise. Indeed, controlled hyper/hypocapnia has been used to challenge and formally quantify cerebral vasoreactivity in concussed athletes (Bailey et al., 2013a, Gardner et al., 2015). We observed that $CVR_{CO2RANGE}$ was suppressed among young players (Figure 44). Retired players exhibited no reduction in $CVR_{CO2RANGE}$ compared to the control group (Figure 44) and moreover, $CVR_{CO2RANGE}$ was 13% higher among retired players (Figure 38B) compared to the young

players during post-season (Figure 33B). This was primarily due to elevated $CVR_{CO2HYPER.}$among retired players. While this was not anticipated, our results conform to the findings of Zhu et al. (2013), who observed lower resting CBF in aged participants , but greater hypercapnic vasoreactivity when compared to young control groups. As resting CBF in retired players was lower compared to young players, the CBF-PET_{CO2} curve undergoes a downward and rightward shift, which attenuates hypocapnic vasoreactivity, but enhances hypercapnic vasoreactivity.

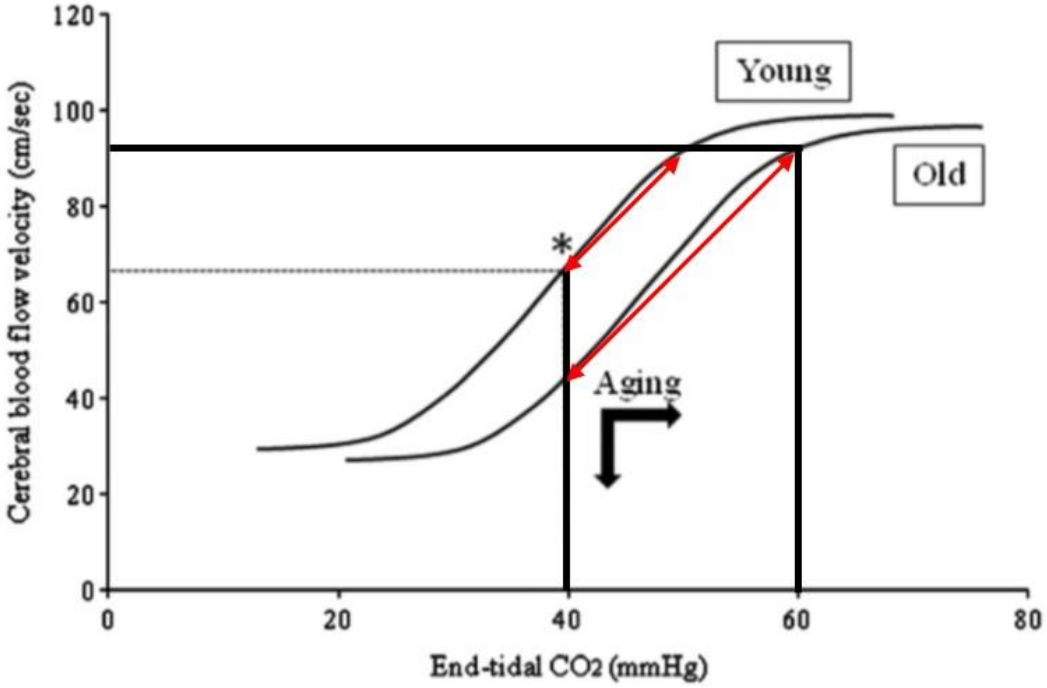

Figure 45: The cerebral blood flow-end tidal carbon dioxide curve of 10 young and 12 aged adults. Values presented as mean ± SD; *, normocapnic cerebral blood flow at rest. Adapted from Zhu et al. (2013).

When observing Figure 45, the Asterix represents resting CBF at a PET_{CO2} of ~40 mmHg for both young and old groups. Hypercapnia increases CBF by 24 cm/s in the young and begins to plateau at a PET_{CO2} of ~50 mmHg. Similarly, hypercapnia increases CBF in the old, however the plateau occurs when PET_{CO2} approaches ~60 mmHg and increases CBF by 52 cm/s, indicating enhanced hypercapnic vasoreactivity. It is therefore apparent that the downward and rightward shift in the CBF-PET_{CO2} curve helps retain cerebral vasoreactivity to hypercapnia. Nonetheless, young players are characterised by suppressed CVR, an albeit indirect reflection of impaired cerebrovascular endothelial function, while the accelerated decline in resting CBF among retired players may increase the risk for stroke (Aries et al., 2010), cognitive impairment (Kelley et al., 1992) and all-cause mortality (Portegies et al., 2014).

Cognition

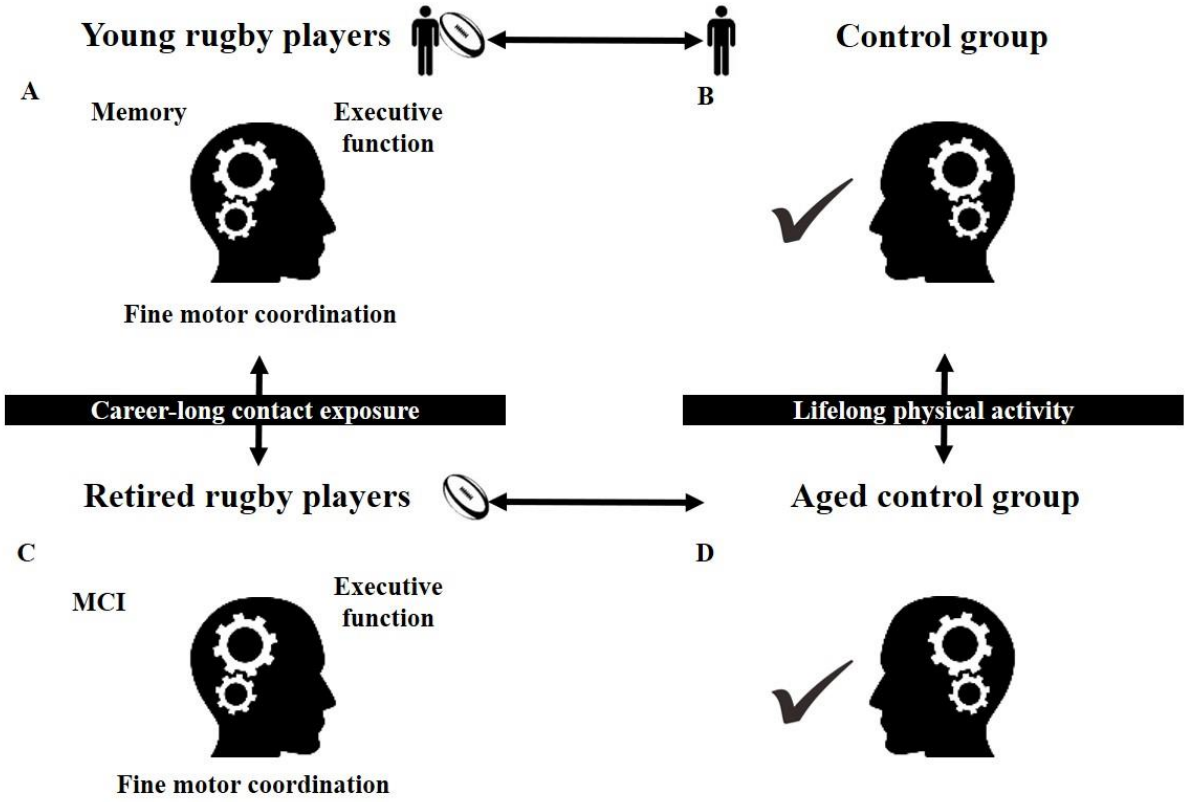

Figure 46: Comparison of observed cognitive impairments in Study 1 and 2. MCI; mild cognitive impairment. Values presented as observed outcome of cognitive assessments; young rugby players (*n* = 21); young control group (*n* = 17); retired rugby players (*n* = 20); aged control group (*n* = 21); ✓, no observed impairments in cognition.

Both young and retired players confirmed an average of three previous concussions over their careers, that may predispose them to a fivefold increased risk of MCI (Guskiewicz et al., 2005). Consistent with the literature (Gardner et al., 2010, Hume et al., 2017), reduced cognition was exhibited by both young and retired players as summarised in Figure 46. Decreased fine motor coordination and executive function were confirmed by slower completion times of the Grooved Pegboard Dexterity Test. Young players were observed with reductions in short-term memory confirmed by lower performance on the RAVLT. Indeed, an age-related decline in cognition was expected (Salthouse, 2009). However, during the post-season completion of the MoCA, young players scored identically to the aged controls of Study 2 (Table 5 and Table 13). On average, the aged controls were 38 years their senior, corresponding to a 2.5-fold increase in the rate of cognitive decline for young players in this assessment. Similarly, retired players presented with MCI compared to their respective control group. To what extent concussion history and impaired cognition across a players lifespan relates to

neurodegeneration remains to be established, however more objective methods of assessment are required to understand relationship.

Cognitive impairment has often been reported among contact sport athletes in the absence of complimentary molecular and haemodynamic measurements (Shuttleworth-Edwards et al., 2014, McMillan et al., 2017). Both young and retired players exhibited evidence of suppressed cerebral haemodynamic function and decreased systemic NO bioavailability. Young players were characterised by suppressed cerebrovascular reactivity, while the decline in resting cerebral perfusion was more marked in retired players. Although cerebral hypoperfusion and suppressed cerebral vasoreactivity are risk factors for cognitive impairment (Kelley et al., 1992, Wolters et al., 2017), the unknown structural consequences of prior exposure to recurrent contact in both young and retired players may also be attributable.

Regional hyperperfusion has been observed following immediate head trauma, causing a heightened neuroinflammatory response (Churchill et al., 2017b), impaired dynamic cerebral autoregulation (dCA, Moir et al., 2018) and increased blood brain barrier (BBB) permeability (Marchi et al., 2013, Marchi et al., 2003). However, regional hypoperfusion may occur in response to impact ~24 hours after injury to decrease neuronal integrity within the cerebral vasculature (Meier et al., 2015). The thalamus is prone to shear stress following head trauma and acts as a relay centre for sensory and motor nervous transmission (Schultz et al., 2018). The thalamus is also functionally connected to the hippocampus, which governs memory function (Stein et al., 2000). Structural damage and hypoperfusion in this brain region would support the findings of Study 1, given that young players were characterised by reduced memory and executive function (Schultz et al., 2018). Similarly, tackling repetitively exposes the frontotemporal region of the brain which partially governs fine-motor processes and is prone to regional hypoperfusion following concussion (Cross et al., 2017, Churchill et al., 2020).

It is important to consider cognitive reserve, a concept which describes the disjunction between the degree of brain damage and clinical impairments in cognition (Stern, 2009). Voss et al. (2011) observed that cognition remained relatively constant between 18-35 years and overall, cognition among the young players seemingly improved over the course of the season despite repetitive head impacts. While this was apparent, Johnson et al. (2012) demonstrated that young concussed athletes may retain cognitive capabilities despite reduced regional brain activity. As the underlying mechanisms are not fully understood, habituation to recurrent

contact cannot be ruled out, though any potential impairments may have been masked given the neuroprotective benefits conferred by high cardiorespiratory fitness (Bailey et al., 2013b). Therefore, the recruitment of a physically active, non-contact, non-concussed control group served as an important comparator as players were consistently outperformed by controls to demonstrate evidence for accelerated cognitive decline among young players.

While an age-related decline in cognition was anticipated from all participants of Study 2, retired players presented with MCI using the MoCA. Moreover, fine-motor coordination and executive function were most notably impaired, which correspond with previous findings among retired athletes (McMillan et al., 2017, Hume et al., 2017, Pearce et al., 2018). Indeed, retired players presented with decreased perfusion of the MCA compared to controls, that supplies blood to the fronto-temporal lobes (Uddin et al., 2017). Prior evidence demonstrated that hypoperfusion in the dorsal mindinsular cortex, right superior temporal sulcus and regions of the frontal lobe positively correlate with the loss of white-matter tracts (Churchill et al., 2020).

This thesis concludes that exposure to prior recurrent contact and concussion may accelerate a rugby union player's trajectory towards cognitive impairment in later life. It is apparent that regardless of age, players exposed to contact and concussion presented with reductions in NO bioactivity, reduced cerebral perfusion or blunted responses to vasoactive stimuli, including reduced cognition compared to non-contact, non-concussed controls

6.3. Limitations

Cerebral perfusion: It is important to acknowledge some limitations to the current works described. First, TCD has been discussed in detail in section 3.8.1.3 and 3.8.1.4 and remains a reputable method of determining CVR_{CO2} (Ainslie and Duffin, 2009). Historically, neuroimaging studies have concluded that MCA diameter remains constant unless fluctuations of 40 mmHg in arterial BP or 20 mmHg in $PaCO_2$ are detected (Serrador et al., 2000a, Giller et al., 1993a, Giller et al., 1993b). Throughout all experimental procedures in both studies, we did not detect fluctuations that breached these limits. More recently, it has been established that MCA diameter can change in response to more subtle alterations in PET_{CO2} (+9 mmHg and -15mmHg, Coverdale et al., 2014, Verbree et al., 2014). PET_{CO2} increased by an average of 10 mmHg during hypercapnia in Studies 1 and 2. Therefore, a degree of caution must be considered when interpreting hypercapnic data as vasodilation would have underestimated

MCAv. During hypocapnia, PET_{CO2} decreased by an average of 12 mmHg in Studies 1 and 2 and we are confident in the validity of the results obtained.

MCI diagnosis: While the MoCA is generally accepted as a more accurate tool for MCI detection compared to the Mini Mental Sate Examination (Dong et al., 2010), the MCI cut off score of 26/30 points remains controversial. Nasreddine et al. (2005) specified that a score of 26 points detected MCI with 90% sensitivity and 87% specificity. In contrast, others have challenged this arguing that this cut-off defined increases the rate of false positive results and have sought to determine more accurate approaches (Waldron-Perrine and Axelrod, 2012). A recent meta-analysis by Carson et al. (2018) recommended that a cut off score of 23/30 points maintained sensitivity (90%) and improved specificity (90%). While we acknowledge that the traditional cut-off score of 26 points for diagnosis of MCI in this thesis and similar peer-reviewed articles may potentially compromise diagnostic specificity (McMillan et al., 2017), we remain confident that recurrent contact contributes to accelerated cognitive decline across the lifespan (Broglio et al., 2012).

Concussion diagnosis: While World Rugby has introduced an operational definition of concussion to support injury surveillance studies (Raftery et al., 2016), diagnosis is still reliant on questionnaire based Head Injury Assessments that fail to incorporate physiological markers of concussion. Concussion history for Studies 1 and 2 were confirmed using the Sports Concussion Assessment Tool (Davis et al., 2017) and cross-checked using medical records available from the young players of Study 1. Medical records were not available for participants of Study 2 and moreover, the definition for concussion used in the present studies was not established when the retired players were still active participants of the game. It is therefore likely that concussion history among retired players was underestimated, given that a formal definition and indeed, methods of reporting and managing concussion in the amateur era of the game had not been established. Cunningham et al. (2020) argues that concussion history remains to be under-reported by both past and present athletes using self-recall. While Studies 1 and 2 conformed to the standards of injury monitoring and reporting (SCAT5 and Head Injury Assessment), it is likely that concussion history had been under-reported by players of both studies.

Exercise neuroprotection: Elevated cardiorespiratory fitness is also known to improve cerebral haemodynamic function and cognition throughout ageing (Colcombe and Kramer, 2003, Bailey et al., 2013b). For Study 1, we were unable to objectively quantify

cardiorespiratory fitness via $\dot{V}O_{2MAX}$ given logistical constraints. Indeed, it was extremely challenging to have access to these top-flight professional players and in order to match physical activity as closely to the professional rugby players, the control group was formed of competitive cyclists, rowers and cross-fit competitors from clubs in the local community. Physical activity status for all participants of Study 1 was indirectly assessed via the Get Active Questionnaire and study specific questionnaire upon recruitment. The Get Active Questionnaire is validated and enabled participants to document weekly physical activity duration (Petrella et al., 2018, Longmuir et al., 2019), while the study specific questionnaire enabled participants to differentiate exercise mode. Players and controls of Study 1 were deemed physically active and reported comparable exercise modes and duration. However, young players completed more resistance exercise compared to controls and this would have been especially difficult to match for. While cognitive benefits have been observed following resistance training in older adults (Liu-Ambrose and Donaldson, 2009), these benefits in younger adults are not clearly established. Participants of Study 2 were matched for cardiorespiratory fitness via $\dot{V}O_{2MAX}$ and completed the Get Active Questionnaire and study specific questionnaire. Estimates of physical activity from both questionnaires correlated well with $\dot{V}O_{2MAX}$. We are therefore confident in the use of questionnaires in Study 1 for determination of physical activity/fitness status. However, as mentioned in section 6.2, the physical activity status of players in Study 1 and 2 likely provided a degree of neuroprotection as aerobic activity across the adult lifespan attenuates the age-related decline in MCAv (Bailey et al., 2013b). Despite the neuroprotective benefits afforded through a physically active lifestyle, retired players were not able to completely 'counter' the cumulative maladaptation to cerebral haemodynamic function incurred through repetitive contact across their career.

6.4. Future directions

Neurovascular unit proteins: While professional rugby union players of the modern era of the game are now heavier, faster and more skilful, concussion incidence continues to rise with disregard to the warning signs accentuated by retired players. However, the relative degree of structural damage present in the brains of these players remains relatively unknown and requires further investigation. To better understand the structural consequences of recurrent contact and concussion history, we are currently investigating serum biomarkers of BBB permeability and neuroglial-vascular damage (Janigro et al., 2021). Data will be collected from participants of Study 1 and 2, serving as the complimentary structural component of the functionally integrated translational model adopted throughout this thesis.

S100 calcium binding protein β (S100β), neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase L1 (UCH-L1) and total tau (T-tau) have been used to assess structural alterations following contact and concussion. S100β is a marker of BBB permeability which can be detected 1-hour following head impacts (O'Connell et al., 2018, Marchi et al., 2013). While the half-life of S100β is between 60-120 minutes (Thelin et al., 2017), some have observed elevated concentrations in rugby union players 36 hours post-injury (Bouvier et al., 2017). Neuron-specific enolase, GFAP and UCH-L1 may be useful for characterising neuronal cell integrity and functionality 30-48 hours after brain injury (Rundgren et al., 2014, Diaz-Arrastia et al., 2014, Shahim et al., 2014, O'Connell et al., 2018). Similarly, total T-tau has been used to assess axonal damage following concussion and correlates with lesion size following more severe instances of TBI (Franz et al., 2003). These biomarkers can be detected rapidly and remain elevated for up to 12 weeks (Neselius et al., 2013, Shahim et al., 2014). Moreover, we will have access automated clinical grade ELISA and Single Molecule Array (Simoa) technology that is considered to be ∼500–1000 fold more sensitive than conventional immunoassays (Bailey et al., 2020). Combined, these biomarkers and more sensitive detection methods may prove useful to assess brain parenchymal damage following injury and help inform safe return-to-play decisions.

Pitch-side biomarkers: The Food and Drug Administration (FDA) recently recognised that elevated GFAP and UCH-L1 following head injury warrant a CT scan to rule out brain haemorrhages (Di Pietro et al., 2021). While CT imaging is useful to rule out gross pathologies, it does not offer diagnostic potential for concussion and other avenues are required to facilitate return-to-play decisions, particularly those that can be performed pitch-side. The Study of Concussion in Rugby Union through MicroRNAs (SCRUM) has recently identified that a select panel of salivary small non-coding RNA's (sncRNA) offer diagnostic potential following a suspected concussion. Di Pietro et al. (2021) revealed that a panel of 14 sncRNA's offered 91% accuracy in differentiating positive and negative results in the Head Injury Assessment. Moreover, the panel of biomarkers was markedly higher in players who returned positive HIA's (i.e. concussed) compared to players who returned negative HIA's, with the 'let-7f-5p' marker particularly sensitive when identifying players with concussion from players without concussion 36-48 hours post-injury.

Not only do the panel of biomarkers identified by Di Pietro et al. (2021) offer a much-improved physiological method for detection of concussion at the pitch side, there are other advantages including the non-invasive nature of salivary sampling methods and readily available analysis

methods. Indeed, these biomarkers have clinical relevance as miR-144 promotes β-amyloid accumulation (Sun et al., 2017), while the let-7 family act as neuroinflammatory markers involved in Alzheimer's disease and other forms of neurodegeneration (Schulte et al., 2011). Therefore, these markers may help to inform clinical management of concussion through improved return-to-play decision making, while also providing mechanistic insights that may predispose contact sport athletes to neurodegeneration in later-life.

Managing contact exposure: While Pollock et al. (2017) has presented evidence for banning the tackle from schoolboy rugby union in a bid to reduce concussion incidence, other potential avenues need to be considered first. The RFU have implemented changes to improve player welfare, particularly around safe tackling technique (Raftery et al., 2021). These include increased sanctions for unsafe tackling, coach interventions to improve tackle technique and law changes to lower tackle height below the line of the armpit (Raftery et al., 2021). Unfortunately, these actions have been unsuccessful in lowering concussion incidence and other avenues need to be considered. For instance, Bailey et al. (2013a) demonstrated that the degree of cerebral haemodynamic impairments among professional boxers were more closely related to sparring intensity and duration than concussion history. As previously discussed, professional rugby union players may experience 11,000 contact events per season, though the number of concussions is far less (Fuller et al., 2007a). Therefore, interventions that seek to lower the number of contact events and concussive injuries should be considered by rugby union's governing bodies.

In 2014, The National Football League was successful in actioning restrictions on the number of full contact training sessions that players undertake. These restrictions limited contact training drills to one-session per-week during the season, or one contact session per-day during team selection camps in a bid to lower overall injury rates. Following the introduction of these restrictions, Broglio et al. (2016) observed a 42% decline in impact exposure across a season of high-school Football, while Pfaller et al. (2019) observed a 57% reduction in concussion incidence. At present, there are no restrictions on the amount or type of training that rugby union teams complete. Therefore, Rugby Union's governing bodies should consider piloting similar strategies to that found in American Football.

Appendix I
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Appendix II
Ethical approval – Study 1 & 2

University of South Wales
Prifysgol De Cymru

Professor Julie E Lydon, Vice-Chancellor
Yr Athro Julie E Lydon, Is-Ganghellor

13/06/2017

Mr Tom Owen
Faculty of Life Sciences and Education
University of South Wales

Dear Tom Owens,

Re: Longitudinal changes in cerebrovascular function in professional rugby union players over the competitive season; from concussion to cognition (0617LSETOE0)

I am writing to confirm that on the 13/06/2017, the Faculty of Life Sciences and Education Ethics Sub Group approved your revised submission for ethical approval via Chair's action.

Please note if you intend on deviating from the approved protocol or documentation you will need to request approval for any changes. I've attached the documents that are approved.
Approval is valid for two years, after this point you must apply for a renewal of this approval. Please note that as Sponsor of this study we will provide insurance and indemnity cover to members of USW staff, USW students and research participants only.

If you need evidence of insurance or indemnity please contact me and I will arrange this to be sent to you.

Sincerely,

Professor Peter McCarthy
Chair of Faculty Ethics Sub Group

University of South Wales
Prifysgol De Cymru

Professor Julie E Lydon OBE, Vice-Chancellor
Yr Athro Julie E Lydon OBE, Is-Ganghellor

2nd July 2018

Mr Tom Owens
C/o Faculty of Life Sciences and Education
University of South Wales

Dear Tom,

Faculty Ethics Sub Group Feedback – 'Neurovascular implications of life-long exposure to recurrent concussion in retired rugby union players; is there a link to accelerated cognitive decline' [2017TO1102]

I am writing to confirm that on the 2nd July 2018, the Faculty of Life Sciences and Education Ethics Sub Group approved your submission for ethical approval.

Please note:

i. Approval is valid for 2 years from the date of issue, you will be notified when approval has expired but you are expected to be mindful of this expiration. Upon the expiration of this ethics approval you may apply for an extension.

ii. The approved documents are attached. If you intend on deviating from the approved protocol, research team, or documentation you will need to seek approval for any changes.

iii. This approval does not confirm that indemnity or insurance are in place for this project.

iv. Please confirm when your research project has closed (a one page closure report highlighting any recruitment issues, adverse events, publications etc. should be appended).

If you have any queries about the committee's decision, please do not hesitate to contact me.

Yours sincerely,

Professor Peter McCarthy
Chair of Faculty Ethics Committee

Appendix III

Research publications and correspondence arising from this thesis

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Experimental Physiology WILEY

RESEARCH PAPER

Contact events in rugby union and the link to reduced cognition: evidence for impaired redox-regulation of cerebrovascular function

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Abstract

Contact events in rugby union remain a public health concern. We determined the molecular, cerebrovascular and cognitive consequences of contact events during a season of professional rugby. Twenty-one male players aged 25 (mean) ± 4 (SD) years were recruited from a professional rugby team comprising forwards (n = 13) and backs (n = 8). Data were collected across the season. Pre- and post-season, venous blood was assayed for the ascorbate free radical ($A^{\bullet-}$, electron paramagnetic resonance spectroscopy) and nitric oxide (NO, reductive ozone-based chemiluminescence) to quantify oxidative–nitrosative stress (OXNOS). Middle cerebral artery velocity (MCAv, Doppler ultrasound) was measured to assess cerebrovascular reactivity (CVR), and cognition was assessed using the Montreal Cognitive Assessment (MoCA). Notational analysis determined contact events over the season. Forwards incurred more collisions (Mean difference [M_D] 7.49; 95% CI, 2.58–12.40; P = 0.005), tackles (M_D 3.49; 95% CI, 0.42–6.56; P = 0.028) and jackals (M_D 2.21; 95% CI, 0.18–4.24; P = 0.034). Forwards suffered five concussions while backs suffered one concussion. An increase in systemic OXNOS, confirmed by elevated $A^{\bullet-}$ ($F_{2,19}$ = 10.589, P = 0.004) and corresponding suppression of NO bioavailability ($F_{2,19}$ = 11.492, P = 0.003) was apparent in forwards and backs across the season. This was accompanied by a reduction in cerebral oxygen delivery (cD_{O_2}, $F_{2,19}$ = 9.440, P = 0.006) and cognition ($F_{2,19}$ = 4.813, P = 0.041). Forwards exhibited a greater decline in the cerebrovascular reactivity range to changes in PET_{CO2} (CVR_{CO_2RANG} compared to backs (M_D 1.378; 95% CI, 0.74–2.02; P < 0.001).

KEYWORDS
cerebral blood flow, cognition, contact, rugby union

1 | INTRODUCTION

Professional rugby union players may be exposed to over 11,000 contact events per-season comprising tackles, collisions, mauls, rucks and scrums (Fuller et al., 2007). Tackles are the most common contact-match event in rugby union and cause 52% of all injuries that have been associated with elevated concussion risk (Cross et al., 2017; England Rugby, 2020).

While the propensity of match-events that cause injury are generally understood, the relationship between player position and the physiological implications of contact remain to be established. Physical trauma promotes an elevation in systemic oxidative–nitrosative stress (OXNOS; Giza & Hovda, 2014; Khatri et al., 2018; Poole et al., 2014; Vagnozzi et al., 2007), defined by a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability, which has been associated with cerebral hypoperfusion, impaired cerebrovascular reactivity (CVR) and cognitive dysfunction (Bailey et al., 2013a, 2019; Meier et al., 2015). Moreover, Breedlove et al. (2012) and Abbas et al. (2015) have demonstrated that contact events correlate with disruption of functional brain networks, while Stewart et al. (2016) and Lee et al. (2019) have speculated that neurodegeneration is an under-recognized consequence of rugby union. Therefore, it is surprising that previously molecular profiling has not been performed and corresponding changes in cerebrovascular function assessed among elite-level players.

Thus, for the first time, the present study sought to determine the molecular, cerebrovascular and cognitive effects associated with contact events over a single season of professional rugby union stratified by playing position and subsequent concussion risk. We hypothesized that compared to backs, an increased number of contact match events encountered by forwards would decrease cognition subsequent to a more pronounced elevation in systemic OXNOS and consequent cerebrovascular dysfunction.

New Findings

- **What is the central question of this study?**
  How does recurrent contact incurred across a season of professional rugby union impact molecular, cerebrovascular and cognitive function?
- **What is the main findings and its importance?**
  A single season of professional rugby union increases systemic oxidative–nitrosative stress (OXNOS) confirmed by a free radical-mediated suppression in nitric oxide bioavailability. Forwards encountered a higher frequency of contact events compared to backs, exhibiting elevated OXNOS and lower cerebrovascular function and cognition. Collectively, these findings provide mechanistic insight into the possible cause of reduced cognition in rugby union subsequent to impairment in the redox regulation of cerebrovascular function.

2 | METHODS

2.1 | Ethical approval

The study was approved by the University of South Wales ethics committee (no. 0617LSETOE0). Participants provided written informed consent before experimentation and the study procedures conformed to standards set forth by the *Declaration of Helsinki*, except for registration in a database.

2.2 | Participants

Twenty-one male players (13 forwards/eight backs) aged 25 (mean) ± 4 (SD) years were recruited from a professional rugby union first team. Playing careers spanned 16 ± 4 years and players self-reported three (IQR: 3) prior concussions (Table 1). All participants were free of disease, non-smokers and not taking any prescribed medication. Ahead of the pre-/post-season assessments, participants refrained from physical activity, caffeine and alcohol, followed a low nitrate/nitrite diet and had completed a 12 h overnight fast prior to experimentation (Bailey et al., 2017). Forwards were identified as props, hookers, locks (second row), flankers and number eights, while backs consisted of half-backs (fly-half and scrum-half), centres, wingers and full backs. Participant demographics are outlined in Table 1.

2.3 | Study design

The study adopted a longitudinal design, over the course of the Guinness PRO14 rugby union season. Data were collected at three time points: pre-season (14 ± 5 days before season), in-season (31 games) and post-season (14 ± 6 days after season).

2.4 | Pre- and post-season experimental procedures

2.4.1 | Concussion history

Life-long concussion history was determined via medical records from the team doctor. Concussion history preceding available medical records was determined via self-recall and cross-checked using the Sport Concussion Assessment Tool (SCAT5) at baseline (Davis et al., 2017).

TABLE 1 Demographics

	Forwards ($n = 13$)			Backs ($n = 8$)			*P* value		
Characteristic	Pre-season	Post-season	Δ	Pre-season	Post-season	Δ	Group	Time point	Interaction
Anthropometrics									
Stature (m)	1.88 ± 0.06	1.88 ± 0.06	0 ± 0.01	1.80 ± 0.07	1.80 ± 0.06	0 ± 0.01	**0.013**	0.380	0.303
Mass (kg)	113 ± 9	113 ± 10	0 ± 2	90 ± 5**	87 ± 6*,**	−3 ± 3**	**<0.001**	**0.045**	**0.021**
BMI (kg/m^2)	31 (30–35)	31 (30–35)	0 (0–0)	27 (26–28)	26 (26–27)	1 (−1 to 0)**	**<0.001**	0.051	0.339
Body fat (%)	22 ± 4	18 ± 4	−3 ± 3	16 ± 4	14 ± 4	−3 ± 2	**0.008**	**<0.001**	0.711
Cognition									
Montreal cognitive assessment (*n*)	28 ± 2	27 ± 1	−1 ± 2	27 ± 3	26 ± 2	−1 ± 2	0.417	**0.004**	0.196
Contact/activity									
Concussions (*n*)	3 (2–5)	3 (3–5)	0 (0–0)	2 (1–3)	2 (1–2)	0 (0–0)	0.164	0.134	0.435
Rugby career (years)	17 ± 4	17 ± 5	0 ± 3	15 ± 3	16 ± 3	1 ± 1	0.210	0.539	0.539
Physical activity (min/week)	425 ± 155	496 ± 184	72 ± 223	458 ± 171	379 ± 172	−79 ± 190	0.491	0.940	0.130

Values presented as means ± SD or median (IQR).
*$P < 0.05$ within groups;
**$P < 0.05$ between groups. Values shown in bold indicate statistical significance. BMI, body mass index.

2.4.2 | Molecular measurements

Blood was collected into vacutainers from an indwelling cannula located in a forearm antecubital vein (Becton, Dickinson and Company, Oxford, UK) and centrifuged at 600 *g* (4°C) for 10 min. Plasma supernatant were decanted into cryogenic vials (Nalgene Labware, Thermo Fisher Scientific Inc., Waltham, MA, USA) and snap-frozen in liquid nitrogen. Samples were thawed at 37°C prior to batch analysis.

2.4.3 | Free radicals

Plasma ascorbate radical ($A^{\bullet-}$) was determined as a direct measure of systemic free radical formation via X-band electron paramagnetic resonance spectroscopy (AquaX, Bruker Daltonics Inc., Billerica, MA, USA) as previously described (Bailey et al., 2019). Plasma NO bioavailability (combined bioactive concentrations of nitrite (NO_2^-) and S-nitrosothiols (RSNO)) was measured via reductive ozone-based chemiluminescence (Sievers NOA 280i, Analytix Ltd, Durham, UK) as previously described by Bailey et al. (2017). Intra- and inter-assay CVs were both <5%.

2.4.4 | Cerebral haemodynamic function

Middle cerebral artery velocity (MCAv) was determined using a 2 MHz pulsed Doppler ultrasound system (TCD, Multi-Dop X4, DWL Elektroniche Systeme GmbH, Sipplingen, Germany). Beat-by-beat mean arterial blood pressure (MAP), heart rate (HR) and cardiac output ($\dot{Q}$) were recorded continuously using finger photoplethysmography (Finometer PRO; Finapres Medical Systems, Amsterdam, The Netherlands). Cerebrovascular and total peripheral resistance (CVRi and TPR) were calculated as MAP/MCAv or $\dot{Q}$, respectively. Cerebrovascular conductance index (CVCi) was calculated as MCAv/MAP and pulsatility index (PI) calculated as systolic MCAv (SMCAv)−diastolic MCAv (DMCAv)/MCAv. Cerebral oxygen delivery (cD_{O_2}) was calculated as MCAv × arterial O_2 content (C_{aO_2}) (1.39 × Hb × S_{aO_2}/100, Bailey et al., 2019). Cerebrovascular reactivity to hypercapnia (CVR_{CO_2HYPER}; 5% CO_2, balanced air) and hypocapnia (CVR_{CO_2HYPO}; hyperventilation at 15 breaths/min) were assessed for 3 min and calculated as the percentage change in MCAv from baseline per mmHg change in end-tidal CO_2 (P_{ETCO_2}) determined via capnography (ML 206, ADInstruments Ltd, Oxford, UK). The fractional sums of CVR_{CO_2HYPER} and CVR_{CO_2HYPO} were used to calculate the CVR range (CVR_{CO_2RANGE}) as previously described (Bailey et al., 2013a). All data were sampled continuously at 1 kHz (Powerlab, ADInstruments, Colorado Springs, CO, USA) and stored for off-line analysis.

2.4.5 | Cognition

Cognition was assessed using the Montreal Cognitive Assessment (MoCA, Nasreddine et al., 2005).

2.4.6 | In-season measurements

Notational analysis was performed for 31 games played during the Guinness PRO14 rugby union season. Match events during each game were recorded using high-speed cameras (Sprinter-FHD, Optronis, Kehl, Germany) and logged (Hudl Sports Code, London, UK) by two analysts that were blinded to study outcomes. Data were transferred to the research team for interpretation and analysed using definitions

from the Rugby Union Video Analysis Consensus (RUVAC) group (Hendricks et al., 2020). Some contact match descriptors were not available from the data received (e.g., a tackle sequence whereby additional defenders join a tackle that is still in progress, before the ruck begins) and therefore not included or discussed in this manuscript.

2.4.7 | Concussion incidence and propensity

The frequency of match events was calculated per game/season. The total number of games and exposure for each player was determined through video analysis. Concussion/head injury incidence was calculated as the mean number of injuries per 1000 match hours and injury propensity calculated per 1000 match events (Rafferty et al. (2019).

2.4.8 | Statistical analysis

Data were analysed using commercially available software (SPSS 26.0, IBM Corp., Armonk, NY, USA). Following confirmation of distribution normality (Shapiro–Wilk *W* tests), two-way (group (forwards vs. backs) × time point (pre- vs. post-season)) repeated measures analyses of variance (ANOVA) was performed to assess differences in demographics, molecular profile, CVR_{CO_2} and cognition. Changes in cerebrovascular function were performed using a three-way (group (forwards vs. backs) × time point (pre- vs. post-season) × condition (normocapnia vs. hyper/hypocapnia)) repeated measures ANOVA. Following the identification of a significant interaction, *post hoc* analyses were performed using Bonferonni corrected pairwise comparisons (paired sample Student's *t*-test within groups; independent sample *t*-test between groups). *Z*-scores were used to determine outliers for all biomarkers and were calculated by subtracting the sample mean from the raw data point and dividing it by the standard deviation. Data points were deemed as outliers if the *Z*-score was >2 or <−2. An independent samples *t*-test compared the frequency of match events between groups (forwards vs. backs). Concussion incidence and match event injury propensity between groups were calculated using rate ratios (RR) and percentile bootstrapped to confirm 95% CIs. Injury incidence/propensity was deemed different if the 95% CI for the RR did not overlap with unity (Rafferty et al., 2019). Data are expressed as means ± standard deviation (SD) or median (IQR) if not normally distributed. Significance for all two-tailed tests was established at $P < 0.05$.

3 | RESULTS

3.1 | Participants

No significant differences in playing career ($F_{2,19} = 0.391$, $P = 0.539$), concussion history ($F_{2,19} = 0.637$, $P = 0.118$), physical activity ($F_{2,19} = 2.510$, $P = 0.325$), or average playing duration (t_{19} −0.197; 95% CI, −9.76 to 8.08; $P = 0.846$) were apparent between forwards and backs across the season. As anticipated, forwards were taller ($F_{2,19} = 7.462$, $P = 0.013$), heavier ($F_{2,19} = 44.210$, $P < 0.001$), had a higher BMI ($F_{2,19} = 17.295$, $P = 0.001$) and body-fat percentage ($F_{2,19} = 8.726$, $P = 0.008$) compared to backs (Table 1).

3.2 | Match events

Over the course of the season, forwards incurred more match events compared to backs, most notably for collisions (mean difference [M_D] 126.60; 95% CI, 25.16–228.05; $P = 0.017$; Table 2). Per game, forwards were involved in more collisions (M_D 7.49; 95% CI, 2.58–12.40; $P = 0.005$), tackles (M_D 3.49; 95% CI, 0.42–6.56; $P = 0.028$) and jackals (M_D 2.21; 95% CI, 0.18–4.24; $P = 0.034$) compared to backs. No significant differences in the average number of ball carries were observed between groups (M_D −0.31; 95% CI, −2.16 to 1.53, $P = 0.725$).

3.3 | Incidence and propensity

Six concussions were incurred during the season (forwards, $n = 5$; backs, $n = 1$) corresponding to a concussion incidence rate of 10/1000 player match hours (95% CI, −0.00 to 28.49). Forwards suffered 12.9 concussions/1000 match hours (95% CI, 0.00–38.46) compared to 4.2/1000 player match hours among backs (95% CI, 0.00–33.44). Concussion propensity resulting from collisions, tackles, jackals and ball carries was not significantly different between groups (Table 3).

3.4 | Molecular function

An increase in $A^{\bullet-}$ ($F_{2,19} = 10.589$, $P = 0.004$) and corresponding reduction in total NO bioavailability ($F_{2,19} = 11.492$, $P = 0.003$) was observed across the season. However, no between-group differences were apparent ($P > 0.05$; Figure 1).

3.5 | Cerebrovascular function

Across the season, cD_{O_2} ($F_{2,19} = 9.440$, $P = 0.006$, Table 4) and CVR_{CO_2HYPER} ($F_{2,19} = 7.272$, $P = 0.014$, Figure 2a) decreased in both groups. However, we observed a more marked reduction in CVR_{CO_2RANGE} among forwards across the season (M_D 1.378; 95% CI, 0.74–2.02; $P < 0.001$; Figure 2b).

3.6 | Cognition

MoCA scores decreased by 5 ± 8% (95% CI, −9.09 to 0.67) in forwards and 2 ± 6% (95% CI, −6.75 to 2.25) in backs across the

TABLE 2 Comparison of contact events

Group	Forwards ($n = 13$)	Backs ($n = 8$)	P	95% CI
Collisions	186 ± 129	67 ± 64	**0.017**	25.16 to 228.05
Tackles	120 ± 98	71 ± 40	0.126	−18.17 to 135.59
Jackals	48 ± 56	9 ± 10	0.059	−1.78 to 83.14
Ball carries	59 ± 55	63 ± 30	0.932	−42.59 to 46.29
Collisions (per game)	12 ± 6	5 ± 3	**0.005**	2.58 to 12.40
Tackles (per game)	7 ± 4	4 ± 1	**0.028**	0.42 to 6.56
Jackals (per game)	3 ± 3	1 ± 0	**0.034**	0.18 to 4.24
Ball carries (per game)	4 ± 2	4 ± 1	0.725	−2.16 to 1.53

Data presented as means ± SD. Values shown in bold indicate statistical significance.

TABLE 3 Injury incidence and match event propensity

Group	Forwards ($n = 13$)	Backs ($n = 8$)	Rate ratio (95% CI)
Concussion (n/season)	5	1	–
Concussion/1000 h	12.9	4.2	3.08 (0.00–28.49)
Concussion/1000 collisions	1.3	7.4	0.18 (0.00–2.46)
Concussion/1000 tackles	1.7	1.0	1.68 (0.00–2.85)
Concussion/1000 jackals	4.4	37	0.12 (0.00–101.52)
Concussion/1000 ball carries	2.5	1.1	2.28 (0.00–5.66)

Data presented as absolute values.

season ($F_{2,19} = 4.813$, $P = 0.041$). No between-group differences were apparent ($P > 0.05$; Table 1).

4 | DISCUSSION

Across one season, professional rugby union players exhibited an elevation in systemic OXNOS confirmed by a free radical-mediated suppression in NO bioavailability. This was accompanied by a decline in indices of cerebrovascular function and cognition. While no significant differences in concussion incidence were reported across the season, forwards were exposed to more contact events that further compounded molecular, cerebrovascular and cognitive dysfunction compared to the backs. Collectively, these findings provide important mechanistic insight into the possible cause of reduced cognition in rugby union players subsequent to impairment in the redox regulation of cerebrovascular function.

Using the most direct analytical techniques for the molecular detection of free radicals (Bailey et al., 2013c), our findings confirmed that players exhibited a systemic elevation in free radicals and a corresponding suppression in NO bioavailability across the season, a molecular cascade that we have collectively coined OXNOS (Bailey et al., 2013c, 2019). We have previously associated OXNOS with cerebrovascular dysfunction and diffuse blood–brain barrier disruption (Bailey et al., 2011). However, while others have explored the relationship between concussion and less robust surrogates of OXNOS within 5 days of injury in rodents (Tavazzi et al., 2007), our data were collected ~14 days after the end of the competitive season and suggest that cumulative exposure to impact incurs a molecular shift towards a systemic elevation in OXNOS.

The reduction in cerebrovascular function across the season further highlights the importance of NO as a mediator of CBF (Lavi et al., 2003). Global cD_{O_2} reduced in both groups and the suppression of CVR_{CO_2HYPER} indicated a reduced capacity of the cerebrovascular bed to respond to dynamic changes in CBF. Moreover, the suppression of CVR_{CO_2HYPER} (Figure 2a) contributed to a 15% reduction in CVR_{CO_2RANGE} among forwards. Given that reductions in both CBF and CVR_{CO_2} have previously been associated with cognitive decline and neurodegeneration (Silvestrini et al., 2006; Sweeney et al., 2018; Wolters et al., 2017), more research is required in order to determine whether these mechanisms may predispose players to accelerated brain ageing. Indeed, the reversibility of OXNOS and suppressed cerebrovascular function remains to be established given the general lack of recovery and unrelenting nature of recurrent contact exposure that defines the modern game (Owens and Bailey, 2021). At a professional level, a player is unlikely to undergo sustained periods of time without exposure to contact events, and therefore it is likely that OXNOS and suppressed cerebrovascular function would remain

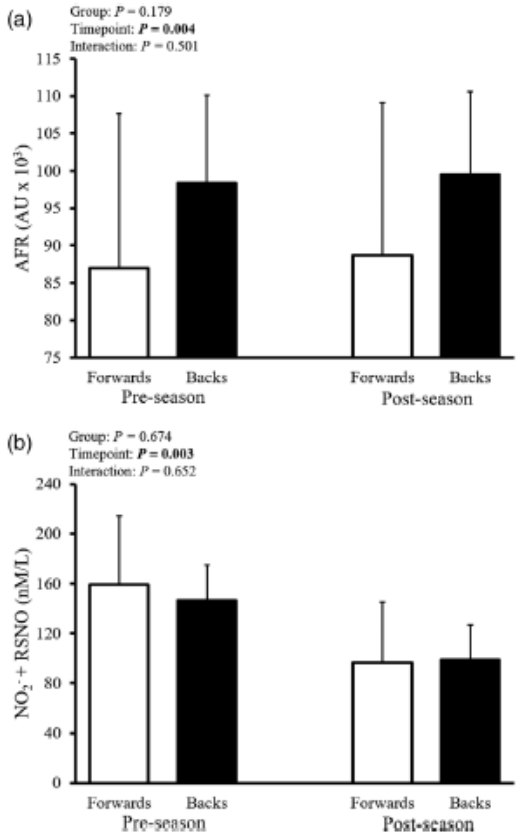

FIGURE 1 Plasma ascorbate free radical (a) and bioactive nitric oxide concentration (b). AFR, ascorbate free radical; AU, arbitrary units; NO_2^-, nitrite; RNSO, *S*-nitrosothiols

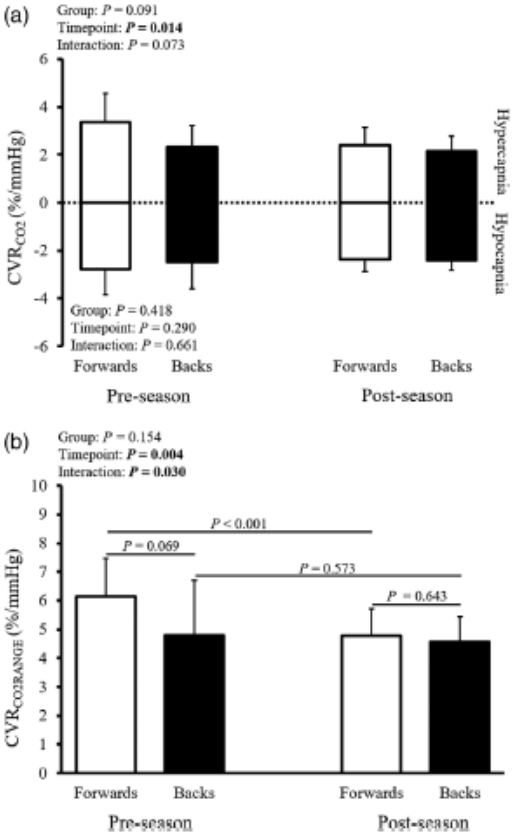

FIGURE 2 Cerebrovascular reactivity (CVR_{CO_2}) to hyper/hypocapnia (a) and cerebrovascular range (CVR_{CO_2RANGE}, b)

apparent, or progress further over time. However, further research is required to better understand these mechanisms.

Cognition decreased across the season in both forwards and backs, but contrary to our original expectations, this was not associated with the increased contact frequency of the forwards. Our results suggest that the reduction in cD_{O_2}, subsequent to elevated systemic OXNOS may have contributed, at least in part, to the observed cognitive decline. While various regions of the head are subject to contact throughout play, previous studies have noted that frontotemporal domains are most adversely affected, notably memory and executive function (McMillan et al., 2017; Meier et al., 2015). Previous studies have identified more marked reductions in brain volume, CBF and fractional anisotropy in these regions (Churchill et al., 2017, 2020), while other modelling studies have found shear retrograde forces concentrated along midline structures, which could similarly affect these cognitive domains (Pellman et al., 2003). However, more research is encouraged to determine whether structural damage to the neurovascular unit occurs across a single season.

Our results concur with the most recent RFU Injury Surveillance Project (England Rugby, 2020), suggesting that injury propensity is not influenced by grouped playing position or the type of match event. Concussion incidence appeared to be three times greater in the forwards compared to the backs, but the wide confidence intervals surrounding the RR varied from substantially higher in the forwards to substantially higher in the backs. Indeed the confidence intervals for all other match event propensities demonstrate wide confidence intervals and we acknowledge that robust conclusions cannot be made using RRs in this study. Moreover, the reporting of injury incidence remains important and serves as an objective measure of whether concussion prevalence is being managed successfully within the game, but it is a blunt tool for measuring the potential for neurological injury in players. Our findings suggest that failure to account for match event frequency and positional differences on the rugby field may lead to an increased susceptibility to molecular, cerebral haemodynamic and

TABLE 4 Cerebrovascular and cardiovascular responses to hypercapnia

Condition	Baseline		Hypercapnia		Baseline		Hypercapnia	
Group	Forwards ($n = 13$)		Forwards ($n = 13$)		Backs ($n = 8$)		Backs ($n = 8$)	
Time point	Pre-season	Post-season	Pre-season	Post-season	Pre-season	Post-season	Pre-season	Post-season
Cerebrovascular								
MCAv (cm/s)	50 ± 6	53 ± 8	70 ± 10	69 ± 13	62 ± 16	63 ± 14	75 ± 19	77 ± 17
Condition: **$P < 0.001$**, time: $P = 0.558$, group: $P = 0.109$, interaction: $P = 0.069$								
SMCAv (cm/s)	83 ± 10	86 ± 12	106 ± 13	104 ± 17	101 ± 25	99 ± 22	117 ± 30	117 ± 28
Condition: **$P < 0.001$**, time: $P = 0.953$, group: $P = 0.095$, interaction: $P = 0.080$								
DMCAv (cm/s)	35 ± 5	36 ± 5	52 ± 8	50 ± 10	43 ± 11	44 ± 11	54 ± 14	56 ± 13
Condition: **$P < 0.001$**, time: $P = 0.794$, group: **$P = 0.035$**, interaction: $P = 0.947$								
CVRi (mmHg/cm/s)	1.68 ± 0.25	1.60 ± 0.36	1.23 ± 0.16	1.37 ± 0.35	0.77 ± 0.43	0.93 ± 0.22	1.29 ± 0.52	1.00 ± 0.14
Condition: **$P < 0.001$**, time: $P = 0.099$, group: $P = 0.075$, interaction: $P = 0.733$								
CVCi (cm/s/mmHg)	0.61 ± 0.11	0.65 ± 0.13	0.83 ± 0.10	0.77 ± 0.18	0.77 ± 0.43	0.93 ± 0.22	0.94 ± 0.49	1.02 ± 0.15
Condition: **$P < 0.001$**, time: $P = 0.342$, group: **$P = 0.033$**, interaction: $P = 0.809$								
PI (AU)	0.96 ± 0.12	0.94 ± 0.09	0.77 ± 0.11	0.80 ± 0.11	0.95 ± 0.12	0.89 ± 0.17	0.86 ± 0.17	0.79 ± 0.15
Condition: **$P < 0.001$**, time: $P = 0.381$, group: $P = 0.894$, interaction: $P = 0.375$								
cD_{O_2} (ml/cm/s)	1080 ± 150	1015 ± 133	1510 ± 251*	1313 ± 239*	1262 ± 324	1160 ± 277	1520 ± 385*	1420 ± 322*
Condition: **$P < 0.001$**, time: **$P = 0.006$**, group: $P = 0.285$, interaction: **$P = 0.037$**								
Cardiopulmonary								
Heart rate (bpm)	58 ± 7	56 ± 8	59 ± 6	59 ± 8	61 ± 8	63 ± 13	62 ± 8	61 ± 10
Condition: $P = 0.341$, time: $P = 0.921$, group: $P = 0.202$, interaction: $P = 0.267$								
MAP (mmHg)	84 ± 11	84 ± 16	86 ± 10	92 ± 15	89 ± 20	69 ± 13	88 ± 21	75 ± 9
Condition: $P = 0.052$, time: $P = 0.154$, group: $P = 0.145$, interaction: $P = 0.770$								
TPR (mmHg/l/min)	14.27 ± 2.79	15.29 ± 3.51	14.23 ± 2.84	15.74 ± 2.86	14.81 ± 3.42	11.40 ± 1.34	14.41 ± 3.71	12.34 ± 1.45
Condition: $P = 0.587$, time: $P = 0.387$, group: $P = 0.076$, interaction: $P = 0.427$								
P_{ETCO_2} (mmHg)	39 ± 9	43 ± 4	52 ± 5	55 ± 4	46 ± 3	44 ± 5	55 ± 3	56 ± 1
Condition: **$P < 0.001$**, time: $P = 0.206$, group: $P = 0.071$, interaction: $P = 0.219$								

Values presented as mean ± SD.

*$P < 0.05$ within groups. Values shown in bold indicate statistical significance. Abbreviations: AU, arbitrary units; cD_{O_2}, cerebral oxygen delivery; CVCi, cerebrovascular conductance index; CVRi, cerebrovascular resistance index; DMCAv, diastolic middle cerebral artery velocity; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; P_{ETCO_2}, end-tidal carbon dioxide; PI, pulsatility index; SMCAv, systolic middle cerebral artery velocity; TPR, total peripheral resistance

cognitive dysfunction across the competitive season. Therefore, more rigorous measures need to be adopted, as we have demonstrated that a player's molecular and cerebral haemodynamic signatures change across the season and are dependent on playing position.

There are limitations to the present study that warrant consideration. First, larger scale follow-up studies with non-contact control groups are encouraged to confirm our findings given the interpretive limitations associated with the small sample sizes employed, including the caveats associated with a type M error (Gelman and Carlin 2014). Second, data for contact events sustained during training were not available, and therefore we cannot rule out the potential contribution this had towards the overall contact load endured. Future studies are encouraged to collect data during both competition and training (perhaps even in response to acute single-contact events) to better understand the relationship between contact load and molecular, cerebral haemodynamic and cognitive function in professional rugby union players. Finally, we observed a 1-point reduction in MoCA scores across the season. However, interpretative caution must be exercised when considering the decline in cognition as previous research indicates that a reliable change index for the MoCA is ±3 points, albeit in an aged sedentary population (Kopecek, 2017) in the absence of intense aerobic strength training known to compound neuroprotection (Bailey et al., 2013b).

In conclusion, it appears that exposure to contact events in rugby promotes a decline in cerebral haemodynamic function and cognition across the season, subsequent to an elevation in systemic OXNOS that may prove the fundamental 'unifying' molecular pathway predisposing to cognitive decline. To better understand the players' physiological phenotype in response to recurrent contact events across a season, future research may consider deploying the measures used in this

TABLE 5 Cerebrovascular and cardiovascular responses to hypocapnia

Condition	Baseline		Hypocapnia		Baseline		Hypocapnia	
Group	Forwards ($n = 13$)		Forwards ($n = 13$)		Backs ($n = 8$)		Backs ($n = 8$)	
Time point	Pre-season	Post-season	Pre-season	Post-season	Pre-season	Post-season	Pre-season	Post-season
Cerebrovascular								
MCAv (cm/s)	52 ± 6	53 ± 9	36 ± 5	38 ± 5	57 ± 15	60 ± 12	40 ± 7	43 ± 9
Condition: **$P < 0.001$**, time: $P = 0.246$, group: $P = 0.128$, interaction: $P = 0.613$								
SMCAv (cm/s)	87 ± 11	86 ± 15	70 ± 11	72 ± 11	97 ± 22	97 ± 18	76 ± 12	78 ± 16
Condition: **$P < 0.001$**, time: $P = 0.802$, group: $P = 0.165$, interaction: $P = 0.954$								
DMCAv (cm/s)	36 ± 4	35 ± 6	22 ± 5	24 ± 3	38 ± 11	41 ± 9	25 ± 5	28 ± 8
Condition: **$P < 0.001$**, time: $P = 0.356$, group: $P = 0.064$, interaction: $P = 0.324$								
CVRi (mmHg/cm/s)	1.61 ± 0.21	1.75 ± 0.38	2.11 ± 0.44	2.28 ± 0.46	1.62 ± 0.64	1.28 ± 0.20	2.02 ± 0.75	1.50 ± 0.30
Condition: **$P < 0.001$**, time: $P = 0.309$, group: **$P = 0.017$**, interaction: $P = 0.234$								
CVCi (cm/s/mmHg)	0.63 ± 0.07	0.59 ± 0.12	0.49 ± 0.10	0.45 ± 0.08	0.75 ± 0.44	0.80 ± 0.11	0.58 ± 0.28	0.70 ± 0.17
Condition: **$P < 0.001$**, time: $P = 0.668$, group: **$P = 0.013$**, interaction: $P = 0.381$								
PI (AU)	0.98 ± 0.13	0.98 ± 0.10	1.32 ± 0.23	1.27 ± 0.18	1.05 ± 0.20	0.94 ± 0.22	1.27 ± 0.16	1.18 ± 0.29
Condition: **$P < 0.001$**, time: $P = 0.145$, group: $P = 0.675$, interaction: $P = 0.213$								
cD_{O_2} (ml/cm/s)	1124 ± 148	1003 ± 164	781 ± 115	724 ± 80	1161 ± 321	1112 ± 256	818 ± 148	792 ± 168
Condition: **$P < 0.001$**, time: $P = 0.066$, group: $P = 0.349$, interaction: $P = 0.485$								
Cardiopulmonary								
Heart rate (bpm)	60 ± 6	58 ± 9	68 ± 10*	60 ± 7*	64 ± 10	62 ± 8	69 ± 8*	67 ± 7*,**
Condition: **$P < 0.001$**, time: $P = 0.109$, group: $P = 0.150$, interaction: **$P = 0.023$**								
MAP (mmHg)	84 ± 9	90 ± 14	75 ± 9	86 ± 12	84 ± 21	75 ± 12	77 ± 22	64 ± 16
Condition: **$P < 0.001$**, time: $P = 0.748$, group: $P = 0.069$, interaction: $P = 0.081$								
TPR (mmHg/l/min)	13.88 ± 2.08	15.55 ± 2.31*	11.01 ± 2.48	14.14 ± 2.38*	13.00 ± 2.84	12.07 ± 1.21*,**	11.10 ± 2.53	9.53 ± 2.30*,**
Condition: **$P < 0.001$**, time: $P = 0.342$, group: **$P = 0.008$**, interaction: **$P = 0.026$**								
P_{ETCO_2} (mmHg)	38 ± 9	43 ± 4	27 ± 8	31 ± 4	43 ± 5	44 ± 4	33 ± 4	32 ± 3
Condition: **$P < 0.001$**, time: $P = 0.109$, group: $P = 0.142$, interaction: $P = 0.635$								

Values presented as mean ± SD.

*$P < 0.05$ within groups;

**$P < 0.05$ between groups. Values shown in bold indicate statistical significance. Abbreviations: AU, arbitrary units; cD_{O_2}, cerebral oxygen delivery; CVCi, cerebrovascular conductance index; CVRi, cerebrovascular resistance index; DMCAv, diastolic middle cerebral artery velocity; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; P_{ETCO_2}, end-tidal carbon dioxide; PI, pulsatility index; SMCAv, systolic middle cerebral artery velocity; TPR, total peripheral resistance

study to evaluate acute single match events at the pitch side, including recovery based on a clearer delineation of the underlying temporal kinetics.

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COMPETING INTERESTS

None.

AUTHOR CONTRIBUTIONS

T.S.O., T.A.C., B.S.S., A.I., L.V., G.R., L.F., H.T., R.M.G.B., G.L.J., C.J.M. and D.M.B. contributed towards the conception and design of the work, acquisition, analysis, and interpretation of data and the drafting of the work, including revisions of critically important intellectual content. All authors have approved the final version of the manuscript, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

Original data arising from this research is available directly from Professor Damian Miles Bailey upon reasonable request.

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THE DYNAMICS OF MODERN RUGBY

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ROUTLEDGE

Chapter 9

Collision and the Effects of Head Injuries

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Introduction

The professionalisation of rugby union has seen significant advances in player size, strength and speed to maintain competitive advantages (Fuller et al., 2013). While this may have enhanced physiological performance adding to the spectacle of the game, a concomitant rise in injuries has also been observed. Since the 2011-12 rugby season, the Professional Rugby Injury Surveillance and Prevention Project (PRISP) has recognised concussion as the most frequent match injury. This remains a public health concern amid fears of increased susceptibility to neurodegeneration in later-life (Lee et al., 2019).

Concussion and current practice in rugby

Concussion is a traumatic brain injury induced by biomechanical forces resulting from head-to-head collision, contact between the head and limbs of two players, or contact between the head and the ground (McCrory et al., 2017). Concussion induces diffuse axonal injury (DAI; Figure 1) and is accompanied by a constellation of short-lived (1hr - 10 days) neurological sequelae including; headache, amnesia, blurred vision and difficulty concentrating (McCrory et al., 2017).

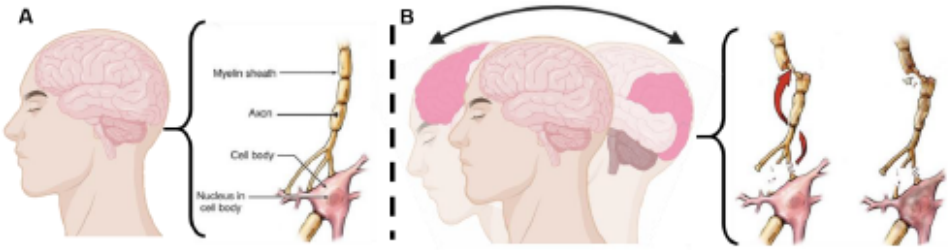

Figure 1: Non-injured brain with structure of intact neuron (A). Sports-related concussion is a diffuse axonal injury that induces torsional rotation in axons leading to mechanical shear and loss of neuronal networks (B).

While most athletes recover within seven days, ~10% develop post-concussive syndrome (PCS), that may last for weeks, months or even years (Hiploylee et al., 2017). To avoid potential long-term neurological complications, World Rugby has devised the graduated return-to-play (gRTP) protocol (World Rugby, 2017). During gRTP, players begin with complete rest (7 days; stage 1) and progress through stages, 2-6 every 24 hours, comprising of light aerobic and sports-specific exercise, non-contact and contact training, before a formal return to sport, provided symptoms do not re-appear (World Rugby, 2017). However, some

athletes (predominantly at elite-level) may return-to-play within seven days provided an advanced level of concussion care is provided (World Rugby, 2017).

Incidence

Concussion incidence has been documented since the 1950's (Owens et al., 2019b). The professionalisation of rugby in 1995 and formation of the Rugby Injury Consensus Group (RICG) in 2007 has ensured an improved means of injury reporting (Fuller et al., 2007), while concussion awareness among players and coaches has improved, leading to a dramatic rise in reported injuries (England Rugby, 2019). It has recently been established that concussion incidence in rugby (0.02 – 8.83/1000 hours across various levels of play) has surpassed that of other team sports, including American football and ice hockey (Prien et al., 2018). The RICG recommend differentiating match and training incidence as a model of best practice (Fuller et al., 2007). However, this is rarely feasible at the lower levels of play due to limited resources and staff and concussion incidence is best captured consistently at elite level. According to the PRISP, concussion incidence has increased from 5 events/1000 match-hours during the 2002-03 English premiership season to a high of 20.9/1000 match-hours during 2016-17 (England Rugby, 2018). However, incidence decreased to 10/1000 match-hours during the 2019 Rugby World Cup, corresponding to a 35% decline compared to the 2017-18 season of 17.9/1000 match-hours (Figure 2; World Rugby, 2019).

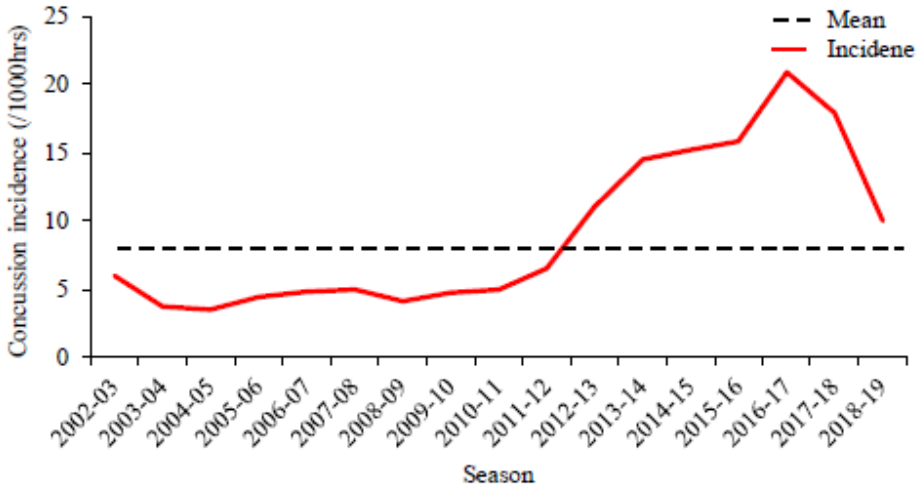

Figure 2: Concussion incidence in English Premiership Rugby from 2002-19. Data obtained from England Rugby (2019) and World Rugby (2019).

Players encounter an average of 26 collision events per-game across a season and the tackle is most associated with concussion incidence, effecting both ball carrier and tackler (Brooks et al., 2005, Gardner et al., 2014, Reardon et al., 2017, England Rugby, 2019). The tackler is most prone to concussion, with the ball carrier less frequently injured at professional level (England Rugby, 2019). Concussion risk is elevated in the event that a tackler accelerates into the tackle, or the tackler travels at higher speed relative to the ball carrier (Cross et al., 2017). Concussion risk is further heightened with head-to-head contact, which often occurs when tackles are made above the line of the armpit, subsequent to an upright tackle position (Cross et al., 2017, Tucker et al., 2017). Conversely, concussion risk is lowered in the event a ball carrier is bent at the waist, lowering the tackle height to below the armpit line (World Rugby, 2019, Tucker et al., 2017). Collisions, rucks, jackals, scrums, mauls and lineouts also cause concussion (Gardner et al., 2014), however injury incidence related specifically to these match events is lower than that of tackling (England Rugby, 2019). Forwards encounter higher frequencies of scrums, lineouts and rucks relative to backs (Brooks et al., 2005, Sedeaud et al., 2012). However, previous studies suggest that playing position does not dramatically effect concussion incidence (Gardner et al., 2014), and emphasis remains on identifying effective risk reduction strategies for tackling at present (Cross et al., 2017, Rafferty et al., 2018).

Injury mechanism; focus on the neurovascular unit (NVU)

Concussion generates linear and rotational acceleration of the brain (Ommaya and Gennarelli, 1974). Several have attempted to identify a 'force threshold' for concussion diagnosis that remains elusive (Guskiewicz et al., 2007). However, advancements in wearable microtechnology now suggest that average collision forces in elite-level rugby are 19*g* (MacLeod et al., 2018), surpassing the 18*g* threshold that triggers the medical warning light used in Formula 1 racing requiring drivers to undergo a medical examination (International Federation of Automobiles, 2020).

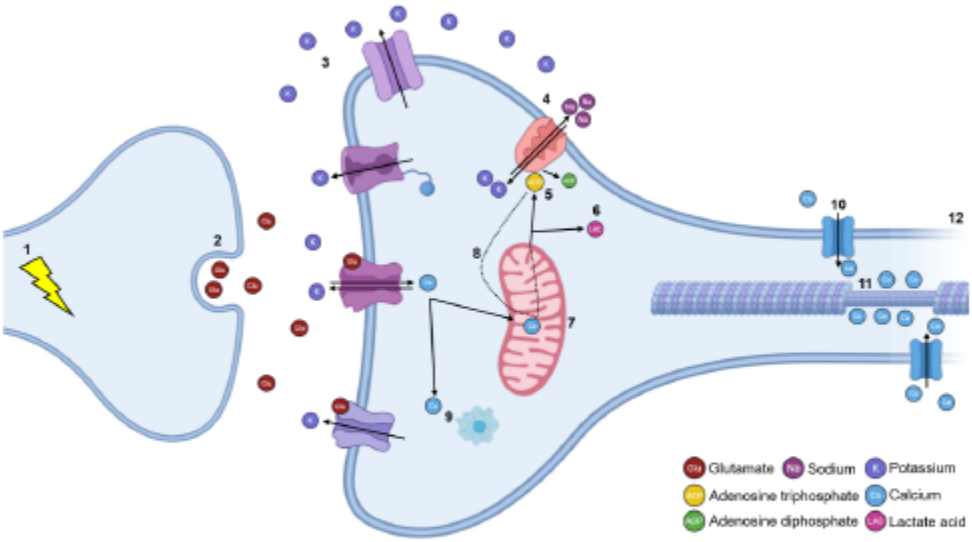

Figure 3: Neurometabolic cascade following concussion. Depolarisation of pre-synaptic neuron causes initiation of action potential (**1**). The release of excitatory neurotransmitters from the pre-synaptic neuron (**2**) bind with receptors of the post-synaptic neuron to generate potassium efflux (**3**). Restoration of homeostasis is achieved by increased activity of sodium-potassium-ATPase pumps (**4**) via hyperglycolysis (**5**) which causes lactate accumulation (**6**). Calcium influx and sequestration in mitochondria causes impaired oxidative metabolism (**7**) and decreased adenosine triphosphate (ATP) production (**8**). Calcium accumulation causes activation of calpain which initiates apoptosis (**9**). Disruption in axon initiates calcium influx (**10**) and phosphorylation which causes neurofilament compaction (**11**) and microtubule disassembly to generate axonal swelling and eventual axotomy (**12**). Recent evidence indicates that a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability adversely impacts the structural integrity, molecular signaling and flow autoregulation across the neurovascular unit, established biomarkers underlying cognitive decline and dementia Adapted from Giza and Hovda (2001) and (Bailey et al., 2020).

Much interest has focused on the neurometabolic cascade associated with concussion (Giza and Hovda, 2001), whereby acceleration of brain tissue provokes an immediate loss of ionic homeostasis, namely intracellular calcium accumulation preceding mitochondrial dysfunction and microtubule disassembly (Figure 3; Giza and Hovda, 2014). Evidence of increased

oxidative-nitrosative stress (OXNOS) subsequent to mitochondrial dysfunction has been identified in the rodent model (Vagnozzi et al., 2007, Tavazzi et al., 2007) and preliminary data from our research group suggest that both current elite-level and retired rugby players are characterised by elevated OXNOS. We have since identified OXNOS, defined by a free radical-mediated reduction in the systemic bioavailability of nitric oxide (NO) predisposes cerebrovascular dysfunction and cognitive decline (Owens et al., 2018, Owens et al., 2019a, Bailey et al., 2019).

Like OXNOS, research is focused on the development and identification of prognostic biomarkers of concussion for point-of-injury (POI) testing and athlete recovery. The integrity of the NVU is compromised by concussion, which allows brain-derived proteins to cross a 'leaky' blood brain barrier (BBB) for detection in peripheral blood, saliva and urine (Figure 4; Zetterberg and Blennow, 2016). While concussion diagnosis capabilities through elevation of single or multiple biomarkers remains elusive at present, elevated concentrations of S100β have been confirmed among concussed rugby players and research is ongoing for detection of salivary micro-RNA following injury (Bouvier et al., 2017, Yakoub et al., 2018). Similarly, evidence of neuronal and astrocytic damage in various contact sport athletes have been confirmed by elevated concentrations of neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1) and tau (Marchi et al., 2013, O'Connell et al., 2018, McCrea et al., 2020). These are significant advances given that the NVU forms the functionally integrated cellular network responsible for maintaining structural integrity of the BBB and autoregulation of cerebral blood flow (CBF) through neurovascular coupling (NVC, Kaplan et al., 2020). This ensures tight coupling between oxygen (O_2)/glucose supply and demand, to which the human brain has evolved heightened sensitivity (Bailey, 2019b). Functional impairments in BBB integrity and NVC have been shown to precede, or at least associate with, cognitive decline and dementia (Kisler et al., 2017).

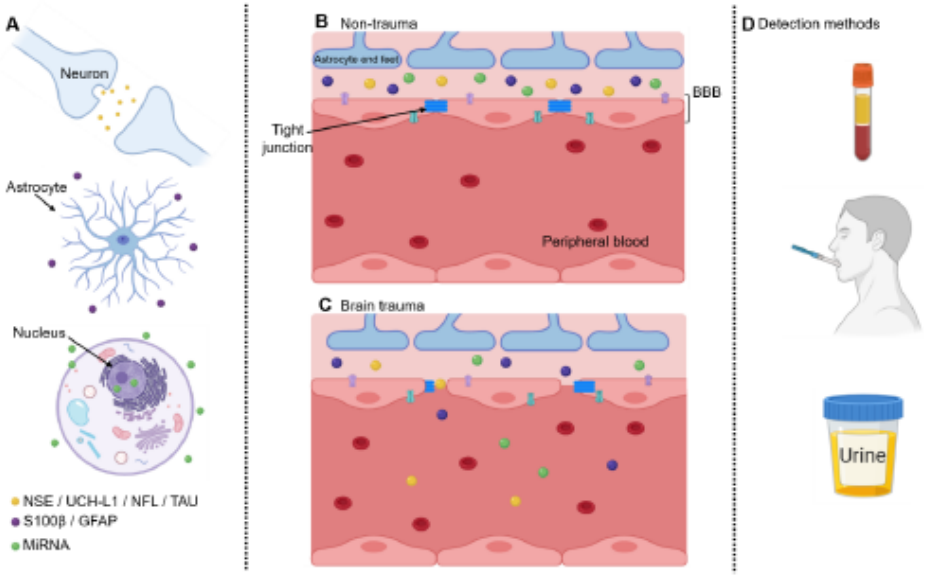

Figure 4: Contemporary biomarkers of concussion. Neurovascular cells are sources of specific proteins (colour coded; **A**), incapable of accessing peripheral blood through an intact blood brain barrier (BBB; **B**). Concussion compromises BBB integrity (**C**), allowing brain-specific proteins to be detected in peripheral blood, saliva and urine (**D**).

Cerebrovascular function

Adequate CBF is vitally important as the brain consumes 20% of the body's energy at rest (Bailey, 2019a, Bailey, 2019b). The cerebrovascular profiles of concussed rugby players have rarely been studied, although emerging evidence suggests that retired rugby players (≥ 3 previous concussions) are characterised by impaired NVC and reduced cortical oxygenation of the middle frontal gyrus (Sharma et al., 2020). Evidence from concussed American footballers suggest that CBF is decreased at baseline and across a competitive season when compared to a non-concussed control group (Churchill et al., 2020), while computational models suggest that the thalamus elicits the greatest shear stress of any brain region, corresponding to a 50% reduction in regional perfusion and subsequent cognitive impairment (Grossman et al., 2013). Similarly, prolonged cerebral hypoperfusion (>1 month post-injury) has been associated with poorer clinical outcome and increased symptomology (Meier et al., 2015), while others have observed that resting perfusion returns to baseline within five days of injury (Len et al., 2011, Bailey et al., 2013, Len et al., 2013). Therefore the true extent to which long-term CBF deficits

prevail after concussion remains unclear given the variability between-subjects and methods of assessment.

Athletes that are apparently asymptomatic at rest within days of concussion may present with headache, dizziness and migraines during exercise (Gardner et al., 2015). Evidence suggests that this is a consequence of impaired cerebrovascular reactivity (CVR), reflecting the (reduced) ability of the vascular bed to vasodilate or constrict in response to fluctuations in arterial carbon dioxide concentration ($PaCO_2$, Ainslie and Duffin, 2009). Impaired CVR is associated with increased susceptibility to neurodegenerative diseases in later-life, therefore examining cerebrovascular response to physiological stressors after a concussion are important for detecting impairments that are not apparent at rest, in effect, forcing the signal out of the noise (Sweeney et al., 2018, Churchill et al., 2019). Concussed rugby players have elicited impaired CVR compared to a non-concussed control group within 7 days of injury (Churchill et al., 2019). Similarly, Len et al. (2011), Bailey et al. (2013) and Len et al. (2013) have demonstrated impaired CVR among hockey players and boxers with no impairment in resting cerebral perfusion 2-5 days after injury.

Cognition

Concussion is often associated with neurological disorders including mild cognitive impairment (MCI), particularly in ageing athletes with a history of multiple concussions (Guskiewicz et al., 2005). Cognitive impairment after concussion is commonly short-lived, lasting between 7-10 days (Figure 5; Belanger and Vanderploeg, 2005, Belanger et al., 2010, Cross et al., 2016) and aligns with the recovery of metabolic and cerebrovascular function (Bailey et al., 2013, Giza and Hovda, 2014). Several research groups have documented frontotemporal impairments in cognition among past and present rugby players. These include deficits in memory (Cross et al., 2016, McMillan et al., 2017), attention (King et al., 2013, Hume et al., 2017), visuomotor coordination and processing speed (Shuttleworth-Edwards et al., 2014, Hume et al., 2017), while others have observed MCI and absence of learning effects (Gardner et al., 2010, Decq et al., 2016).

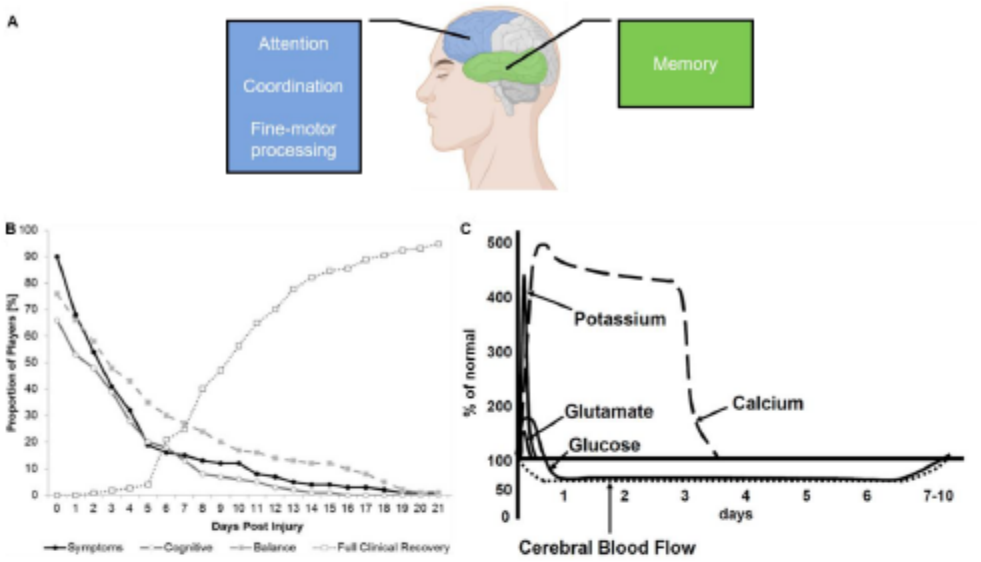

Figure 5: Frontotemporal cognitive impairments are most commonly reported among current and retired rugby players with concussion history (**A**). Cognitive impairments in the majority of concussed elite rugby union players resolve within 7-10 days of injury (n = 100) (**B**); corresponding with the recovery of ionic imbalances and cerebral blood flow subsequent to the neurometabolic cascade (**C**). Adapted from Giza and Hovda (2014) and Cross et al. (2016).

Neurodegeneration

Retired contact sport athletes with concussion history are at an increased risk of developing chronic traumatic encephalopathy (CTE), a unique form of neurodegeneration that presents 10-20 years following retirement from contact sports (McKee et al., 2009, McKee et al., 2016). Brain atrophy is a hallmark of CTE, which is associated with a subset of behavioural changes and functional disorders including depression and subsequent suicide tendency (Omalu et al., 2006). Some research groups have confirmed CTE pathology in up to 99% of brains donated by deceased National Football League players (Mez et al., 2017), however few cases of CTE have been confirmed in former rugby players despite comparable concussion incidence between both sports (Prien et al., 2018).

Stewart et al. (2016) suggests that CTE is an under-recognised consequence of rugby, while Lee et al. (2019) performed brain autopsies in four deceased retired rugby union players diagnosed with dementia and confirmed CTE pathology among three. Common macroscopic

observations include atrophy of the frontal and temporal lobes, while microscopic inspection commonly uncovers perivascular hyperphosphorylated tau (p-tau) lesions, neurites and thorn shaped astrocytes clustered around cortical vessels, including β-amyloid and TAR DNA-binding protein 43 deposition (Stewart et al., 2016, Lee et al., 2019). CTE has further been confirmed in rugby league and Australian Rules players (McKee et al., 2014, Buckland et al., 2019), however the mechanisms that underpin development of the disease remain largely unknown, given that only a minority of athletes present with these unique behavioural and pathological alterations.

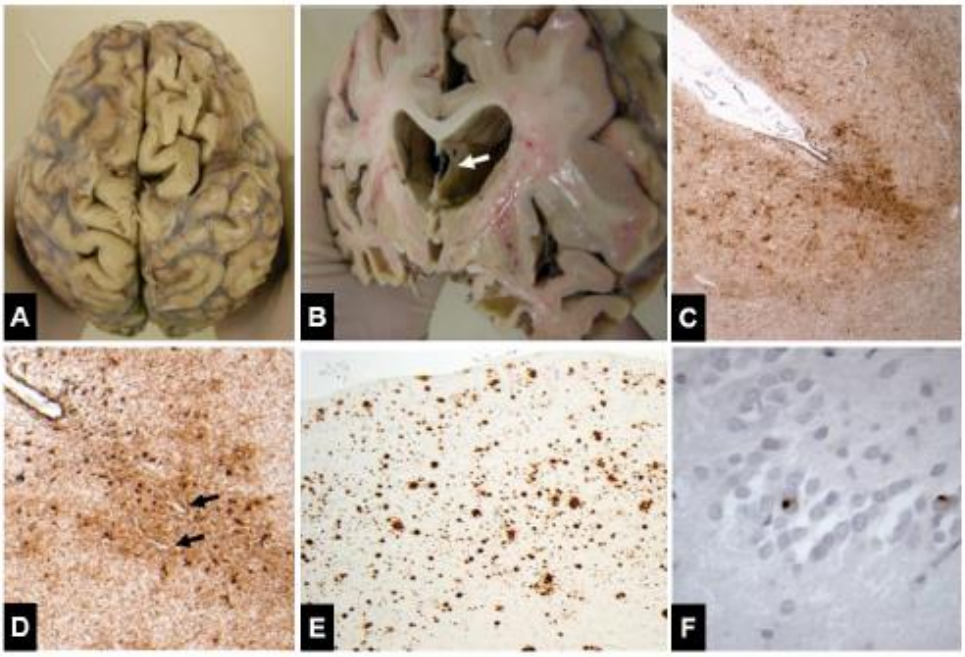

Figure 6: Representative pathology of chronic traumatic encephalopathy in rugby union players. Frontal lobe atrophy and sulcal thinning (**A**) associated with ventriculomegally and fenestration of the septum pellucidum (white arrow; **B**); hyperphosphorylated tau (p-tau) at the depths of the cortical sulci (**C**) clustered around small blood vessels (black arrows; **D**); β-amyloid plaques (**E**) and TAR DNA-binding protein 43 (TDP-43) deposition have also been observed (**F**). Sourced from (Stewart et al., 2015) and (Lee et al., 2019).

Perspectives

Rugby is a gladiatorial sport with a Janus face and while there is growing public concern over the perceived risk of early-onset dementia in former rugby union players, it is important not to

lose sight of the neuroprotective benefits conferred by regular physical activity. There is a clear need for more research to elucidate the integrated molecular-haemodynamic-structural mechanisms and associated biomarkers underpinning neurodegeneration, to better inform clinical management of players.

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Original Article

The changing nature of concussion in rugby union: Looking back to look forward

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Abstract

Introduction: Concussion is regularly observed in rugby union and has generated a growing public health concern, yet remains one of the least understood injuries facing the sports medicine community. Evidence suggests that multiple concussions may increase susceptibility to long-term neurological complications that present decades after the initial injury for reasons that remain unclear. We aimed to determine the incidence rate and risk factors for concussion amongst community-level rugby union-15s players active during the 1980s given that it may help to better understand the risks and mechanisms of injury.

Methods: Injury data were collected from clubs by the coach at the time of injury in players using a 15-item questionnaire (1982–1984).

Results: Seventy games were recorded throughout 1982–1983 and 1983–1984 rugby union seasons. Forty-two documented concussions accounted for ~6% of injuries corresponding to an incidence rate of 0.64 per 1000 playing hours, more than a third lower than the 'modern-day' equivalent. Tackling (relative risk 1.60, $p < 0.05$), collisions (relative risk 0.95, $p < 0.05$) and gum shield use (relative risk 1.69, $p < 0.05$) were independently associated with concussion whereas no associations were observed for ground condition, quarter of play or players playing out of position ($p > 0.05$).

Conclusion: Despite limitations due to the retrospective focus and reliance on questionnaire data notwithstanding raised awareness of concussion, the incidence rate of concussion during the 1980s appears to be appreciably lower compared to the present-day game. This is the likely outcome of improvements in the clinical understanding of concussion, data collection tools, reporting methods and clinical management of concussive injuries, including changes to both player and game. However, the findings of this study help better understand the risks and mechanisms of injury once encountered by rugby union players active during the 1980s, of which some of those risks are still apparent.

Keywords

Concussion, traumatic brain injury, incidence, risk factors

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Introduction

Concussive injury has generated a growing public health concern yet remains one of the least understood injuries facing the sports medicine community today. Emerging evidence suggests that multiple concussions sustained during young adulthood may increase susceptibility to long-term neurological complications that typically present decades after the initial injury for reasons that remain unclear.[1] These complications include accelerated cognitive decline, chronic traumatic encephalopathy (CTE), depression, Parkinson's syndrome and Alzheimer's disease.[2,3] Subsequently in recent years, focus has centred towards retired contact sport athletes who may be more susceptible to neurological sequela as a result of recurrent concussion.[1]

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Since the advent of professionalism was introduced to rugby union in 1995, participation throughout the United Kingdom (UK) has risen to 2.5 million players.[4] A recent meta-analysis of community sub-elite rugby union players identified a concussion incidence rate of 2.08/1000 player match hours and speculated this trend would continue to rise with the current reporting methods.[5] Furthermore, professional rugby union players in the UK are more likely than not to sustain a concussion within 25 games,[6] thus generating future health concerns among the medical community and players alike.

A number of intrinsic and extrinsic factors contribute towards concussion risk including: playing position, tackling technique, use of protective head/mouth apparatus, neck strength, warm-up strategy, foul play, quarter of play, ground condition and weather, including various others.[6–10] Given that the majority of the available literature relating to concussion and risk of neurological impairment centres towards retired contact sports athletes aged between 50 and 80,[1] it is seldom supported by injury data from those cohorts, other than individual recall of past events[11] and questions whether today's athletes are exposed to the same risks as once encountered by their senior counterparts. This uncertainty is likely due to the primitive recorded injury data throughout the 'amateur years' of rugby union. However, some rugby union injury data throughout this period in both the adult and schoolboy levels exists. Durkin[12] observed injures in British adult rugby union players over the course of the 1972–1976 seasons and observed that 5.6% of all injures were concussions. Sparks[13] recorded over half a million hours of schoolboy rugby between 1950 and 1980 and observed 9885 injuries, of which 513 (5.2%) were concussion. South African school boy rugby injuries were reported by Nathan et al.[14] and Roux et al.[15] who observed concussion in 22% and 12% of all injuries respectively.

In addition, the understanding of concussion has improved, thus improving the standard of clinical care provided to those with suspected injury and making comparisons between the 'amateur' and present game difficult. For instance, throughout the beginning of the 1980s, a concussion was clinically defined as a loss of consciousness or a loss of awareness following a blow to the head,[16] which was later evolved by Cantu[17] into three categories (mild, moderate or severe). The long-term neurological consequences of concussion were poorly understood and while literature had documented dementia pugilistica[18] among boxing cohorts and later described as CTE,[19] no such evidence existed in rugby union.

Data collection for all injuries in rugby union have improved drastically in the modern day due to the consensus definitions and methodologies to standardise the recording of injuries and reporting of studies which was introduced by the Rugby Injury Consensus Group (RICG) in 2007.[20] This is accompanied by a research determined definition of concussion that is a 'traumatic brain injury induced by biomechanical forces' which is accompanied by a number of symptoms including headache, dizziness, balance/gait abnormalities, confusion, amnesia and various others which can occur without loss of consciousness.[21] Clinical questionnaires specific to concussion including the Sports Concussion Assessment Tool 5th edition and the Head Injury Assessment further allow certified athletic trainers and medical professionals alike to recognise concussion and remove athletes from play, while governing bodies have pre-defined return to play protocols to ensure athletes have recovered adequately before returning to competition.[22] Despite these comparative difficulties, the importance of a detailed injury history has time again been emphasised to be of relevance when diagnosing neurological disorders.[11]

Given the evidence presented, retrospective injury data from the period may be of relevance to retired contact sports athletes and clinicians, to better understand the risks and mechanisms of injuries once encountered. We sought to determine the incidence and corresponding in-game variables and risk factors for concussion among Welsh rugby union players who were active during the 1980s.

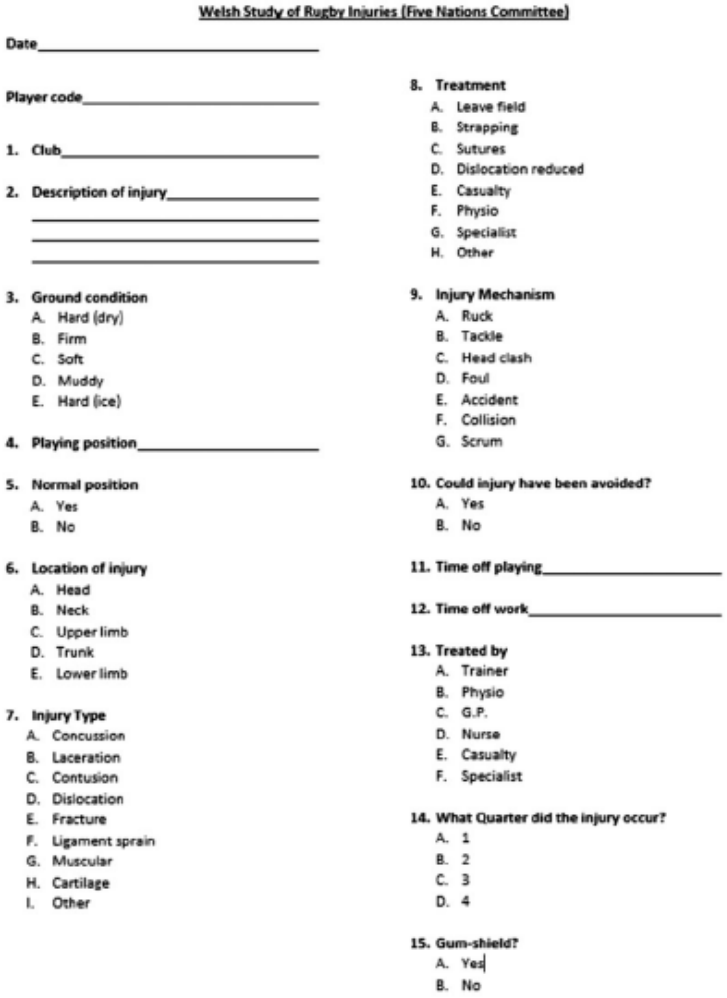
Methods

Participants

Information was obtained from a total of 708 college and senior level rugby union-15s players from clubs across Wales using a 15-item questionnaire (Figure 1) at the time of injury by the team coach between the 1982–1983 and 1983–1984 rugby union seasons. All players and coaches from the selected clubs were invited to participate. All players and coaches who participated provided written and verbal informed consent with data collection overseen by a general practitioner and consultant orthopaedic surgeon-player.

Procedures

Questionnaires included intrinsic and extrinsic factors associated with injuries and each player was assigned an identification code with anonymised datasets subsequently uploaded to a computer database for analysis. Concussion was defined by loss of consciousness or a loss of awareness following a blow to the head,[16] including symptoms of amnesia (personal



Welsh Study of Rugby Injuries (Five Nations Committee)

Date_____

Player code_____

1. **Club**_____
2. **Description of injury**_____
3. **Ground condition**
   A. Hard (dry)
   B. Firm
   C. Soft
   D. Muddy
   E. Hard (ice)
4. **Playing position**_____
5. **Normal position**
   A. Yes
   B. No
6. **Location of injury**
   A. Head
   B. Neck
   C. Upper limb
   D. Trunk
   E. Lower limb
7. **Injury Type**
   A. Concussion
   B. Laceration
   C. Contusion
   D. Dislocation
   E. Fracture
   F. Ligament sprain
   G. Muscular
   H. Cartilage
   I. Other
8. **Treatment**
   A. Leave field
   B. Strapping
   C. Sutures
   D. Dislocation reduced
   E. Casualty
   F. Physio
   G. Specialist
   H. Other
9. **Injury Mechanism**
   A. Ruck
   B. Tackle
   C. Head clash
   D. Foul
   E. Accident
   F. Collision
   G. Scrum
10. **Could injury have been avoided?**
    A. Yes
    B. No
11. **Time off playing**_____
12. **Time off work**_____
13. **Treated by**
    A. Trainer
    B. Physio
    C. G.P.
    D. Nurse
    E. Casualty
    F. Specialist
14. **What Quarter did the injury occur?**
    A. 1
    B. 2
    C. 3
    D. 4
15. **Gum-shield?**
    A. Yes
    B. No

Figure 1. Fifteen-item questionnaire for injury reporting.

communication with general practitioner). The incidence rate was subsequently calculated as

$$\frac{\text{Total number of players with concussion}}{\left\{\begin{array}{l}\text{Exposure (number of matches}\\ \times\text{match duration} \times \text{number of players)}\end{array}\right\}} \times 1000(\text{h})$$

Statistical analysis

Statistical analyses were completed using the Statistics Package for Social Scientists (Version 23.0). Data were first categorised into concussion or other injuries for each category observed via the 15-item questionnaire. To determine association of injury, 2 × 2 Pearson chi square (χ) tests were used however in the event that more than 20% of variables had expected cell counts below five, likelihood ratios (LH) were calculated as a surrogate measure.[23] Throughout association tests, relative risk (RR) of injury were computed simultaneously and incidence rates were calculated for all variables manually. Players with cases of missing data for the observed factor were excluded from the overall analyses.

Results

Seventy games were observed throughout 1982–1983 and 1983–1984 rugby union seasons among 708 players. A total of 178 injuries were classified as head

Table 1. Factors associated with concussion incidence.[a]

	Frequency	Percent	IR	RR	95% CI	p value
Playing position						
Front row	4	11	0.06	0.32	0.12–0.84	**0.001**
Lock	6	17	0.09	1.40	0.58–3.41	0.47
Loose forward	4	11	0.06	0.50	0.19–1.36	0.15
Inside back	9	26	0.14	1.73	0.84–3.58	0.14
Midfield back	3	9	0.05	1.09	0.31–3.84	0.89
Outside back	9	26	0.13	1.28	0.65–2.54	0.48
Cause of injury						
Tackle[b]	21	50	0.32	1.60	1.08–2.36	**0.02**
Foul	2	5	0.03	0.18	0.05–0.73	**0.001**
Collision[c]	2	5	0.03	0.95	0.89–1.02	**0.02**
Ruck	11	26	0.16	0.98	0.55–1.74	0.93
Head clash[d]	4	10	0.06	0.91	0.32–2.62	0.86
Accident[e]	1	2	0.02	1.06	0.11–9.96	0.96
Scrum	1	2	0.02	0.80	0.09–6.94	0.83
Gum shield						
Yes	18	69	0.27	1.69	1.12–2.51	**0.02**
No	8	31	0.12	0.52	0.28–0.96	
Ground condition						
Hard	6	16	0.09	0.85	0.37–1.93	0.69
Firm	11	29	0.16	0.70	0.41–1.12	0.17
Soft	15	39	0.23	1.41	0.87–2.30	0.18
Muddy	5	13	0.08	1.09	0.43–2.80	0.86
Icy	1	3	0.02	0.77	0.09–6.68	0.81
Quarter of play						
1	5	16	0.08	0.95	0.37–2.48	0.92
2	10	32	0.15	1.20	0.66–2.18	0.55
3	7	23	0.11	0.70	0.34–1.45	0.32
4	9	29	0.13	1.37	0.68–2.80	0.39
Usual playing position						
Yes	35	92	0.53	1.03	0.93–1.16	0.58
No	3	8	0.05	0.72	0.22–2.38	

IR: incidence rate (per 1000 playing hours); RR: relative risk; CI: confidence interval.
[a]Percentages may not total to 100% due to rounding;
[b]Tackle defined as a collision where opposing player uses arms to ground player in possession of the ball.
[c]Collision, collision where opposing player does not use arms to ground player in possession of the ball.
[d]Head clash, contact of heads when a player was in possession/not in possession of the ball.
[e]Accident, an unintended collision while a player was in possession/not in possession of the ball. The values given in bold highlight the significant findings ($p < 0.05$).

injuries (26% of all injuries). We observed 42 concussions (~6%) corresponding to an incidence rate of 0.64 per 1000 playing hours (~1 concussion every 1.7 games).

Injury data are outlined in Table 1. Tackling ($\chi = 4.84$, $p < 0.05$, RR, 1.60, 95% CI, 1.08–2.36), collisions (LH = 5.81, $p < 0.05$, RR 0.95, 95% CI, 0.89–1.02) and gum shield use ($\chi = 5.82$, $p < 0.05$, RR 1.69, 95% CI, 1.12–2.51) were independently associated with concussion. In contrast, front row players were at lowest risk of injury compared to the backs ($\chi = 7.12$, $p < 0.05$, RR 0.32, 95% CI, 0.12–0.84) and fouling posed the lowest risk of concussion ($\chi = 8.78$, $p < 0.05$, RR 0.18, 95% CI, 0.05–0.73). No associations were observed between concussion and ground condition (LH = 2.27, $p > 0.05$), quarter of play ($\chi = 1.34$, $p > 0.05$) and players playing out of position (LH = 0.31, $p > 0.05$).

Discussion

Our descriptive findings have provided a unique insight into the changing nature of concussion and associated risk factors from rugby union during the 1980s against the modern day game. Notwithstanding the limitations of the current investigation, the incidence rate of concussion nearly four decades ago aligned closely to other injury data available from rugby union players during

the 1980s. Furthermore, this retrospective data have identified risk factors once encountered by past athletes, of which some of those risks are still apparent in the modern era. Comparatively, concussive incidence was seen to be appreciably lower and some risk factors were not entirely consistent with what has been reported in the published literature during the modern era. This is a result of greater clinical management of concussion in modern rugby union, assisted by methods that better recognise and remove an athlete from play safely following injury.

Historical comparisons

In the present study, head injuries were shown to account for approximately one quarter of all injuries corresponding to an incidence rate of 0.64 concussions per 1000 playing hours. We further calculated that concussive injury accounted for 6% of all injures which replicates the earlier findings of Durkin.[12] Additionally, Sparks[13] documented that 16.9% of all injuries recorded were to the head and neck which is appreciably lower than our observations, however the overall percentage of concussive injuries were similar (5%). Our observations of concussion were substantially lower than the 22% and 12% documented by Nathan et al.[14] and Roux et al.[15] in South African school-level rugby union. However, our results align with data from other southern hemisphere regions during that period as Davidson[24] observed 24.5% of injuries to the head and neck among Australian rugby union players.

While data for factors associated with concussion were primitive, previous literature acknowledged that tackling was the primary mechanism for injury[13,15] and front row players were at lower risk of concussion relative to the backs,[15] which corresponds with our findings. However, our observations revealed that hard ground did not increase the risk of concussion, contrary to the findings of others.[13,15] The discrepancies between these studies are likely due to the variation of data collection tools, study sizes, definition of concussion and subsequent clinical management provided following injury, including international differences in health care procedures.

Modern comparisons

Given the inevitable discrepancies in injury definition across studies, our calculated incidence rate is appreciably lower than the 'modern-day' equivalent of 2.08 (range of 1.2–6.9) cited in a recent meta-analysis of players at a similar standard (community, sub-elite 15s) who are at greatest risk of injury.[5] This more than tripling in incidence is the likely consequence of changes to knowledge, identification, reporting and management of concussion within modern day rugby union.[20,21]

Principally, the introduction of the consensus definitions and methodologies to standardise the recording of injuries and reporting of studies[20] has altered injury reporting within rugby union to great effect. Injuries are defined and data are now collected in accordance to whether an injury is: recurrent, non-fatal or catastrophic, and classified by severity, location, type, diagnosis and cause. All injuries are further recorded in relation to training and match exposures, providing detailed medical records for all athletes, thereby allowing qualified health professionals and coaches to better recognise concussive injuries and typical severity characteristics. Moreover, as reporting and recognition of concussion has developed among health professionals and coaching staff, athlete under-reporting of concussive injury has been identified as a key area of improvement.[25] In turn, concussion awareness and education programmes are now utilised to varying degrees from school level onwards, in a bid to enable athletes to better recognise and self-report concussive symptoms.[26]

During the 1980s, no such tools or consensus agreements had been formed, thus highlighting the limitations of this investigation. However (to the best of our knowledge), the 15-item questionnaire utilised throughout the 1982–1984 rugby union seasons in the current investigation was the first of its kind within the United Kingdom and indeed may be of relevance to better understand the mechanisms of previous injuries that may apply to a number of retired athletes. For example, the 15-item questionnaire (Figure 1) shares eight similarities between the Injury Report Form for Rugby Union as constructed by the RICG,[20] which was introduced some 25 years later and still utilised today.

Indeed with the advent of professionalism, training methods have changed such that the 'modern game' now sees players who are more skilful, powerful, conditioned and heavier[27] with increased speed and force of contact events, duration of time the ball is in play and number of tackles/rucks per game[28] that collectively increase concussion risk. In support, tackling was identified as one of the primary risk factors for concussion and continues to prevail in the modern game especially, with the number of tackles seen to quadruple following the advent of professionalism.[28] Front row players were at a lower risk compared to the backs, again consistent with the published literature,[8] likely due to limited opportunity to run with the ball and fewer tacking incidents.[29]

However, some of the risk factors identified in the 1980s were not entirely consistent with what has been reported in the modern game.[5] Fouling has previously

been associated with an increased risk of concussion[30] whereas we observed the contrary. Likewise, we failed to confirm previous reports of an increased incidence of concussion during the third quarter of play (40–60 min) subsequent to insufficient warm up following the half-time break[31] and play on hard ground.[30] Finally, gum shield use that was beginning to be actively encouraged during the 1980s (personal communication personal communication JPR Williams) increased concussion risk in contrast to recent findings.[30] With consideration towards the biomechanics and attendant forces during rugby events, the extent that gum shields could reduce the incidence of brain injury and concussion remains unclear.

Limitations

There are inevitable limitations to this study given its retrospective focus and reliance on questionnaire data. The understanding of concussion from the period of data collection to the modern day has evolved to the extent that the definitions used to diagnosis are different and may highlight the underreporting of such injuries throughout the 'amateur years'. Although medical assessments were carried out by qualified clinicians following injury, data collection forms were populated via the team coach and may subsequently overlook relevant medical information relating to an athlete's injury. Furthermore, we were not in a position to record player demographics including concussion history thus information on concussion severity, residual symptoms from any prior concussions reported and medical clearance to return-to-play were not captured. Finally, we did not assess the long-term functional alterations in these players that would have allowed us to determine to what extent, if indeed any, enduring cumulative cognitive, cerebrovascular and motor function impairments were incurred as a result of concussions sustained decades earlier.

Conclusion

The present findings highlight the changing nature of concussion incidence rates in rugby union since the 1980s. The incidence rate of concussion during the 1980s appears to be appreciably lower compared to that reported in the modern (present-day) game,[5] the likely outcome of improvements in the clinical understanding of concussion, data collection tools, reporting methods and clinical management of concussion,[20] including changes to both player and game.[27] From a clinical perspective, this report allows us to better understand the risks and mechanisms of injury once encountered by rugby union players that were active during the 'amateur period' of the sport, of which some of those risks are still apparent in the modern era and may be priming athletes for future neurological symptoms.

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ORIGINAL ARTICLE

WILEY

Impaired cerebral blood flow regulation and cognition in male football players

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Football players are at increased risk of neurodegeneration, the likely consequence of repetitive mechanical trauma caused by heading the ball. However, to what extent a history of heading the ball affects cerebral blood flow (CBF) regulation and its potential relationship to cognitive impairment is unknown. To address this, we recruited 16 concussion-free male amateur football players (age: 25 ± 6 y) with a history of heading the ball (18 ± 6 y) and 18 sex, age, education, and activity-matched controls with no prior history of contact sport participation or concussion. Cerebral perfusion was measured at rest and in response to both hyper/hypocapnia to determine cerebrovascular reactivity to carbon dioxide ($CVR_{CO2HYPER/HYPO}$) using transcranial Doppler ultrasound and capnography, with the sum reflecting the cerebral vasomotor range. Cognition and visuomotor coordination were assessed using the Montreal cognitive assessment (MoCA) and the Grooved Pegboard Dexterity Test (GPD), respectively. While no differences in cerebral perfusion were observed ($p = 0.938$), $CVR_{CO2HYPER/HYPO}$ ($p = 0.038/p = 0.025$), cerebral vasomotor range ($p = 0.002$), MoCA ($p = 0.027$), and GPD performance (dominant hand, $P \leq 0.001$) were consistently lower in the players compared to controls. These findings are the first to demonstrate that CBF regulation and cognition are collectively impaired in male football players with history of heading the ball, which may contribute to neurodegeneration.

KEYWORDS
cerebral blood flow, cognition, football, soccer, traumatic brain injury

1 | INTRODUCTION

Football players are at increased risk of neurodegeneration including dementia in later life,[1] the likely consequence of repetitive mechanical trauma caused by heading the ball.[2] While research has traditionally focused on structural abnormalities,[3] there has been less attention focused on potential impairments in cerebral blood flow (CBF) regulation to which cerebrovascular reactivity to carbon dioxide (CVR_{CO2}) is a primary determinant.

The CVR_{CO2} of blood vessels supplying the frontotemporal regions of the brain [ie, served by the middle cerebral artery (MCA)] may be particularly vulnerable due to direct contact with the ball during heading. In support, a recent study identified a suppression of MCAv-derived neurovascular coupling during a visual task after heading the ball, with (anterior) flow suppression corresponding specifically to the site of mechanical impact.[4] This is of potential concern given that impaired CVR_{CO2} is an established risk factor for both stroke[5] and neurodegenerative[6]

diseases. Indeed, Svaldi and colleagues have reported impaired CVR_{CO2} in female high school football players compared to non-collision sport controls,[7] as well as following a season of playing football.[8] This may be the consequence of increased oxidative-inflammatory-nitrosative stress (OXINOS) induced by the mechanical trauma caused by heading, that we have previously linked to impaired cerebrovascular and cognitive function, albeit in different cohorts.[9] However, to what extent heading the ball impacts CVR_{CO2} and cognition in apparently healthy, physically active male football players remains to be investigated. To address this knowledge gap, the present exploratory study was designed to provide preliminary insight into what extent a history of heading the ball has on CBF regulation and cognition in male players.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

The study was approved by the University of South Wales ethics committee (approval #19CM0901HR) and conformed to the procedures set forth by the Declaration of Helsinki, except for registration in a database. All participants were informed of the requirements of the study and provided written informed consent.

2.2 | Design and participants

A cross-sectional study design was utilized to compare select determinants of CBF regulation and cognitive function in male amateur football players ($n = 16$) and non-contact controls ($n = 18$). The players consisted of 7 defenders, 2 midfielders, and 7 strikers, while the control group consisted of recreational endurance athletes (eg, cyclists, runners, rowers). Groups were matched for key demographics (Table 1), with none of the participants reporting a prior history of concussion. All participants were free of cardiovascular, pulmonary, and cerebrovascular disease and were not taking any nutritional supplements including over-the-counter antioxidant or anti-inflammatory medications. They were advised to refrain from physical activity, caffeine, and alcohol and to follow a low nitrate/nitrite diet 24 h prior to formal experimentation.[10] Participants attended the laboratory following a 12-h overnight fast.

2.3 | Procedures

2.3.1 | Playing history and physical activity levels

Playing history was self-reported via a non-validated study-specific questionnaire, asking players to recall their playing position, experience, and heading history. Physical

TABLE 1 Participant demographics and cerebrovascular function

	Controls ($n = 18$)	Players ($n = 16$)	*P* Values	*d* Values
Demographics				
Age (y)	26 ± 6	25 ± 6	0.646	−0.15
Body mass index ($kg.m^{-2}$)	23.4 ± 3.1	25.3 ± 3.2	0.087	0.61
Total education (y)	16 ± 2	16 ± 2	0.772	−0.05
Physical activity (min.week)	360 ± 124	354 ± 106	0.875	−0.05
Playing history (y)	0 ± 0	18 ± 6†	<0.001	6.50
Heading frequency (per game)	0 ± 0	9 ± 4†	<0.001	4.08
Cerebrovascular				
MCAv ($cm.s^{-1}$)	60 ± 12	61 ± 10	0.938	0.03
MAP (mm Hg)	94 ± 11	91 ± 11	0.450	−0.25
CVCi ($cm.s^{-1}.mm\ Hg^{-1}$)	0.64 ± 0.12	0.67 ± 0.14	0.972	0.22
CVRi ($mm\ Hg^{-1}.cm.s^{-1}$)	1.55 ± 0.26	1.54 ± 0.31	0.479	−0.01
HR (bpm)	61 ± 10	64 ± 8	0.359	0.34
Baseline PET_{CO2} (mm Hg)	42 ± 6	41 ± 3	0.624	−0.18
$PET_{CO2HYPER}$ (mm Hg)	52 ± 5	51 ± 3	0.465	−0.33
$PET_{CO2HYPO}$ (mm Hg)	29 ± 7	27 ± 4	0.691	−0.42

Note: Values are mean ± SD.

Abbreviations: CVCi/CVRi, cerebrovascular conductance/resistance indices; HR, heart rate; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; PET_{CO2}, partial pressure of end-tidal carbon dioxide; $PET_{CO2HYPER/HYPO}$, partial pressure of end-tidal carbon dioxide during hypercapnia/hypocapnia, respectively.

†$p < 0.050$ vs. control.

activity was self-reported using the validated Get Active Questionnaire[11] and reflected the volume of physical activity the participants completed in a typical week.

2.3.2 | Cerebrovascular function

A lead II electrocardiogram (Dual BioAmp; ADInstruments, Oxford, UK) was used to measure heart rate (HR). Finger photoplethysmography (Finometer PRO, Finapres Medical Systems) was used to measure beat-by-beat mean arterial blood pressure (MAP). Blood velocity in the middle cerebral artery (MCAv) was insonated through the right (or left if the right was not possible) temporal window at a depth of ~1 cm distal to the MCA-anterior cerebral artery bifurcation using standardized search techniques[12] with a 2 MHz pulsed transcranial Doppler ultrasound (TCD; Multi-Dop X4, DWL Elektronische Systeme GmbH, Germany). Cerebrovascular conductance and resistance indices were calculated as MCAv/MAP and MAP/MCAv, respectively. Capnography (ML 206, ADInstruments Ltd) was used to measure the partial pressure of end-tidal carbon dioxide (PET_{CO2}). Measurements were performed in an upright seated position at rest and in response to hyper/hypocapnia to assess $CVR_{CO2HYPER/HYPO}$. Following 10 min of seated rest, $CVR_{CO2HYPER}$ was assessed by breathing an inspiration of 5% CO_2 with 21% O_2 (balanced nitrogen) for 3 min to minimize inter-individual responses.[13] Following a 5-min recovery breathing room air to allow for baseline values to return, $CVR_{CO2HYPO}$ was determined following 3 min of controlled hyperventilation (15 breaths min^{-1}) where participants were verbally instructed to increase their tidal volume in order to reduce PET_{CO2}.

2.3.3 | Data sampling/analysis

Beat-by-beat data were continuously sampled at 1 kHz using an analogue-to-digital converter (Powerlab/16SP ML795, ADInstruments Ltd) and stored for offline analysis (Lab Chart v8.1.17). During analysis, baseline values were obtained by averaging the last 30 s of data collection following 10 min of rest. Both $CVR_{CO2HYPER/HYPO}$ were calculated as the percentage change in MCAv from baseline per 1 mm Hg change in PET_{CO2} recorded during the final 30 s (average taken) of the respective challenge once steady-state had been achieved:

$$CVR_{CO2HYPER/HYPO}\left(\%.\,mm\,Hg^{-1}\right) = 100\,x\,\frac{MCAv\ (final) - MCAv\ (baseline)}{MCAv\ (baseline)} / \left[PET_{CO2}\,(final) - PET_{CO2}\,(baseline)\right]$$

$CVR_{CO2HYPER/HYPO}$ were subsequently used to calculate the cerebral vasomotor range as detailed below[14]:

$$\text{Cerebral vasomotor range}\,(\%.\,mm\,Hg^{-1}) = CVR_{CO2Hyper}(\%.\,mm\,Hg^{-1}) + CVR_{CO2Hypo}(\%.\,mm\,Hg^{-1})$$

2.3.4 | Cognitive function

Cognitive function was determined using the Montreal Cognitive Assessment (MoCA)[15] and visuomotor coordination assessed using the Grooved Pegboard Dexterity Test (GPD)[16] using both dominant and non-dominant hands (Lafayette Instruments). All tests were completed in a quiet room free from distraction and administered by a trained investigator.

2.4 | Statistical analysis

2.4.1 | Power calculation

Sample size was determined using G* Power (version 3.1). Assuming a comparable difference in our primary outcome measure CVR_{CO2} as observed in our previous work with professional boxers,[14] it was determined that the present study required a (minimum) sample size of 26 participants (13 per group) in order to achieve (minimum) power of 0.80 at $p < 0.05$. We chose to further inflate this to the current sample size in the event of any potential technical complications during data collection.

2.4.2 | Inferential statistics

Following confirmation of distribution normality (Shapiro-Wilk *W* tests), independent samples *t tests* were used to determine differences between groups. Where data were not normally distributed (age, education, playing/heading history, cerebrovascular conductance index and $PET_{CO2HYPER/HYPO}$), the Mann-Whitney *U* test was used to determine differences between groups. Effect size for each reported outcome was retrospectively calculated using Cohen's equation[17] and reported as a *d* value. The relationship (pooled data) between cerebral vasomotor range and cognition (MoCA) was determined using the Pearson Product Moment correlation. SPSS (version 27.0; IBM) was used and statistical significance set at $p < 0.050$. All data are presented as mean ± SD.

3 | RESULTS

The findings of the study are outlined in Table 1 and Figure 1. By design, the players had extensive experience of playing football and heading the ball. While we observed no differences in basal cerebral perfusion ($p = 0.938$), $CVR_{CO2HYPER}$ (controls = 3.37 ± 1.21 vs. players = 2.67% ± 0.61%.mm Hg^{-1}, $p = 0.038$), $CVR_{CO2HYPO}$ (controls = 2.90 ± 0.78 vs. players = 2.35% ± 0.53%.mm Hg^{-1}, $p = 0.025$), cerebral vasomotor range (controls = 6.27 ± 1.17 vs. players = 5.02% ± 1.01%.mm Hg^{-1}, $p = 0.002$), MoCA (controls = 28 ± 2 vs. players = 26 ± 2 points, $p = 0.027$), and GPD performance (dominant hand, controls = 60 ± 7 vs. players = 72 ± 10 s, $P \leq 0.001$) were consistently lower in the players compared to controls. Reduced cerebral vasomotor range was associated with poorer MoCA performance ($r = 0.39$; $p = 0.021$).

4 | DISCUSSION

These findings are the first to demonstrate that CBF regulation and cognition are collectively impaired in male football players. Given that the players had no history of concussion, our findings suggest that the neurological deficits observed are the likely consequence of repetitive head trauma inflicted over the course of a player's career.

Our findings concur with previous studies highlighting impaired cognition in male football players.[18,19] Importantly, our study extends these findings providing mechanistic insight into the underlying mechanisms that may predispose to an accelerated trajectory toward neurodegeneration.[2] While no differences in cerebral perfusion were observed, players were characterized by impaired CVR_{CO2}, an established hemodynamic risk factor for cerebrovascular[5] and neurogenerative[6] disease. These findings are consistent with those

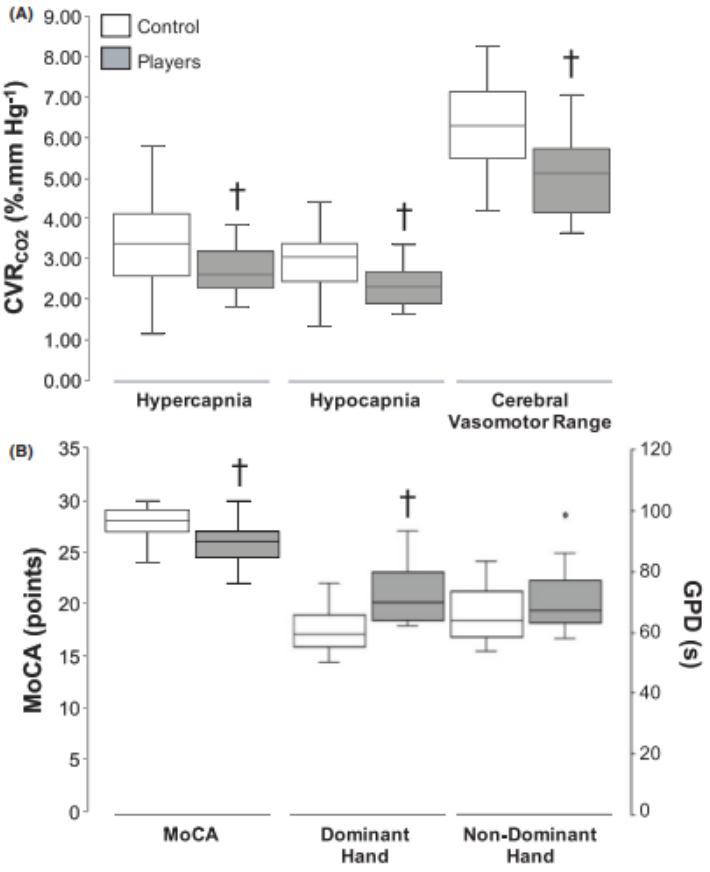

FIGURE 1 Boxplots for CVR_{CO2} (Panel A) and cognitive function (Panel B) data. CVR_{CO2}, cerebrovascular reactivity to changes in CO_2; MoCA, Montreal cognitive assessment; GPD, grooved pegboard dexterity test. †$p < 0.050$ vs. control

reported in female high school football players[7,8] and provide further evidence of impaired CBF regulation as a result of heading the ball.[4] Reduced CVR_{CO2} was also associated with poorer cognitive performance, highlighting the potential contribution of impaired cerebrovascular function to neurodegenerative risk.

To put this deficit into clearer clinical perspective, the magnitude of impairment in cerebral vasomotor range was identical to what we have previously documented in world-class professional boxers with 13 ± 4 years of international boxing.[14] Therefore, the simple act of repetitively heading a ball across a player's career may "outweigh" any neuroprotective benefit conferred by physical activity.[20,21] However, to what extent these vascular deficits are the cause or simply the consequence of structural abnormalities previously reported in football players[3] remains to be established.

Though speculative, the suppression of cerebral vasomotor range observed in the players may potentially be the consequence of elevated OXINOS, reflected by a free radical-mediated suppression of vascular nitric oxide bioavailability induced by repeated head trauma[22] experienced over the course of the playing career. In support, we have previously shown OXINOS to be associated with impaired CBF regulation[9,23] and cognition,[9] albeit in different clinical settings. Alternatively, it may be a prolonged consequence of the neurometabolic cascade initiated upon head injury and known to cause increased calcium influx, mitochondrial dysfunction, and impaired cerebral bioenergetics,[24] that collectively suppress CVR_{CO2}. However, further research is required to better understand the precise mechanisms that underpin our findings.

These functional impairments translated into impaired cognition in the form of a lower MoCA score (~7%) and slower GPD completion time (~18%), with five of the players recording MoCA scores diagnostic of mild cognitive impairment (< 26 points).[15] Moreover, the time taken to complete the GPD with the dominant hand was longer than the normal time expected for those aged between 20 and 29 years (63.4 s),[16] placing the neurological deficits observed into further context.

However, there are several limitations to the present study that warrant careful consideration. The first relates to the cross-sectional nature of our study design that unlike randomized controlled (interventional) trials fail to fully establish causality. Second, we implemented a self-recall questionnaire/interview so that participants could report playing history. Albeit an unavoidable caveat, this would have likely been confounded by recall bias further justifying follow-up interventional or longitudinal studies with formal assessment of football heading frequency. Third, we did not objectively measure cardiorespiratory fitness (CRF); despite our best efforts during recruitment and reliance on physical activity questionnaires for the "matching" of CRF across groups, we cannot exclude the possibility that some degree of (CRF-induced) neuroprotective bias[20,21] may have confounded comparative analyses. We also need to exercise caution when interpreting our findings constrained by small sample sizes (even in the face of adequate power) given the caveats associated with a Type M error[25] and the implementation of hand-based (rather than foot-based) tasks to assess cognition that may prove less "task specific" for footballers resulting in a potential bias. Finally, we restricted our comparative analyses to young, albeit experienced players, although we speculate that the observed impairments would have been further compounded in older and especially retired sedentary professional players given more exposure to the (adverse) mechanical stimulus of heading the ball combined with the "neuroprotection-neutralizing" effects of physical inactivity. Indeed, future studies are warranted to determine the underlying molecular precedents (e.g. elevated OXINOS) and whether impairments in CBF regulation persist and further amplified in later life that may contribute at least in part to a player's accelerated trajectory toward neurodegeneration. Given these limitations, it is important that our findings do not deter individuals from participating in football given the established physical, psychological, and social benefits of playing the game,[26] until future randomized control trials are able to substantiate our preliminary findings.

In conclusion, CBF regulation and cognition are collectively impaired in male football players that may be related to the repetitive act of heading the ball across a player's career. This appears to outweigh any neuroprotective benefits conferred by physical activity and may accelerate the brain's trajectory toward cognitive decline and neurodegeneration in later life.

4.1 | Perspectives

This study highlights the probable implications of regularly heading the ball across the playing career for cerebrovascular and cognitive health in male football players and provides mechanistic insight into why players may be at increased risk of neurodegenerative disease in later life.[1] Therefore, our findings support recent governing body decisions to reduce the number of headers that players make across the lifetime.[27,28] Future research should focus on quantifying the number of head impacts that can be sustained while achieving the established physical, psychological, and social benefits of playing the game,[26] without compromising brain health.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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NEUROSCIENCE
RESEARCH ARTICLE
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Long-term Exercise Confers Equivalent Neuroprotection in Females Despite Lower Cardiorespiratory Fitness

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Abstract—Females are more prone to cognitive decline, stroke and neurodegenerative disease, possibly due to more marked reductions in cerebral blood flow and cerebrovascular reactivity to CO_2 ($CVR_{CO2HYPER}$) in later life. To what extent regular exercise confers selective neuroprotection in females remains unestablished. To examine this, 73 adults were prospectively assigned to 1 of 4 groups based on sex (male, ♂ *vs.* female, ♀) and physical activity status (trained, ≥150 min of moderate-vigorous intensity aerobic exercise/week; $n = 18$♂ *vs.* 18♀ *vs.* untrained, no formal exercise; $n = 18$♂ *vs.* 19♀). Middle cerebral artery velocity (MCAv, transcranial Doppler ultrasound), mean arterial pressure (MAP, finger photoplethysmography) and end-tidal CO_2 (capnography) were assessed at rest during normocapnea and hypercapnea (5% CO_2) enabling $CVR_{CO2HYPER}$ to be assessed. Cerebrovascular resistance/conductance indices (CVRi/CVCi) were calculated as MAP/MCAv and MCAv/MAP. Maximal oxygen uptake (VO_{2MAX}) was determined during incremental semi-recumbent cycling ergometry to volitional exhaustion. Despite having a lower VO_{2MAX}, females were characterized by selective elevations in MCAv, $CVR_{CO2HYPER}$ and lower CVRi ($P < 0.05$), but the training responses were similar across sexes. Linear relationships were observed between VO_{2MAX} and $CVR_{CO2HYPER}$ (pooled untrained and trained data; ♂ $r = 0.70$, ♀ $r = 0.51$; both $P < 0.05$) with a consistent elevation in the latter equivalent to ~1.50%.mmHg^{-1} compared to males across the spectrum of cardiorespiratory fitness. These findings indicate that despite having comparatively lower levels of cardiorespiratory fitness, the neuroprotective benefits of regular exercise translate into females and may help combat cerebrovascular disease in later life.

Key words: cerebral haemodynamics, exercise, neuroprotection, sex.

INTRODUCTION

Regular aerobic exercise is cardioprotective (Soares-Miranda et al., 2016), but emerging evidence attests to its neuroprotective benefits helping reduce the incidence of stroke (Prestgaard et al., 2018) and neurodegenerative disease (Gorelick et al., 2011). While the underlying mechanisms remain to be established, studies in males suggest that it is associated with elevations in cerebral blood flow (CBF) (Ainslie et al., 2008) and cerebrovascular reactivity to carbon dioxide (CVR_{CO2}) (Bailey et al., 2013b), with the net effect of increased O_2 and glucose delivery to support the high bioenergetic demands of the brain (Bailey, 2019). This is important since reductions in both CBF and CVR_{CO2} are considered important risk factors for stroke (Markus and Cullinane, 2001; Moskowitz et al., 2010) and neurodegenerative diseases (Lim et al., 2018; Wolters et al., 2017).

It is well established that compared to males, females exhibit elevated CBF (Tegeler et al., 2013; Tomoto et al., 2019) and CVR_{CO2} (Kastrup et al., 1998, 1997) albeit until menopause. Thereafter, these gains in cerebrovascular function diminish (Kastrup et al., 1998), largely owing to a reduction in estrogen, which is known to have protective effects on the vasculature (Krause et al., 2006). This may explain, at least in part, why females are more prone to

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Abbreviations: CBF, cerebral blood flow; CRF, cardiorespiratory fitness; CVCi, conductance indices; CVR_{CO2}, cerebrovascular reactivity to carbon dioxide; CVRi, Cerebrovascular resistance; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; VO_{2MAX}, maximal oxygen consumption.

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stroke (Writing Group et al., 2016) and neurodegenerative disease (Andersen et al., 1999) in later life. Surprisingly however, there are few studies that have examined to what extent exercise confers neuroprotection in females compared to males. Therefore, we designed a comparative study with trained and untrained adults to determine if incidental cardiorespiratory fitness (CRF) and corresponding exercise-induced cerebrovascular adaptation is comparable, or indeed not, between sexes. Given the established relationship between CRF and cerebrovascular function in males (Ainslie et al., 2008; Bailey et al., 2013a) and that females are characterized as having a lower CRF compared to their physical activity-matched male counterparts (Cureton et al., 1986), we hypothesised that regular exercise would confer greater neuroprotection in females, owing to greater capacity for improvements in CRF.

EXPERIMENTAL PROCEDURES

Ethical approval

The experimental protocol was approved by the The University of South Wales ethics committee (#07/01/2013 and #2017TC1201). All participants were informed of the purpose/risks of the experiment and provided written informed consent. All procedures adhered to guidelines set forth in the Declaration of Helsinki.

Participants

Males and females (aged 18–35 years) were recruited who according to self-report physical activity levels were either trained (≥150 min of moderate to vigerous intensity aerobic exercise/week) or untrained (no formal aerobic exercise outside of everyday living) (Bailey et al., 2013b). All participants were free of any cardiovascular, cerebral or pulmonary disease and were non-smokers. None of the participants were taking nutritional supplements including over-the-counter antioxidant or anti-inflammatory medications know to affect cerebrovascular function.

Assignment

Seventy-three participants were considered eligible for the study. They were prospectively assigned in a balanced fashion to 1 of 4 groups based on their sex, physical activity levels and body mass index (for given training status) and categorised as: male trained (n = 18), male untrained (n = 18), female trained (n = 18) and female untrained (n = 19). Prior to testing, all participants were asked to refrain from partaking in vigorous exercise and consuming alcohol 24 h prior to the experimental day. They were also asked to avoid consuming caffeine on the day of testing.

Procedures

Cerebrovascular function. Middle cerebral artery velocity (MCAv) was determined using a 2 MHz pulsed Doppler ultrasound system (Multi-Dop X4, DWL Elektroniche Systeme GmbH, Sipplingen, Germany) as previously described (Bailey et al., 2013a). Mean arterial pressure (MAP) was monitored using finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). Cerebrovascular resistance (CVRi) and conductance (CVCi) indices were calculated as MAP/MCAv and MCAv/MAP. Following 30 min of breathing room air at rest, CVR_{CO2} to hypercapnea (5% CO_2, balanced air; $CVR_{CO2HYPER}$) was assessed for 3 min and calculated as the percentage increase in MCAv from baseline per mmHg increase in end-tidal CO_2 determined via capnography (ML 206, ADInstruments Ltd, Oxford, UK). Data were sampled continuously at 1 kHz and stored for off-line analysis.

Cardiorespiratory fitness. Maximal oxygen uptake (VO_{2MAX}) was determined during an incremental cycling test to volitional exhaustion using on-line breath-by-breath respiratory gas analysis (MedGraphics Ultima Series, Minnesota, USA). VO_{2MAX} was confirmed according to established criteria described previously (Ainslie et al., 2008). To account for differences in body size and mass, data were allometrically scaled to the exponent of 0.67 (Nevill et al., 2003).

Statistical analysis

Distribution of normality was assessed using Shapiro-*W*-Wilk tests and equal variance confirmed using Levene's (homogeneity of variances) tests. Data were analysed using a 2-way (Sex: male *vs.* female × Training status: trained *vs.* untrained) analysis of variance (ANOVA). Where an interaction effect was identified, differences were located using a one-way ANOVA and post-hoc Tukey's Honestly Significant Difference test or Games-Howell test depending on whether data were of equal (BMI and all cerebrovascular variables) or unequal (age and CRF variables) variance, respectively. Relationships were determined using Pearson Product Moment correlations. Significance was established at $P < 0.05$ and data expressed as mean ± SD.

RESULTS

Main effects for sex identified that females exhibited higher MCAv ($F_{1, 69} = 13.7$, $P = 0.000$), CVCi ($F_{1, 69} = 10.6$, $P = 0.002$), $CVR_{CO2HYPER}$ ($F_{1, 69} = 10.3$, $P = 0.002$) and lower CVRi ($F_{1, 69} = 13.2$, $P = 0.001$) compared to males, despite presenting with a lower VO_{2MAX} (scaled, $F_{1, 69} = 32.5$, $P = 0.000$ and unscaled, $F_{1, 69} = 54.1$, $P = 0.000$; Table 1). As anticipated, main effects for activity were identified and showed that VO_{2MAX} was elevated as a functioning of training ($F_{1, 69} = 117.7$, $P = 0.000$) and was associated with a lower MAP ($F_{1, 69} = 4.3$, $P = 0.042$), CVRi ($F_{1, 69} = 6.0$, $P = 0.017$) and BMI ($F_{1, 69} = 14.9$, $P = 0.000$) and elevated $CVR_{CO2HYPER}$ ($F_{1, 69} = 43.9$, $P = 0.000$) and MCAv ($F_{1, 69} = 6.7$, $P = 0.012$), the latter selectively confined to the males following confirmation of an interaction effect (Table 1; $P < 0.05$). Linear relationships were observed between VO_{2MAX} and

Table 1. Participant characteristics

Sex	Male			Female			Significance (*P* Values)		
Activity	Untrained (n = 18)	Trained (n = 18)	Δ	Untrained (n = 19)	Trained (n = 18)	Δ	Sex	Activity	Interaction
Demographics									
Age (yr)	24 ± 6	22 ± 3	–	22 ± 4	22 ± 3	–	0.264	0.756	0.240
BMI ($kg.m^2$)	25.8 ± 3.2	23.4 ± 2.3	−2.4	25.5 ± 2.9	22.6 ± 3.0	−2.9	0.447	0.000*	0.701
Cardiopulmonary									
VO_{2MAX} ($L.min^{-1}$)	3.18 ± 0.65	4.59 ± 0.61*	1.41	2.14 ± 0.31†‡	2.94 ± 0.78*†	0.8	0.000*	0.000*	0.035*
VO_{2MAX} ($mL.kg^{-1}.min^{-1}$)	38 ± 7	61 ± 10	23	31 ± 4	47 ± 9	16	0.000*	0.000*	0.098
VO_{2MAX} ($mL.kg^{-0.67}.min^{-1}$)	164 ± 27	252 ± 34	88	125 ± 16	184 ± 42	59	0.000*	0.000*	0.051
Cerebrovascular									
MCAv ($cm.s^{-1}$)	55 ± 11	66 ± 12*	11	73 ± 11†	69 ± 13‡	−4	0.000*	0.187	0.012*
MAP (mmHg)	89 ± 12	83 ± 7	−6	86 ± 10	82 ± 10	−4	0.415	0.042*	0.564
CVRi ($mmHg^{-1}.cm.s^{-1}$)	1.68 ± 0.41	1.30 ± 0.30*	−0.38	1.21 ± 0.26†	1.23 ± 0.27‡	0.02	0.001*	0.017*	0.009*
CVCi ($cm.s^{-1}.mmHg^{-1}$)	0.63 ± 0.17	0.80 ± 0.16*	0.17	0.86 ± 0.20†	0.86 ± 0.21‡	0	0.002*	0.064	0.042*
$CVR_{CO2HYPER}$ ($\%.mmHg^{-1}$)	1.90 ± 0.67	3.43 ± 0.95	1.53	2.69 ± 1.02	4.03 ± 1.02	1.34	0.002*	0.000*	0.651

Mean ± SD; BMI, body mass index; VO_{2MAX}, maximal oxygen uptake; MCAv, middle cerebral artery velocity; MAP, mean arterial pressure; CVRi/CVCi, cerebrovascular resistance/conductance indicies; $CVR_{CO2HYPER}$, cerebrovascular reactivity to carbon dioxide; */† = Different within/between sex for given activity; ‡ = Different between sex and given activity ($P < 0.05$).

$CVR_{CO2HYPER}$ for both sexes (Fig. 1, Panel A) and between VO_{2MAX} and MCAv in males (Fig. 1 Panel B). Based on the mean VO_{2MAX} of 181 $mL.kg^{-0.67}.min^{-1}$ (pooled data), the corresponding elevation in $CVR_{CO2HYPER}$ in females equated to ~1.50%.$mmHg^{-1}$ maintained across the spectrum of cardiorespiratory fitness (Fig. 1, Panel A).

DISCUSSION

The present study has identified that despite having a lower VO_{2MAX}, females were characterized by elevated cerebral perfusion, CVR_{CO2} and lower CVRi compared to males. Moreover, contrary to our hypothesis, we show that the training response was equivalent across sexes as demonstrated in the form of similar elevations in CVR_{CO2}. Nonetheless, these findings highlight for the first time that the neuroprotective benefits of regular exercise previously observed in males translate into females and could help offset the accelerated cerebrovascular decline observed during later life, thereby delaying the trajectory towards stroke and neurodegeneration.

To the best of our knowledge, this is the first cross-sectional study that has investigated to what extent exercise-induced cerebrovascular adaptation compares between sexes. Our findings agree with previous studies that have reported exercise-induced improvements in both CBF (Ainslie et al., 2008) and $CVR_{CO2HYPER}$ (Bailey et al., 2013b) across the adult lifespan in males. Importantly, our study extends these findings to the female brain and demonstrates that regular exercise is also associated with elevations in $CVR_{CO2HYPER}$, in a dose–response manner; that is, the observed gains were in direct proportion to CRF in the form of elevated VO_{2MAX}, further demonstrating its neuroprotective effects. However, the training responses between sexes were equiavlent.

The clinical significance of our findings primarily relate to the elevated $CVR_{CO2HYPER}$ observed with increases in cardiorespiratory fitness and its potential to combat the inexorable and accelerated decline in cerebrovascular function known to occur in later life in females (Kastrup et al., 1998). The $CVR_{CO2HYPER}$ is a surrogate measure of regional cerebrovascular endothelial function (Lavi et al., 2006) and its selective impairment is associated with neurodegeneration (Gorelick et al., 2011) and increased stroke risk (Gupta et al., 2012). While $CVR_{CO2HYPER}$ is selectivley elevated in females during early adulthood, as confirmed in the present study, it is thought to later decrease by ~20% following menopause (Kastrup et al., 1998). This change has traditionally been attributed to a reduction in the circulating concentration of estrogen and corresponding increase in oxidative-nitrosative stress defined by a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability considered the primary mechanistic basis for systemic and regional vascular dysfunction (Strehlow et al., 2003). Thus, it is conceivable that the antioxidant protection afforded through regular exercise (de Sousa et al., 2017) may serve to improve not only systemic, but also regional cerebrovascular reactivity subsequent to a reduction in oxidative-nitrosative stress, helping explain the observed elevation in $CVR_{CO2HYPER}$ which is known to be NO-dependent (Lavi et al., 2006). Moreover, estrogen is associated with increased sensitivity to NO and reduced oxidative stress (Krause et al., 2006), which may explain why cerebrovascular function was suprerior in females compared to males.

In the untrained state, females exhibited elevated cerebral perfusion, equivalent to ~18 cm/s. Based on prior research defining the inverse linear relationship observed between MCAv and age in sedentary participants (MCAv = 87.8–0.73 age, $P < 0.05$) (Ainslie et al., 2008) the observed difference in cerebral perfusion would have equated to a "gain" of ~25 yr in the female

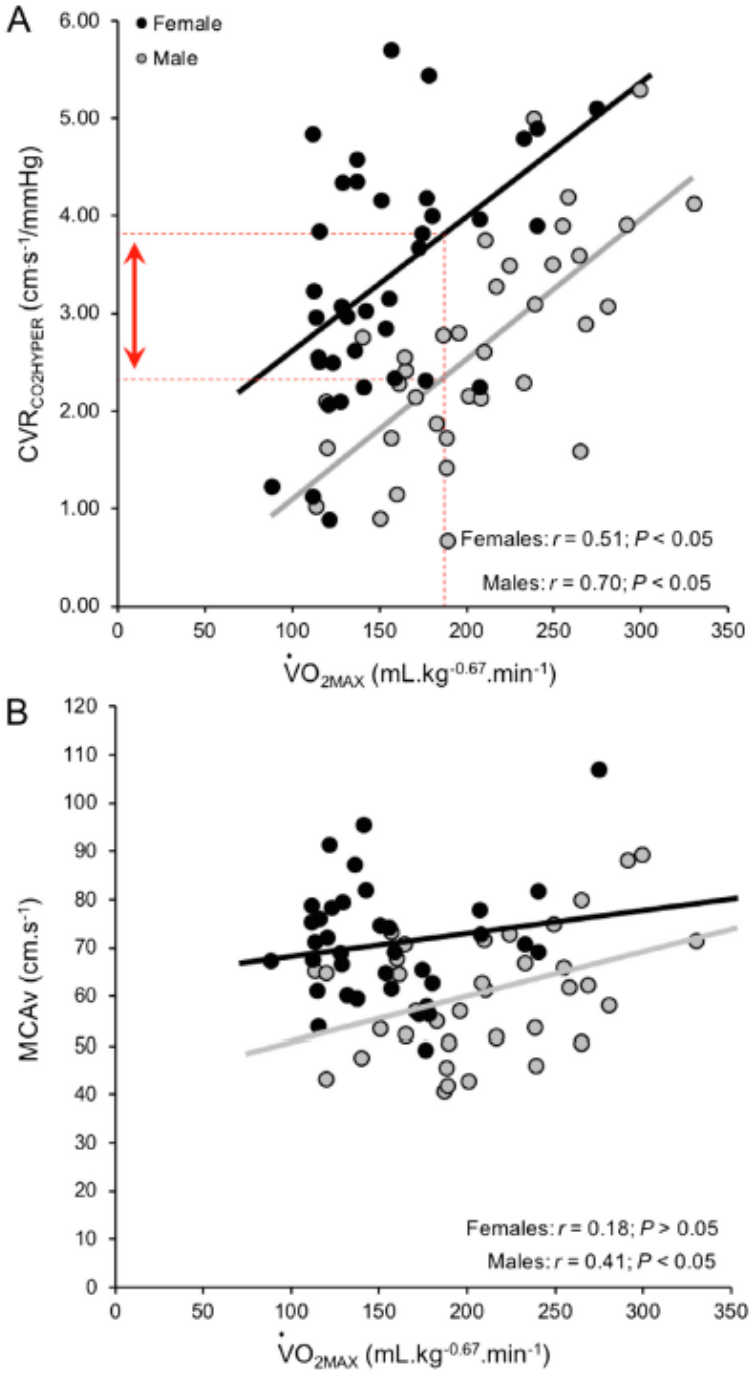

Fig. 1. Relationships between cardiorespiratory fitness and $CVR_{CO2HYPER}$ (Panel **A**)/MCAv (Panel **B**) as a function of sex (pooled untrained and trained). Note that for an average $\dot{V}O_{2MAX}$ (pooled data), the corresponding elevation in $CVR_{CO2HYPER}$ in females is ~1.50%.mmHg^{-1} (Panel **A**). $\dot{V}O_{2MAX}$, maximal oxygen uptake; $CVR_{CO2HYPER}$, cerebrovascular reactivity to carbon dioxide; MCAv, middle cerebral artery velocity.

brain's functional hemodynamic (as opposed to chronological) age. Likewise, a comparable gain in functional age (~20 yr) would have been conferred by the consistent elevation observed in $CVR_{CO2HYPER}$ by ~1.50%.mmHg^{-1} (given CVR_{CO2} = 2.4944–0.0155 age, P < 0.05) (Bailey et al., 2013b). Thus, highlighting the extent by which regular exercise confers neuroprotection that may help offset cerebrovascular disease in later life.

Importantly and in contrast to males, exercise training was not associated with a corresponding elevation in MCAv, which suggests that there is an uncoupling of cardiorespiratory fitness and cerebral perfusion. Furthermore, it also suggest the intriguing possibility that there may be an "upper-limit" to exercise-induced improvements in cerebral perfusion, albeit in the young, healthy female brain. However, we cannot exclude that differences may have occurred within the cohort of trained females and thus a randomised control trial is necessary to confirm these observations. Nonetheless, the fact that other metrics of cerebrovascular function, notably cerebrovascular reactivity, was shown to respond favorably to training stands further testament for the need to physiologically "stress" the cerebrovasculature in order to reveal neuroprotective benefits that would have otherwise gone undetected (Brugniaux et al., 2014).

It must be noted, that the present study has some limitations, which warrant further discussion. Only young participants were recruited for the study and therefore the results cannot be generalized to other populations including older adults, who are more vulnerable to stroke and neurodegenerative diseases. We were unable to control for the effects that the menstrual cycle may have had on our measures of cerebrovascular function. Therefore, some caution

must be taken when interepreting our findings, given the effect that varying levels of estrogen can have on CBF (Nevo et al., 2007). BMI was elevated in the untrained compared to the trained, which is commonly seen as a natural consequence of a sedentary lifestyle. However, this was consistent for both sexes. Moreover, the untrained BMI was was only marginally higher than the cut-off for the "healthy" weight range, which suggests it would have unlikely had an effect. We also used TCD to determine MCAv as a surrogate measure of CBF, a limitation that is well established. While it is considered a reliable indicator of cerebral perfusion at rest (Serrador et al., 2005), we cannot rule out changes in the MCA diameter when assessing CVR_{CO2}, which could effect MCAv. Indeed, magnetic reasonance imagining studies have reported conflicting findings that suggest CRF and CVR_{CO2} are inversely related (Furby et al., 2019; Intzandt et al., 2019), possibly due to improved MCA compliance. However, irrespective of this, CVR_{CO2} assessed using TCD remains a useful method to assess cerebrovascular health given its association with neurodegenerative disease (Lim et al., 2018) and stroke risk (Markus and Cullinane, 2001).

To address these limitations, future research should focus on ensuring participants are matched for BMI and that the stage of menstrual cycle/menopause is controlled for when testing females. Moreover, while the present study identified that the neuroprotective benefits of exercise extend to females, further research is required to determine whether this is maintained with advancing age, as observed in males (Bailey et al., 2013b). It is also important to recognize that CVR_{CO2} is only one of several cerebrovascular variables that can characterize neuroprotection (Willie et al., 2011). For example, to what extent regular exercise and sex impact dynamic cerebral autoregulation is still not well understood. While it has been reported that elevated CRF may attenuate dynamic cerebral autoregulation (Drapeau et al., 2019; Labrecque et al., 2017; Lind-Holst et al., 2011) and that it may be further compounded in females (Labrecque et al., 2019), most studies have examined each independent variable in isolation. Thus, future studies using a similar experimental design as the present study are required to establish the complex interaction between CRF and sex. Similarly, the extent to which regular exercise impacts neurovascular coupling and the posterior cerebral circulation also remains largely unknown and should be of future focus. Collectively, this would allow for a more comprehensive mechanistic insight into the neuroprotective changes that may occur in response to regular exercise.

In conclusion, the present findings are the first to highlight that while the less fit female brain is characterized by elevated cerebral perfusion, CVR_{CO2} and lower CVR compared to their male counterparts, it exhibits equivalent neuroplasticity to regular exercise as identified by improvements in CVR_{CO2}. Given the inexorable and accelelerated decline in cerebrovascular function following menopause, these findings highlight the potential neuroprophylactic benefits of regular exercise to combat cerebrovascular disease in later life.

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AUTHOR CONTRIBUTIONS

All experimentation was completed in the Neurovascular Research Laboratory at the University of South Wales. CJM and DMB conceived the concept and design of the study, led the acquisition/analysis/interpretation of the data and wrote the original manuscript. JVB, DD, TAC, TSO, BSS and HT facilitated the design of the study and made substantial contributions to data acquisition. SO and PNA contributed to the design of the study and facilitated the interepretation of data. All authors revised the manuscript for important intellectual content, approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

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THE LANCET
Psychiatry

Correspondence to "Long-term risk of dementia among people with traumatic brain injury in Denmark: a population-based observational cohort study"

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To the Editor,

We read with interest the recent publication by Fann and colleagues[1] highlighting the association between traumatic brain injury (TBI) and long-term risk of dementia in Danish citizens. The authors are to be congratulated on an impressive sample and experimental design that avoided recall bias/reverse causation confounds associated with the more traditional reliance on a patient's memory during the assessment of TBI exposure. However, there are a number of important points that we would like to bring to the authors' attention that warrant consideration.

Firstly, the acute impact of TBI on dementia onset was up to two-times greater six months following injury (Figure 1), which was subsequently normalised within two years[1]. The rapid 'kinetic' of dementia onset following TBI is inconsistent to the progressive trajectory towards neurodegeneration typically outlined in the established literature[2] and indirectly suggests that dementia and CTE do not share the same pathophysiology, despite comparable neurological sequelae consistent with previous arguments[3].

Secondly, while the prevalence of later-life dementia is greater for females[4], males possess greater dementia risk following TBI[1] suggesting that females are better protected from neurodegeneration after TBI.

It is unfortunate that the medical comorbidities forming the basis of the Model Two analysis were not quantified. This is particularly important given that 73.4% of Danish population reach the recommended physical activity levels and the prevalence of obesity and diabetes are among the lowest of any European country[5]. Therefore, the current findings may use a somewhat biased sample that underestimates post TBI vulnerability to dementia in other populations that possess more risk factors.

Smiloga and colleagues[6] have specified that some findings in epidemiological studies cannot be explained using physiological rigor or remain coincidental, citing that the presence of animal logos on American football jerseys were associated with reduced concussion risk. Using large sample sizes, associations are easier to observe and the findings of Fann and colleagues[1] may have been misinterpreted by media outlets for greater headline appeal, thus failing to recognise the extent to which TBI increases the risk of dementia. For instance, 95% of those who developed dementia had never sustained a head injury. However, many outlets recognised those with five or more TBI's were at double the risk of dementia compared to those with one TBI[1].

Given the current lack of curative treatments for dementia, it is important that resources are prioritised on other, arguably more important modifiable risk factors, notably physical inactivity, hypertension and smoking[7].

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Physical activity, cognitive decline, and risk of dementia: 28 year follow-up of Whitehall II cohort study

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Rapid Response:

Re: Physical activity, cognitive decline, and risk of dementia: 28 year follow-up of Whitehall II cohort study

To the Editor,

A decline in cognitive function is often synonymous with aging and leaves the elderly population at greater risk of developing dementia1. This is of particular significance as the elderly population is growing at a rapid rate given the advances in healthcare provision and medicine2, placing a greater strain on healthcare services. As a result, attention has shifted towards prevention with a focus on modifiable risk factors such as exercise. It has previously been reported that various forms of exercise, such as aerobic and resistance training, have the capacity to improve cognitive function3. With this in mind, it is reasonable to predict that the prevalence of dementia decreases in accordance with increased physical activity, an assumption that has been supported in the past4. However, the findings reported by Sabia and colleagues5 suggest the contrary. While this study is an impressive contribution to the growing body of literature, like many others who have responded to this article, we propose that some of the results need to be interpreted with caution for fear that the "brain-train" message becomes "lost in translation". Interestingly, the authors conclude that reverse causation can explain the reduction in time spent exercising during the 9-years prior to dementia diagnosis. In other words, physical activity levels decrease during this time period due to patients being in a pre-clinical phase of dementia.

Sabia and colleagues5 have critiqued several other prospective cohort studies that claim a reduction in activity levels as a pre-cursor for dementia. In spite of this, we would like to highlight several physiological factors that may underlie the onset of dementia and how exercise can in fact delay the onset of this disease. The first of these concerns cerebral blood flow and underlying vasoreactivity, that decrease inexorably with age6. In support, a recent prospective cohort study has shown that an accelerated decline in cerebral blood flow is a risk-factor for developing dementia7. It has also been demonstrated that physical activity has the capacity to attenuate this decline, favourably reducing the brains "functional age" by over a decade6. Attention should also be directed to the effect exercise has on brain volume and structure. Neurodegeneration and a reduction in brain volume are hallmark features of dementia4. While this decline can prove to be fatal, exercise has been shown to induce neurogenesis within the hippocampus and increase brain volume4 8. We are not arguing that exercise has the capacity to reverse the progression of dementia once diagnosed. However, collectively, these studies strongly suggest that physical activity constitutes a potentially important neuroprotective countermeasure.

A finding from the present study that warrants further discussion relates to the effect exercise has on cognition with ageing. Sabia and colleagues5 reported that the difference in the cognitive scores between those that completed the recommended levels of physical activity and those that did not, was four times as large at the age of 80 years than at 50 years. It is interesting that this result was only considered very briefly, as we propose that it highlights the importance of physical activity with increasing age and the associated neuroprotective benefits. Even more significantly, cognitive decline has major financial and social implications, amplified if the condition develops into dementia9. As a result of the societal impact that this finding may have, it could be beneficial to explore the result further. It should also be noted that physical activity levels were obtained through self-report measures once every four years on average5. While we recognise the difficulties with obtaining data from a cohort of this size, collecting the questionnaires annually may provide a more accurate representation of physical activity levels where physiological measurements are not feasible.

On a final note, we are interested in ascertaining the interaction between the covariates and the risk of dementia. Livingston and colleagues8 recently published results that weighed the impact of various risk-factors for dementia. While a sedentary lifestyle predisposed to dementia, other factors such as hypertension, obesity and diabetes were also deemed contributory. Physical activity has the collective capacity to attenuate all of these risk-factors and therefore, the impact of exercise may be greater than first thought (the whole being more than simply the sum of the parts!). It would be interesting to know if Sabia and colleagues5 considered how exercise, or indeed the lack of, affected the covariates described in their study.

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Competing interests: No competing interests

Circulation

LETTER TO THE EDITOR

Letter by Bailey et al Regarding Article, "Cerebral Perfusion and the Risk of Dementia: A Population-Based Study"

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To the Editor:

We read with interest the recent publication by Wolters et al[1] highlighting cerebral hypoperfusion as an independent risk factor for accelerated cognitive decline and increased risk of dementia in the general population. Because curative treatments for dementia are currently unavailable, future efforts that seek to promote lifelong brain health need to focus on prevention with a specific emphasis on modifiable risk factors. Thus, it was unfortunate that the authors did not consider baseline cardiorespiratory fitness as an additional input variable in their overall analysis given the potential for exercise to restore the inexorable decline in cerebral perfusion that defines sedentary ageing. Here, we take the opportunity to further translate their important findings by briefly exploring the vascular-hemodynamic basis underlying exercise neuroprotection.

Although it is well established that regular physical activity and associated improvements in cardiorespiratory fitness can reduce the risk of cardiovascular disease and all-cause mortality, emerging evidence also suggests translational benefits for enhanced cognitive function in older adults with healthy cognition, subjective memory complaints, mild cognitive impairment, and dementia.[2] It is important to note that improvements in cardiorespiratory fitness maintained over the human lifespan have been shown to increase cerebral perfusion and carbon dioxide vasoreactivity, reducing the brain's hemodynamic age by more than a decade.[3]

How might enhanced cerebrovascular function, in the form of elevated perfusion, improve neural activity and cognition? The evidence suggests that the overall potential for improved nutrient (oxygen and glucose) delivery to brain parenchyma may be enhanced, thus supporting improved neural function. Indeed, elevated cardiorespiratory fitness has been associated with improved cerebrovascular function subsequent to a reduction in free radical–mediated oxidative stress mediated by an upregulation of antioxidant defenses and the corresponding liberation of nitric oxide (NO) from the vascular endothelium.[4] In further support, endothelium-derived NO known to be impaired in Alzheimer disease (AD) and vascular dementia has been shown to inhibit the expression of amyloid precursor protein and its conversion to Aβ in microvessels and brain parenchyma. In addition, increasing vascular NO bioavailability and decreasing oxidative stress through regular exercise training may also act synergistically to enhance the brain-derived neurotrophic factor pathway providing a molecular basis to the exercise-induced improvements in neurogenesis, brain plasticity, and cognitive gains reported in the literature.[2] Collectively, these findings indicate that endothelial NO protects against increases in Aβ, that endothelial dysfunction contributes mechanistically to AD, and that improving endothelial NO-mediated function may delay an individual's clinical trajectory toward AD.

Although the optimal exercise stimulus (mode, duration, frequency, and intensity) and underlying mechanisms remain to be established, there is an emerging consensus

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that cerebral perfusion and cognitive processes that display a substantial age-related decline can improve through the simple act of daily exercise. To put these benefits into clearer perspective, physical inactivity was shown to contribute to 13% of all cases of AD worldwide (accounting for ≈4.3 million) and reducing inactivity by as little as 10% to 25% could potentially translate into 380 000 to 1 million fewer cases of AD globally.[5] Thus, the hemodynamic findings reported by Wolters et al[1] provide additional justification for brain train as a potential neuroprotective countermeasure to combat age-related brain drain.

ARTICLE INFORMATION

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VIEWPOINT

Commentaries on Viewpoint: "Tighter fit" theory—physiologists explain why "higher altitude" and jugular occlusion are unlikely to reduce risks for sports concussion and brain injuries

ALL'S SWELL WITH THE BRAIN; ON THE SIGNIFICANCE OF BLOOD-BRAIN BARRIER DISRUPTION IN HYPOXIA

TO THE EDITOR: The hypothesis that cerebral venous engorgement induced via inspiratory hypoxia or jugular occlusion could promote a "tighter fit" of the brain thereby reducing slosh-induced injury similar to the neuroanatomical idiosyncracies of the woodpecker has attracted criticism. Although we agree with much of the concerns raised by Smoliga and Zavorsky (4), the implications of vasogenic brain swelling at least in the absence of elevated intracranial pressure in the healthy athlete remain unclear and to state that it "opposes basic principles of altitude medicine" is premature. Indeed, we and others have provided molecular ($\uparrow$ S100β) and MRI diffusion-weighted ($\uparrow T_2 + \uparrow$ ADC) evidence for a subtle breach of the blood-brain barrier (BBB) not only in hypoxia but also during acute exercise subsequent to a redox-regulated impairment in dynamic cerebral autoregulation (2, 3). Could it be that priming of the extracellular space (ECS) subsequent to BBB disruption reflects an adaptive neuroprotective response? An increase in the ECS would serve to buffer any rise in the interstitial concentration of neurotransmitters such as glutamate and glycine reducing tonic activation of NMDA receptors and attenuate extracellular K^+ that could threaten to depolarize the brain (1), metabolic events known to be triggered by concussion. Indeed, an expanded ECS has been tentatively linked to improved hypoxia tolerance in neonates (15–25% of the whole brain compared with 10–20% in the human adult) and helps explain at least in part, the extraordinary anoxia tolerance in the goldfish and crucian carp (1, 5). To swell or not to swell, that remains the question!

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Proceedings from American College of Sports Medicine Conference 2020, virtual event – Board 187

History Of Heading In Soccer Impairs Cognition But Not Cerebral Perfusion In Young Amateur Players

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Heading the ball in soccer has been linked to impaired cognition and may increase the risk of neurodegenerative disease. This may be explained by an accelerated decline in cerebral perfusion, a major risk factor for cognitive impairment, stroke and dementia, for reasons that remain unclear.
Purpose To determine if a history of recurrent heading of a football predisposes to cerebral hypoperfusion and cognitive impairment.
Methods Twenty-nine amateur male soccer players (age: 28 ± 6 yrs) with a playing history of 15 ± 6 yrs and a self-reported heading frequency of 9 ± 4 balls per game were recruited for the study. They were compared to 32 age and fitness-matched controls who had not participated in contact sports with no history of concussion. All participants completed a battery of psychometric tests that assessed learning and memory (Rey-Auditory Verbal Learning Test), working memory (Repetition of Digits Backwards; Trail Making Test B) and attention and information processing (Repetition of Digits Forwards; Trail Making Test A; Digit Symbol Substitution Test). A sample of the soccer players (n = 13) and controls (n = 22) also completed a cerebrovascular screening whereby middle cerebral artery velocity (MCAv) and mean arterial blood pressure (MAP) were assessed using transcranial Doppler ultrasound and finger photoplethysmography, respectively. Cerebrovascular conductance/resistance were calculated as MCAv/MAP and MAP/MCAv.
Results Soccer players were characterized by impaired learning and memory, and attention and information processing compared to controls ($P < 0.05$; Table 1). However, no between group differences were observed in MCAv, CVC or CVR between groups ($P > 0.05$; Table 1).
Conclusion Heading the ball in soccer is associated with impaired cognition that appears to be independent of cerebral hypoperfusion.

Table 1. Cognitive function and cerebral perfusion

	Controls	**Soccer Players**	***P* Values**
MCAv ($cm.s^{-1}$)	59 ± 12	61 ± 10	0.587
MAP (mmHg)	88 ± 20	90 ± 11	0.620
CVC ($cm.s^{-1}$.mmHg)	0.71 ± 0.20	0.69 ± 0.15	0.959
CVR (mmHg. $cm.s^{-1}$)	1.49 ± 0.32	1.51 ± 0.33	0.922
Rey Auditory Verbal Learning Test A1-A5 (*n*)	53 ± 8	46 ± 9*	**0.002**
Rey Auditory Verbal Learning Test B1 (*n*)	7 ± 2	5 ± 2*	**0.001**
Rey Auditory Verbal Learning Test A6 (*n*)	12 ± 3	10 ± 2*	**0.002**
Rey Auditory Verbal Learning Test A6-A5 (*n*)	-1 ± 2	-1 ± 1	0.450
Repetition of Digits Backwards (*n*)	6 ± 2	5 ± 2	0.429
Trail Making Test B (*s*)	57 ± 14	62 ± 13	0.186
Repetition of Digits Forwards (*n*)	8 ± 2	6 ± 2*	**0.001**
Trail Making Test A (*s*)	26 ± 6	30 ± 9	0.066
Digit Symbol Substitution Test (*n*)	61 ± 10	57 ± 10	0.134

Proceedings from Physiology 2019, Aberdeen, UK – PC105

Poster Communications: Recurrent concussion in retired rugby union players is associated with cerebral hypoperfusion and cognitive impairment

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Sports-related concussion (SRC) represents a significant and growing public health concern yet remain one of the least understood injuries facing the sports medicine community today. There is increasing concern that prior recurrent concussion may contribute to long-term neurologic sequelae in later-life as retired contact sport athletes with three or more SRC's present with a fivefold prevalence of mild cognitive impairment (MCI, 1). To what extent this relates to an accelerated decline in cerebral perfusion, a major risk factor for cognitive decline and dementia, remains to be explored. We recruited 40 aged participants who were divided into two distinct groups; 20 retired rugby union players aged 65 (mean) ± 7 (SD) years with a self-report history of 3 (mean) concussions ± 3 (SD) incurred over the course of their playing careers and 20 non-concussed, non-contact, age, education and fitness-matched controls aged 63 ± 6 years. Middle cerebral artery blood flow velocity (MCAv) was measured via Doppler ultrasomography at rest and in response to hypercapnia/hypocapnia (+/- 5mmHg change in end-tidal PCO2) with cerebral oxygen content (CDO2) calculated retrospectively(2). Ongoing symptoms of concussion were reporting using the Sports Concussion Assessment Tool 5 (SCAT5). Cognitive function was assessed via neuropsychometric testing and screened for MCI using the Montreal Cognitive Assessment (MoCA). Rugby players had played for 23 ± 8 years and reported between 3 – 10 previous concussions with ongoing, persistent symptoms related to concussion (7 ± 6 vs. 3 ± 4, P = 0.01) with greater severity (16 ± 13 vs. 4 ± 8, P = 0.00). Resting cerebral perfusion was lower in rugby players (45 ± 9 cm.s-1 vs. 51 ± 7 cm.s-1, P = 0.01) and in response to hypercapnia (57 ± 10 cm.s-1 vs. 69 ± 15 cm.s-1, P = 0.01). Subsequently, CDO2 was lower in rugby players at rest (1048 ± 170 ml/cm/s vs. 878 ± 184 ml/cm/s, P = 0.00), throughout hypercapnia (1406 ± 289 ml/cm/s vs. 1118 ± 242 ml/cm/s P = 0.00) and hypocapnia (683 ± 136 ml/cm/s vs. 577 ± 139 ml/cm/s P = 0.02). Executive motor function was slower in rugby players using the non-dominant hand on the Grooved Pegboard (92 ± 18 vs. 79 ± 15 seconds, P = 0.02) in addition to lower MoCA scores (24 ± 3 vs. 26 ± 2, P = 0.02). These findings indicate that neurological complications associated with SRC persist after retirement from rugby union. Cerebral hypoperfusion is identified as a haemodynamic risk factor that may precede the observed cognitive impairment that in later-life, may accelerate a (former) player's trajectory towards neurodegeneration(3, 4). Our prior research has identified that the cerebral hypoperfusion characteristic of SRC, albeit in the younger demographic, is associated with a free radical-mediated reduction in nitric oxide bioavailability(5).

Proceedings from Europhysiology 2018, London, UK – PCA180

Poster Communications: Elevated systemic oxidative-nitrosative stress and cerebrovascular function in professional rugby union players: the link to impaired cognition

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Sports-related concussion (SRC) represents a growing public health concern in rugby union, yet remains one of the least understood injuries facing the community today. There is concern that prior SRC may contribute to long-term neurologic sequelae in later-life(1). This may be due to an accelerated decline in cerebral perfusion, a major risk factor for neurocognitive decline (2), though the underlying mechanisms remain inconclusive. It was hypothesised that recurrent SRC in current professional rugby union players would result in elevated systemic oxidative-nitrosative stress(3), reflected by a free radical-mediated reduction in bioactive nitric oxide (NO) metabolite bioavailability and impaired cerebrovascular and cognitive function. A longitudinal study design was adopted across the 2017-2018 rugby season. Ethical approval was obtained from the University of South Wales Ethics Committee. Data collection is ongoing and the current report documents result from the pre-season data collection. Participants were divided into two separate groups; 23 professional rugby union players (aged 26 ± 5 years) and 22, age- and physical activity-matched non-concussed controls (27 ± 8 years). Pre-season measurements were performed for cerebrovascular function (Doppler ultrasound of middle cerebral artery velocity (MCAv) at rest and in response to hypocapnia/hypercapnia), venous concentrations of the ascorbate radical (electron paramagnetic resonance spectroscopy), and bioactive NO metabolites (nitrite and S-nitrosothiols, ozone-based chemiluminescence) including cognition (neuropsychometric tests). The rugby players expressed greater oxidative-nitrosative stress confirmed by a systemic elevation in ascorbate radical ($P < 0.05$ vs. control) and reduction in cumulative bioactive NO ($P < 0.05$ vs. control). The players performed worse in the Rey Auditory Verbal Learning Test B (learning and memory) and the Grooved Pegboard test using both the dominant and non-dominant hands (visuomotor coordination, $P < 0.05$ vs. control). No between-group differences in cerebral perfusion at rest (MCAv:54 ± 13 cm.s-1 vs. 59 ± 12 cm.s-1, $P > 0.05$) or in response to the CO2 challenges were observed (CVRCO2Hypo: 2.58 ± 1.01 cm.s-1 vs. 2.58 ± 0.75 cm.s-1, $P > 0.05$ and CVRCO2Hyper: 2.69 ± 1.07 cm.s-1 vs. 3.35 ± 1.28 cm.s-1, $P > 0.05$) The present study identified that the rugby players are characterised by impaired cognitive function subsequent to elevated systemic-oxidative-nitrosative stress. This appears to be independent of any functional impairment in cerebrovascular function. Given the potential long term trajectory towards accelerated cognitive decline following SRC, prophylaxis to increase NO bioavailability warrants consideration (4).

Proceedings from the International Conference on Concussion and Sports Neurology 2018, Venice, Italy

Elevated Systemic Oxidative-Nitrosative Stress and Cerebrovascular Function in Professional Rugby Union Players: The Link to Impaired Cognition

T. S. Owens, T. A. Calverley, B. S. Stacey, C. J. Marley, G. Rose, L. Fall, G. L. Jones, P. Williams, J.P.R. Williams, M. Steggall, D. M. Bailey

Abstract—
Introduction and aims: Sports-related concussion (SRC) represents a significant and growing public health concern in rugby union, yet remains one of the least understood injuries facing the health community today. Alongside increasing SRC incidence rates, there is concern that prior recurrent concussion may contribute to long-term neurologic sequelae in later-life. This may be due to an accelerated decline in cerebral perfusion, a major risk factor for neurocognitive decline and neurodegeneration, though the underlying mechanisms remain to be established. The present study hypothesised that recurrent concussion in current professional rugby union players would result in elevated systemic oxidative-nitrosative stress, reflected by a free radical-mediated reduction in nitric oxide (NO) bioavailability and impaired cerebrovascular and cognitive function.
Methodology: A longitudinal study design was adopted across the 2017-2018 rugby union season. Ethical approval was obtained from the University of South Wales Ethics Committee. Data collection is ongoing and therefore the current report documents result from the pre-season and first half of the in-season data collection. Participants were initially divided into two subgroups; 23 professional rugby union players (aged 26 ± 5 years) and 22 non-concussed controls (27 ± 8 years). Pre-season measurements were performed for cerebrovascular function (Doppler ultrasound of middle cerebral artery velocity (MCAv) in response to hypocapnia/normocapnia/hypercapnia), cephalic venous concentrations of the ascorbate radical ($A^{\bullet-}$, electron paramagnetic resonance spectroscopy), NO (ozone-based chemiluminescence) and cognition (neuropsychometric tests). Notational analysis was performed to assess contact in the rugby group throughout each competitive game.
Results: 1001 tackles and 62 injuries, including three concussions were observed across the first half of the season. However, no associations were apparent between number of tackles and any injury type ($P > 0.05$). The rugby group expressed greater oxidative stress as indicated by increased $A^{\bullet-}$ ($P < 0.05$ vs. control) and a subsequent decrease in NO bioavailability ($P < 0.05$ vs. control). The rugby group performed worse in the Rey Auditory Verbal Learning Test B (RAVLT-B, learning and memory) and the Grooved Pegboard test using both the dominant and non-dominant hands (visuomotor coordination, $P < 0.05$ vs. control). There were no between-group differences in cerebral perfusion at baseline (MCAv: 54 ± 13 vs. 59 ± 12, $P > 0.05$). Likewise, no between-group differences in $CVR_{CO2Hypo}$ (2.58 ± 1.01 vs. 2.58 ± 0.75, $P > 0.05$) or $CVR_{CO2Hyper}$ (2.69 ± 1.07 vs. 3.35 ± 1.28, $P > 0.05$) were observed. Conclusion: The present study identified that the rugby union players are characterized by impaired cognitive function subsequent to elevated systemic-oxidative-nitrosative stress. However, this appears to be independent of any functional impairment in cerebrovascular function. Given the potential long term trajectory towards accelerated cognitive decline in populations exposed to SRC, prophylaxis to increase NO bioavailability warrants consideration.

Keywords— cognition, concussion, mild traumatic brain injury, rugby.

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Concussion can accelerate ageing of the brain – research from the rugby pitch

October 15, 2020 2.44pm BST

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The serious harm caused by concussion in sport first became apparent among the "punch-drunk" boxers who suffered repetitive blows to the head over the course of their fighting careers. A related form of brain damage is known to affect a range of other sports and professions, where repeated head injuries kill brain cells and gradually cause the brain to shrink.

By working closely with elite rugby union players, our research has now helped us to understand the impact of concussion on the brain as we get older. We have shown that brain function in a young player with a history of concussion is on a par with someone in their 60s. In simple terms, concussion seems to accelerate biological brain ageing by as much as three decades.

One important discovery was that concussed rugby union players have more "free radicals" – unstable, cell-damaging molecules – in their blood. They also have less nitric oxide, a beneficial molecule which allows more oxygen and glucose to get to the brain. As a result, blood vessels in the concussed brain react sluggishly to changes in blood flow, a condition known as "cerebrovascular impairment".

Cerebrovascular impairment has been linked to cognitive dysfunction, and can have a negative effect on the way a person thinks, concentrates, formulates ideas and remembers. It can also make them more vulnerable to dementia in later life. In our analysis, these impairments were mostly seen in the regions of the brain where head contact is most frequently made during play.

Both active and retired elite rugby union players were found to be cognitively impaired compared with people of a similar age and fitness, who had not been concussed or participated in contact sports. The effects also appeared to be related to a player's position in the rugby team. They were particularly prevalent among forwards, who usually experience more tackles and collisions in a game compared to the backs.

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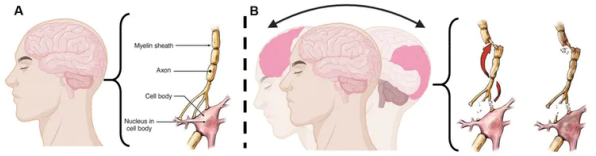
Game changer?

Positive steps have been taken in the world of rugby as awareness has grown from the negative effects that concussion has on the brain. Indeed, we have come a long way since 2005, when a neuropathologist working in a Pittsburgh coroner's office became the first to identify a form of neurodegeneration called "chronic traumatic encephalopathy" (CTE) in an American Footballer who had suffered repetitive brain injuries.

Read more: Will the law strike a knockout blow on concussion in rugby?

But the problem has not been solved. And the increasingly gladiatorial nature of the modern game, with players whose physiques resemble those of a bodybuilder, mean the risks could be worsening. So what can be done to reduce the potentially life-altering effects of concussion in rugby players?

One approach involves changing the rules of the game. While some adjustments have been proposed, such as waist height tackling to reduce the number of head-to-head collisions, some argue that the tackle should be banned altogether. Others, including ourselves, have focused on establishing effective molecular (blood, saliva, urine) and cerebrovascular (brain blood flow) assessments for pitchside detection of concussion to enable swifter treatment and recovery.



Non-injured brain with structure of intact neuron (A). Concussion causes the tail of a neuron known as an axon to tear, which eventually leads to the death of the neuron and reduces our ability to send messages across the brain (B). Artery Studios, Author provided (no reuse)

While these remain in development, there are methods that rugby players – and anyone else – can use to help our brains fight off the risk of dementia. These include high-intensity interval training (HIIT) and regular aerobic exercise.

This is because exercise helps maintain a healthy body weight and promotes the release of nitric oxide, which improves the way our blood vessels function and contributes to better brain health.

For now, though, concussion across all sports remains a prominent and potentially life-altering injury, with the true consequences often only noticed when it is too late. There are almost 10 million rugby players around the world, and professional players are more likely than not to sustain a concussion within 25 games.

And while modern treatments and management protocols for concussion have improved with increased monitoring and neurological testing, the mechanisms which increase an athlete's susceptibility to CTE and other neurological complications remain poorly understood.

Meanwhile, rugby union's governing bodies have faced criticism for the lack of concussion management. But perhaps this should come as little surprise given that not very long ago, in the amateur era of the game, the traditional treatment for a knock to the head was a "magic sponge" doused in cold water.

Appendix IV
Awards and achievements

Awards and Achievements

Best presentation – 'Concussion in Rugby Union; an accelerated trajectory towards cognitive decline.' University of South Wales Virtual Postgraduate Research Conference, July 29th, 2020.

Research Student of the Year - University of South Wales IMPACT Awards, November 14th, 2019.

Best presentation – 'Elevated systemic oxidative-nitrosative stress and cerebrovascular function in professional rugby union players; the link to impaired cognition.' University of South Wales FLSE Conference, April 25th, 2018.

Best presentation – 'Elevated systemic oxidative-nitrosative stress and cerebrovascular function in professional rugby union players; the link to impaired cognition.' International Conference on Concussion and Sports Neurology 2018, April 12-13th 2018, Venice, Italy.


INTERNATIONAL SCIENTIFIC RESEARCH AND EXPERIMENTAL DEVELOPMENT

CERTIFICATE OF BEST PRESENTATION AWARD

ICCSN 2018: 20th International Conference on Concussion and Sports Neurology

hereby certifies that the below mentioned paper has been selected as the best presentation

Elevated Systemic Oxidative-Nitrosative Stress and Cerebrovascular Function in Professional Rugby Union Players: The Link to Impaired Cognition

Tom S. Owens, Tom A. Calverley, Benjamin S. Stacey, Christopher J. Marley, George Rose, Lewis Fall, Gareth L. Jones, Priscilla Williams, John P. R. Williams, Martin Steggall, Damian J. Bailey

Venice, Italy — April 12-13, 2018

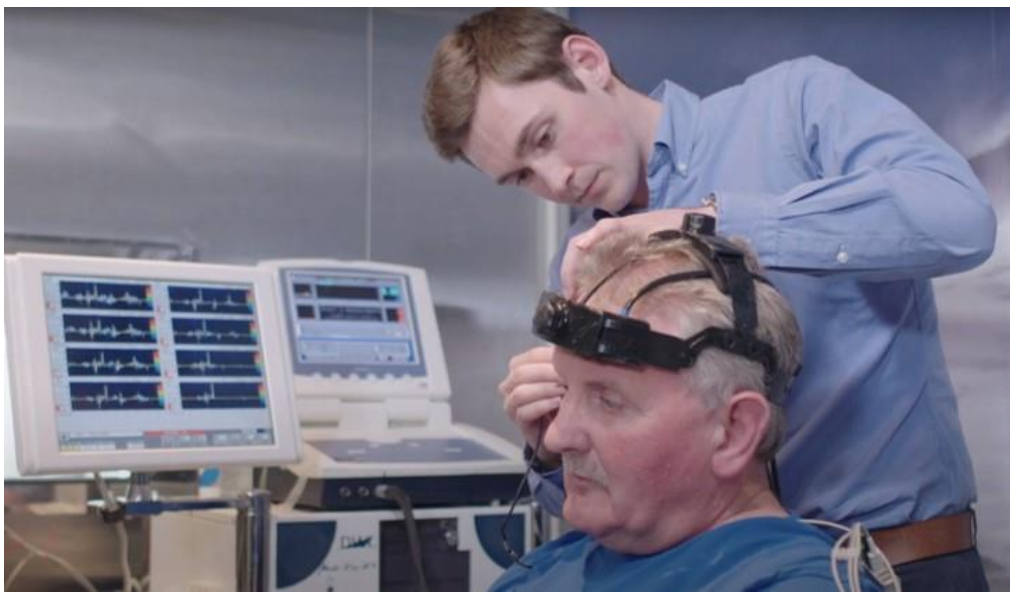
2nd prize presentation – 'Lessons from the eighties: incidence and risk factors of concussion in rugby union.' University of South Wales Postgraduate Student Presentation Day, July 15th, 2017.

Appendix V
Media engagement

Rugby Union: The Invisible Injury – June 14th, 2020.

This short documentary follows the life of former professional Rugby Union Player Adam Hughes, who was forced to retire from the game following repetitive concussions. During the documentary, Adam visits the Neurovascular Research Laboratory and underwent a series of measures carried out during Study 1 and 2 of this thesis. The documentary further serves to increase awareness for concussion in sport and how technology may protect players in future.

Linking Concussion and Dementia: USW Research IMPACT - November 13th, 2019.



This short video was filmed and prepared for the 2019 University of South Wales Impact Awards and sought to provide an overview of the research investigating concussion and the link to accelerated cognitive decline in rugby union players.

Developing our understanding of concussion in sport – December 8th, 2018.

Written for the USW Sports Degree web page, we highlighted that the Neurovascular Research Laboratory was pursuing research that sought to determine the physiological implications associated with concussion in current and retired rugby union players.

Research into sports-related concussion – December 6th, 2018.

This short video highlighted the Neurovascular Research Laboratory's research direction to investigate accelerated brain ageing as a result of concussion among current and retired rugby union players.