The acute effect of heading on neurovascular coupling, optic nerve sheath diameter and memory recall in women footballers

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I certify that all material in this thesis which is not my own work has been identified and that no material had previously been submitted and approved for the award of a degree by this or any other University.

Abstract

Purpose: Retired male footballers are at 3.5 times greater risk of neurodegenerative disease, with repeated heading suggested to contribute to the increased risk. Currently, no such cohort data exist in women players. However, the women's game is growing, so studies are needed to understand whether heading acutely alters outcomes potentially linked to neurodegenerative disease in this group. Alterations in neurovascular coupling (NVC) may precede the cognitive impairment associated with neurological disease, and evidence suggests that NVC is acutely altered following heading in men. Increased intracranial pressure (ICP) is linked with unfavourable patient outcomes following mild traumatic brain injury. This study assessed whether exposure to a realistic number of football headers acutely influenced NVC, ICP and cognitive function in women footballers. **Methods:** 19 women footballers completed a heading trial consisting of 6 headers at 40 ± 5 km/h, evenly spaced across an hour, and a time-matched seated control trial. Trials were performed on separate days, spaced a least 7 days apart. The posterior cerebral artery blood velocity response to a visual search task was measured via transcranial Doppler ultrasonography and used to quantify NVC. ICP was evaluated by measuring the optic nerve sheath diameter (ONSD). Finally, cognitive performance was determined using a modified version of the international shopping list test. Each outcome was assessed before and after the heading and control trials. **Results:** No significant time by trial interaction was present for any metric of NVC (P>0.14, η_p^2<0.16), ONSD (P= 0.65, η_p^2 = 0.01) or cognitive function (P = 0.053, η_p^2 = 0.19). **Conclusion:** Our data suggests that NVC, ICP and cognitive function is not altered following six headers in women footballers. Future studies should examine how these outcomes are effected by repeated exposure across a season and career.

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Symbols and Abbreviations

CTE	Chronic traumatic encephalopathy
FA	Football Association
UEFA	Union of European Football Associations
NVC	Neurovascular coupling
ACA	Anterior cerebral artery
MCA	Middle cerebral artery
PCA	Posterior cerebral artery
CVR_{CO2}	Cerebrovascular reactivity to carbon dioxide
$PaCO_2$	Partial Pressure of arterial carbon dioxide
CA	Cerebral Autoregulation
CBF	Cerebral blood flow
AD	Alzheimer's disease
MRI	Magnetic resonance imaging
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
TCD	Transcranial Doppler ultrasonography
CB_v	Cerebral blood velocity
$P_{ET}CO_2$	Partial end tidal carbon dioxide
AUC	Area under the curve
mTBI	Mild traumatic brain injury
ICP	Intracranial pressure
ONSD	Optic nerve sheath diameter
PCA_v	Posterior cerebral artery

TMD	Tympanic membrane displacement
τ	Tau
η_p^2	Partial eta Squared
Δ	Delta
g	Gravitational accelerations

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CHAPTER 1: Introduction and Research Aims

1.1. Introduction

Football is the most popular sport in the world, with an estimated 265 million active players worldwide (FIFA, 2007). Women's football has also seen exceptional growth to reach 29 million players globally, a 32% increase in participation over the last 10 years (Manson et al., 2014). Furthermore, in 2016 the 'FIFA 2.0' strategy was released, detailing plans to double female participation to 60 million by 2026 which indicates continued investment and ambition to grow the women's game (FIFA, 2016). Football participation is associated with a plethora of long term health benefits including reduced mortality from ischemic heart disease (Mackay et al., 2019) cancer and cardiovascular disease (Taioli, 2007). In addition, the team nature of football improves psychological and social health (Andersen et al., 2019). Despite these reported benefits, there is growing concern regarding the link between football participation and an increased risk of developing neurodegenerative disease (Mackay et al., 2019, Russell et al., 2021). The majority of concern centres on the idea that repetitive heading and incidence of concussion accrued over a football career maybe a major contributing factor to the increased risk of neurodegenerative disease (Mackay et al., 2019, Russell et al., 2021). Given the vast number of people that could be affected, the economic costs of dementia care alone totalling £34.7 billion in 2015 and the social costs to families that provide an estimated £13.9 billion worth of free care(Wittenberg et al., 2019), this is clearly an area that warrants further investigation.

Recent concerns regarding player welfare and concussion prompted the International Football Association Board (IFAB) to introduce concussion

substitutions, in January 2021, across all competitions. However, in comparison to other contact sports such as Rugby, American Football and Boxing, concussion incidence remains low in football (Van Pelt et al., 2021). A review by Spiotta et al. (2012) reported an incident rate of 1.8 per thousand match hours in colligate female and 1.38 per thousand match hours in colligate males. Despite the low occurrence, a recent seminal study demonstrated that retired male footballers were at ~3.5 time's greater risk of developing neurodegenerative disease and almost 5 times more likely to be prescribed dementia related medication compared to controls matched for age, sex and social standing (Mackay et al., 2019). Subsequent analysis from the same group revealed that neurodegenerative disease risk varied depending on player position (Russell et al., 2021). Specifically, defenders had the greatest risk, and this position is associated with the greatest number of headers during a match (Cassoudesalle et al., 2020). Importantly, goalkeepers who's heading incidences is extremely low, posed no increased risk of neurodegenerative disease compared to general population controls (Russell et al., 2021). This has led to speculation that the repetitive sub-concussive impact of heading maybe responsible, at least in part, for the increased risk of neurodegenerative disease.

The current evidence base linking football, and by extension heading, to an increased risk of neurodegenerative disease is solely driven by data in former male footballers (Mackay et al., 2019, Russell et al., 2021), largely because no suitable female evidence base is available. Bretzin *et al*. (2017), however demonstrated that women experience greater rotational head accelerations (1416.13 ± 507.63 rad/s^2) than men (774.60 ± 501.13 rad/s^2) when heading footballs projected at the same velocity (40 mph). This was later corroborated by Caccese et al. (2018) who in

addition to rotational differences observed that women experience greater linear head accelerations compare to men (40.9 ± 13.3g and 27.6 ± 8.5g, respectively). This may explain why female footballers matched for heading exposure experience greater white matter micro trauma than males (Rubin et al., 2018). This may mean that heading imposes a greater risk of neurodegenerative disease in women compared to males.

Whilst a direct causal link between heading and neurodegenerative disease is yet to be established, sufficient concern for player welfare prompted the Football association (FA) to update heading guidance across all levels of the footballing pyramid, including banning heading during the foundation phase (FA, 2021a). However, with limited scientific evidence the FA conceded that this is a precautionary measure and alongside the Union of European Football Associations (UEFA) call for further independent research into the potential cause of neurodegenerative disorders seen in footballers (FA, 2021b, UEFA, 2017).

Implementing an acute interventional model where parameters linked to neurodegenerative disease progression are tracked pre and post heading, enables rapid scrutinisation of potential causal links. Whilst a precise mechanistic link between heading and neurodegeneration remains elusive (Gouttebarge, 2021, Neal et al., 2022) reduced neurovascular coupling (NVC) and cognitive function are both hallmarks of neurodegenerative disease (Rombouts et al., 2000, Tarantini et al., 2017). Additionally, altered NVC is thought to be a sentinel event in the development of neurodegenerative disease (Ruitenberg et al., 2005, Zlokovic, 2011). The vascular hypothesis of dementia also suggests that disrupted vascular mechanisms give rise to the breakdown of the blood brain barrier, which leads to neurodegeneration due to the accumulation of neurotoxins (Beishon and Panerai, 2021). Given that the NVC

response is shown to be subtly altered following a single bout of 40 headers (Smirl *et al.*, 2020) and post-concussion (Broglio et al., 2007, McCrea et al., 2003, Wright et al., 2017b) it seems pertinent to investigate further how these parameters are effected by heading in order to improve our understanding of potential links to neurodegenerative disease.

Acute interventional work exploring the potential links between heading and future neurodegenerative disease is in its relative infancy. Previous work has utilised an unrepresentative heading stimulus – for example 20 (Ashton et al., 2021, Di Virgilio et al., 2016) or 40 (Smirl et al., 2022, Smirl et al., 2020, Wallace et al., 2018) in less than 20 minutes to explore acute changes post heading. Whilst this approach may allow inferences to be made regarding the long term pathological effects, it risks sensationalising any findings which may result in misconceptions regarding the danger of heading. Additionally this previous work almost exclusively examined male footballers. Therefore work is needed to explore the subsequent pathological effects of heading in females, who maybe more susceptible to heading induce trauma (Lipton *et al*., 2013, Rubin *et al*., 2018). An acute interventional study will provide information on the effects of each exposure and allows us to quickly scrutinise the effects that heading has on parameters believed to be involved in the development of neurodegenerative disease. This may shed light on potential causal relationships which will guide further work and aid in the protection against the development of such diseases.

1.2. Research Aims

The current work aimed to address a critical gap in the understanding of how heading may effect player welfare in the women's game. This is particularly

important given the growth of the women's game, the greater head movement and increased micro trauma seen in female footballers (Rubin et al., 2018). Specifically this study aimed to quantify changes in: NVC response; optic nerve sheath diameter as a surrogate measure of intracranial pressure and cognitive function in female footballers following a heading protocol which is more representative of the typical exposure experienced in a match or training session. This project aimed to provide insight into the acute effect of heading on parameters linked to neurodegenerative disease progression and in doing so permit rapid scrutinisation of potential causal links.

CHAPTER 2: Literature review

2.1. Anatomy of Cerebral Circulation

The brain accounts for only 2% of total body mass, yet is responsible for ~20% of resting metabolism (Zlokovic, 2011). Despite the immense metabolic demand the brain has very limited intracellular energy stores, therefore adequate fuel delivery is critical (Phillips et al., 2016). Should cerebral blood flow (CBF) be interrupted, normal brain function would cease within seconds and irreversible damage to the brains cellular constructs would take hold within minutes (Kisler et al., 2017). Evolution therefore has resulted in the development of a number of control measures and an anatomical infrastructure that ensures adequate cerebral perfusion despite changes in arterial carbon dioxide (cerebrovascular reactivity), blood pressure (cerebral autoregulation) and cerebral metabolism (neurovascular coupling).

The circle of Willis (Figure 2.1) is the arterial network found at the base of the brain which consolidates the brains blood supply (Pascalau et al., 2018). The primary function of the circle of Willis is to provide collateral circulation through the anterior, middle and posterior cerebral arteries (ACA, MCA and PCA, respectively). This means that should an artery become occluded, perfusion to all brain areas can be maintained via an alternative route (Jones et al., 2021). Studies report wide variation within the structure with a complete circle of Willis present in between 4.8 and 57.6% of the general population (Jones et al., 2021). A recent study by Thomas et al. (2020) demonstrated that monozygotic twins display significant structural differences in the circle of Willis, suggesting that variations maybe driven by environmental factors as opposed to genetics. Those displaying an incomplete circle of Willis are less well equipped to divert blood via an alternative path (De Silva et al., 2009),

however, this may not manifest with any overt symptoms or alterations in brain function, which demonstrates that this has little impact of daily living.

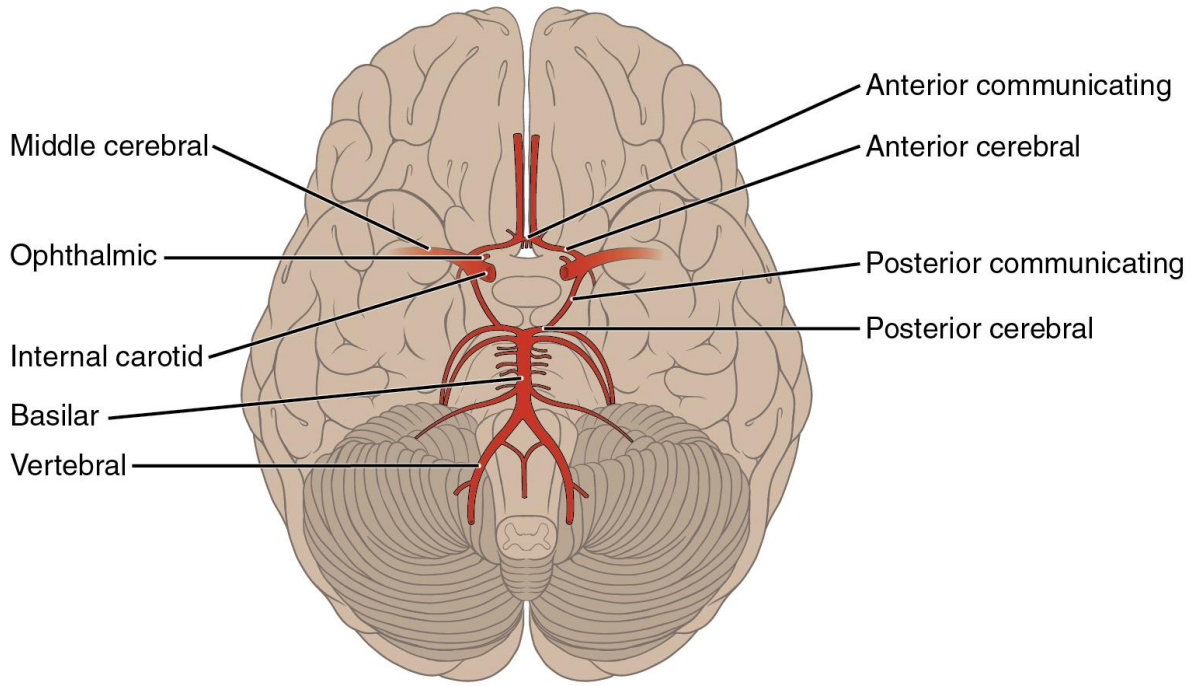

Figure 2.1 – An inferior view of the circle of Willis. Figure replicated with permission from OpenStax College, Radiopaedia.org, Radiopaedia ID: 42608

2.2. Control of Cerebral blood flow

2.2.1 Cerebrovascular reactivity

Cerebrovascular reactivity to carbon dioxide (CVR_{CO2}) refers to the change in vessel diameter in response to alterations in the partial pressure of arterial CO_2 ($PaCO_2$). Elevations in $PaCO_2$ (hypercapnia) has a vasodilator effect on vascular smooth muscle, whereas, decreases in $PaCO_2$ (hypocapnia) has a vasoconstrictive effect (Wei et al., 1980). According to Poiseuille's law (Equation 2.1), blood flow is directly

proportional to the fourth power of the vessels radius. Brain blood flow therefore is highly sensitive to changes in $PaCO_2$ as small alterations in vessel diameter result in larger changes in blood flow. The influence of Co2 is shown to be profound throughout the cerebrovascular tree (Carr et al., 2021, Ainslie and Hoiland, 2014)

Equation 2.1 Poiseuille's law:

$$Blood\ flow = \frac{\Delta P \pi r^4}{8ln}$$

ΔP = Driving pressure, r = vessel radius, L = vessel length, n = Blood viscosity

2.2.2 Cerebral Autoregulation (CA)

CA denotes the ability of cerebrovasculature to maintain cerebral perfusion in response to alterations in blood pressure (Brassard et al., 2021). As shown by Poiseuille's Law (equation 2.1), adjustments in vessel diameter can counteract changes in pressure. To achieve constant perfusion, arteries which perfuse the brain actively constrict or dilate in reaction to increased or decreased blood pressure, respectively (Cipolla et al., 2014, Fog, 1938, Toth et al., 2017).

Despite not being the focus of this thesis CVR_{CO2} and CA are key regulatory factors and it is important to understand how they impact the control of CBF. It is also important to consider these control mechanisms as much of what we currently know regarding the effects of head impacts and cerebrovascular health comes from these fields.

2.2.3 Neurovascular coupling (NVC)

NVC refers to the adjustment of CBF in response to changes in neuronal activity to meet the local metabolic demand (Hosford and Gourine, 2019). As discussed

previously (Chapter 2.1) the brain has very limited energy reserves and therefore the ability to match CBF to changes in local metabolic demand is imperative to brain functioning.

A negative feedback model was originally proposed to drive the NVC response, whereby metabolic needs of the tissue stimulated changes in blood flow. Whilst by products of brain activity include potent vasodilators which could trigger a flow response (Freeman and Li, 2016), Fox and Raichle (1986) demonstrated that increases in CBF exceed the oxygen demand of the tissue. Furthermore increased CBF is shown to occur under conditions of excess oxygen and glucose (Reviewed in Attwell and Iadecola, 2002), suggesting that changes in flow are not driven by the tissues metabolic state. This evidence led Iadecola (2017)to the hypothesis that the NVC response is driven by a feedforward mechanism in which increased CBF is stimulated through the release of vasoactive by products of synaptic activity. However, microvascular work demonstrates that at the onset of neural activity oxygen and/or glucose levels are reduced prior to CBF increases (Freeman and Li, 2016), implying that these metabolic factors may play a role in the NVC response. Collectively this evidence has led to the belief that both models work in tandem, with feedforward mechanisms resulting in exaggerated flow response and feedback mechanisms adjusting CBF to better match the metabolic needs of the tissue (Beishon et al., 2021). This hypothesis is broadly supported by the shape of the blood flow response during sustained neural activity, which often peaks shortly after onset, before subsequently being modified toward a lower level (Freeman and Li, 2016).

2.3. Mediators of the neurovascular coupling response

2.3.1 Local NVC response

The NVC response is driven by a sequence of highly coordinated multicellular events, which initiates a local response in micro-vessels near the metabolically active site and a remote response in the larger upstream vasculature (Iadecola, 2017). The local response is thought to be mediated by activation of excitatory and interneurons. Excitatory neurons have a powerful effect on local neural activity (Ma et al., 2016) and drive the haemodynamic response by mediating the release of neurotransmitters, vasoactive ions and by-products of neural activity such as adenosine and arachidonic acid metabolites (Schaeffer and Iadecola, 2021) (Figure 2.2). Meanwhile, interneurons stimulate changes in the vasculature through direct neural connections by realising vasoactive neuropeptides (Schaeffer and Iadecola, 2021) and nitric oxide (Yang et al., 2000).

2.3.2 Remote NVC response

The remote response to increased neural activity in the larger upstream vasculature is thought to be induced by retrograde propagation of vasodilation and local autoregulatory adjustments to intravascular pressure changes caused by downstream vasodilation (Iadecola, 2017). Retrograde propagation is proposed to occur via hyperpolarisation of endothelia cells. Neural activity results in the release of potassium ions (K^+) which activate KIR 2.1 channels, hyperpolarising the endothelial cell (Longden et al., 2017). Hyperpolarisation spreads to adjacent endothelial cells through inter-endothelial gap junctions (Zechariah et al., 2020) and

is transmitted to contractile mural cells (Smooth muscle cells and pericytes) probably through myoendothelial junctions and K_{IR} channels (Schaeffer and Iadecola, 2021). Hyperpolarisation of mural cells suppresses activity of voltage-gated Ca^{2+} channels, leading to intracellular Ca^{2+} depletion which in turn results in vasodilation through relaxation of the contractile apparatus. This relaxation spreads to adjacent mural cells through gap junctions, propagating vasodilation to reach pial arterioles on the brains surface (Schaeffer and Iadecola, 2021).

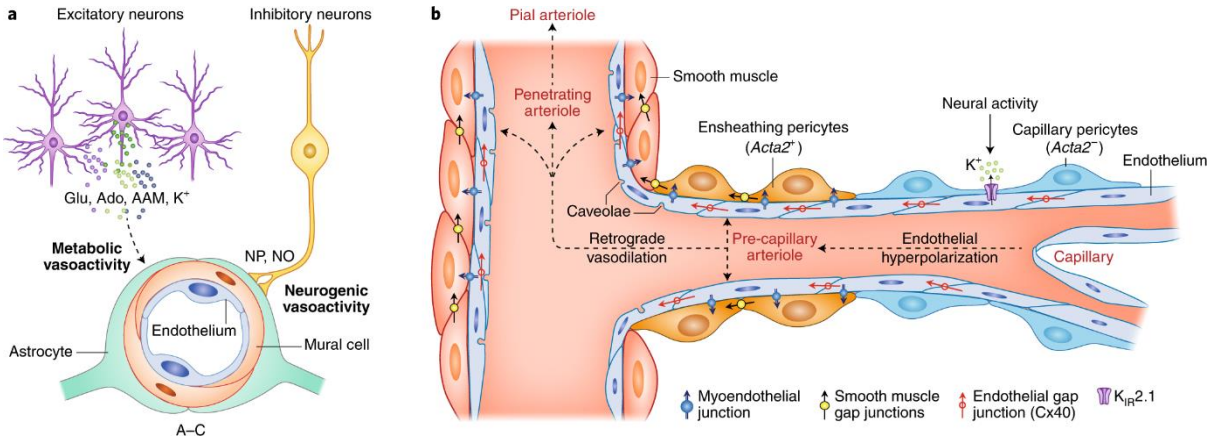

Figure 2.2 – The local and remote neurovascular coupling response. a, excitatory neurons mediate the release of vasoactive ions (K^+) and by products of neural activity such arachidonic acid metabolites (AAMs) and adenosine (Ado) while inhibitory neurons (interneurons) act directly on mural cells by releasing nitric oxide (NO) neuropeptides to bring about the local haemodynamic response. **b,** The remote response is triggered by neural activity which causes hyperpolarisation of endothelial cells. Retrograde propagation of hyperpolarisation via gap junctions results in vasodilation of upstream pial arterioles. Permission for image use was obtained from springer Nature licensing, License number: 5361891472675

2.4 The potential clinical importance of NVC

Impaired NVC is present in Alzheimer's disease (AD) patients (Kisler et al., 2017, Rombouts et al., 2000). Evidence suggests that flow abnormalities occur prior to

overt neuronal loss (Ruitenberg et al., 2005, Zlokovic, 2011). Diminished CBF reduces the delivery of essential nutrients and, importantly, impairs the clearance of neurotoxic metabolites which may result in neural loss and explain the increased risk of neurodegenerative disease associated with impair NVC (Beishon et al., 2017). Additionally animal models demonstrated a causal relationship between pharmacologically induce impairments in NVC and cognitive decline (Tarantini et al., 2015). Moreover restoration of the NVC response in aged rodent models resulted in significantly improve cognition (Tarantini et al., 2017, Tarantini et al., 2019, Tarantini et al., 2018). It should be highlighted that all this data is from animal models and therefore care should be taken when implying these changes also occur in humans, especially given that diseases such as Alzheimer's present differently in animals (Veening-Griffioen et al., 2019). Never the less this evidence has led to the view that impaired NVC maybe a sentinel event in the pathogenesis of AD (Cai et al., 2017).

2.5. Measurement of cerebral blood flow

Interest in measuring CBF and metabolism first began in the 19th century, however initial work was based on observation of pressure changes within brain tissue (Roy and Sherrington, 1890) and later on alterations in local tissue temperature (Wolff, 1936). The first quantitative method of measuring CBF was developed by Dumke and Schmidt (1943) and known as the bubble-flow meter method. This involved cannulation of the common carotid arteries, allowing blood to pass through the meter. Flow was measured by timing the passage of an injected bubble over a known distance. However the extensive surgical intervention required for this method meant that it was only used in monkeys under general anaesthesia. Shortly after this Kety and Schmidt (1945) revolutionised the field with the nitrous oxide method which enabled quantitative assessment of CBF in conscious humans. This method involved

inhalation of nitric oxide, the concentration of which was then measured in blood drawn from the arterial and venous cerebral blood supplies. The time taken to reach equilibrium between arterial and venous concentrations following commencement of nitric oxide inhalation is inversely related to CBF. A major limitation of this method however is that it could only be used to assess average CBF in the brain as a whole.

Development of the ^{133}Xenon or ^{85}Krypton technique allowed researchers to estimate regional CBF (Lassen and Ingvar, 1961, Høedt-Rasmussen et al., 1967). Isotope clearance was recorded to estimate regional flow using strategically positioned scintillation counters (Paulson et al., 2012). This method led to the establishment of the first international meeting of 'regional CBF' due to the influx of work within the area. However this method is limited by its poor temporal resolution making it impossible to track dynamic changes in CBF.

Modern technology now permits dynamic assessment of CBF via methods such as positron emission tomography (PET) (Portnow et al., 2013), single photon emission computed tomography (SPECT) (Iida et al., 1994) and functional magnetic resonance imaging (MRI) (Jueptner and Weiller, 1995). Whilst varying in application each measure can be used to quantify dynamic changes in regional and global CBF, providing important insight into cerebral haemodynamic. While all these methods offer excellent temporal and special resolution there are some major draw backs. Firstly, equipment needed is expensive and requires experts with years of experience to operate and interpret correctly. Further to this the magnetically labelled water used for MRI imaging adds significant additional costs. Secondly, intravenous injection or inhalation of contrast agents required for PET and SPECT combined with the fact that subjects are required to remain in the supine position for up to 30 minutes contributes to significant participant burden. Additionally for accurate results

these measurement techniques must be performed in a highly controlled environment and protocol restrictions such as lack of movement mean that these measurement techniques often lack ecological validity. See Wintermark et al. (2005) for a full review of each of these measurement techniques.

Transcranial Doppler ultrasonography (TCD) is an alternative method that provides relatively cheap, rapid, non-invasive estimation of CBF, by measuring blood velocity (Purkayastha and Sorond, 2013, Willie et al., 2011a). Examination is carried out using 2 MHz ultrasound probe placed over the thin pieces of bone in the temporal region of the skull, termed an acoustic window. These windows allow observation of the Doppler effect, a principle used to calculate cerebral blood velocity (CB_V). Ultrasound waves are transmitted and focused into the large cerebral arteries (ACA, MCA and PCA) where they are reflected by the movement of red blood cells. The frequency difference between emitted and reflected waves, termed the Doppler shift, is used to calculate the speed of the red blood cells. Equation 2.2 describes how flow velocity is calculated from the Doppler shift. Note that the $COS\theta$ also effects flow velocity calculations and therefore maintaining the same insonation angle throughout and between testing is of critical importance.

Equation 2.2. Doppler principle used to calculate flow velocity

$$Flow\ Velocity = \frac{(Doppler\ Shift) \times Propagation\ speed}{2\ \times Incident\ frequency\ \times COS\theta}$$

TCD is shown to have excellent intra-rater reproducibility (McDonnell et al., 2013, Willie et al., 2011a), due to the lack of B mode, vessel identification is solely based on the characteristics of the Doppler waveform (Willie et al., 2011a). Thus, the use of TCD requires continued practice and extensive training (Shen et al., 1999); however, operation of MRI or SPECT equipment requires even greater specialisation. Although TCD provides excellent temporal resolution allowing dynamic assessment of cerebrovascular function only one vessel can be insonated by the Doppler probe,

offering relatively poor spatial resolution. As a result TCD data should be interpreted with regard to the brain area supplied by the insonated vessel.

For accurate blood flow estimation TCD relies on the assumption that CBv is reflective of CBF. For this to remain true the diameter of the vessel must remain constant (See equation 2.1). As discussed previously arterial diameter in influenced by both $PaCO_2$ and alterations in blood pressure, see section 1.2 and 1.3 respectively. It is therefore important to understand if fluctuations in these parameters effect vessel diameter when collecting TCD data.

Primary work by Serrador examined how vessel diameter changes in response to alterations in end-tidal CO_2 ($P_{ET}CO_2$), which is strongly associated with $PaCO_2$ (McSwain et al., 2010). Authors observed no changes in vessel diameter in response to either the moderate hypercapnic or hypocapnic conditions studied. These findings have later been supported by Verbree et al. (2014) who illustrated that small changes in $P_{ET}CO_2$ (±7.5mmHg) resulted in no significant change in vessel diameter. However, results also demonstrated that $P_{ET}CO_2$ elevations above 15mmHg caused significant dilation (6.8% increase in vessel diameter). Despite this compelling evidence that moderate hypercapnia and hypocapnia does not affect vessel diameter, Coverdale et al. (2014) displayed opposing findings using a similar MRI protocol. It should be noted however that Verbree et al. (2014) used a far more sensitive MR unit (7T) compared Coverdale et al. (2014) (3T), providing stronger evidence that diameter likely doesn't change following small fluctuations in $P_{ET}CO_2$. Ainslie and Hoiland (2014) provides an in-depth review of these contrasting findings. Briefly, it is believed that a sigmoidal relationship exists between $PaCO_2$ and MCA diameter meaning small fluctuations within a given range are unlikely to affect diameter.

Currently the effect of blood pressure alterations on the diameter of cerebral arties is poorly understood. Serrador et al. (2000) also employed a lower body negative pressure technique, which decreases systemic blood pressure via the frank-starling mechanism (Goswami et al., 2019), and observed no alteration in vessel diameter. This study however used a 1.5T MRI scanner which has relatively poor resolution compared to the more modern 3T and 7T scanners. It is therefore possible that spatial resolution was insufficient to detect subtle changes in vessel diameter. Despite this Ainslie and Hoiland (2014) argued that vessel diameter changes which remain undetected due to a lack of magnetic resonance imaging resolution likely have a negligible effect on the discrepancy between blood flow and velocity.

2.6. Assessment of NVC

Presentation of a visual stimulus is known to increase blood flow to the visual processing areas of the cerebral cortex which are primarily supplied through the PCA (Wiedensohler et al., 2004, Azevedo et al., 2007). Following the onset of visual stimulation, blood flow through the PCA increases whilst flow through the MCA remains relatively unchanged (Azevedo et al., 2007). Traditionally during TCD assessment participants are presented with stimuli such as reading (Azevedo et al., 2007, Phillips et al., 2014, Willie et al., 2011b), viewing checkerboard patterns (Rosengarten et al., 2001, Rosengarten et al., 2006, Zaletel et al., 2004) and turning on the lights (Aaslid, 1987, Sturzenegger et al., 1996) to evoke an NVC response. A major limitation of these approaches is the moderate CBv changes elicited. This is likely down to task simplicity, as they require little eye movement or involve the eyes following a systematic and predicative layout, for example when reading (Smirl et al., 2016). With only a moderate response, the signal to noise ratio is reduced, which

means that changes in CB_V may be more vulnerable to alterations in $PaCO_2$ and/or Blood pressure (Smirl et al., 2016).

Unlike traditionally used tasks, a visual search for a specific object obscured within a field of similar shapes and colours represents a greater visual search challenge (Nodine and Krupinski, 1998, Smith and Henderson, 2011). This is because the task requires greater saccadic eye movement and longer fixation durations compared to similar tasks such as reading (Henderson and Luke, 2014, Rayner, 2009).

In an attempt to improve on traditional methods Smirl et al. (2016) examined how differing task complexities effected the NVC response. To test this, 3 visual search tasks of varying difficulties were presented on a monitor (27" apple mac) positioned ~60cm away from the participant. CB_v was measured in the MCA and PCA via transcranial ultrasound. Baseline CB_v was attained from 2 minutes of eyes open and two minutes eyes closed seated rest. Following this subject performed 5 cycles of 20s eyes closed and 40s of eyes open visual search. The simplest search paradigm consisted of viewing coloured dots, which randomly appeared on the left or right side of the screen. During the moderate complexity paradigm participants were asked to read an article of interest to them. The most complex visual task utilised a where's Wally search where the object of interest is hidden within a felid of distractors of similar appearance. Results showed elevations in CB_v in both MCA and PCA across all 3 paradigms. Whilst small CB_v increases were consistent across all 3 tasks in the MCA, the larger PCA elevations where stimulus specific. Compared to the simple and moderate paradigm the most complicated task (Where's Wally) evoked the greatest percentage increase in CB_v above baseline (16.6%, 20.1% and 30.4%,

respectively). This presumably occurred due to a greater metabolic demand associated with increased task complexity. Additional the more complex task may benefit from improved participant engagement (Gitelman et al., 2002), which is associated with a greater NVC response (Burma et al., 2021). Authors concluded that previously used visual challenges provide a less robust NVC response that may fail to capture the full relationship between neural activity and changes in PCA CBv.

Despite the potential influence of $PaCO_2$ and blood pressure on CBF, the above work demonstrated that $PaCO_2$ was maintained at eucapnia throughout each of the visual search protocols (Smirl et al., 2016). Whilst small oscillations in blood pressure were observed during each task, work by Serrador et al. (2000) demonstrates that this is unlikely to significantly affect vessel diameter (Smirl et al., 2016). Additionally, during a *Where's Wally* search task the TCD signal to noise ratio is maximised, consequently reducing any potential impact of blood pressure oscillations (Smirl et al., 2016). Later work also shows excellent with-in and between day reliability using this search task, advocating the use of at least 5 search trials, which are then ensemble averaged to maximise this reliability (Burma et al., 2022). This suggests that is method can be used effectively to detect change.

2.6.1 Analysis of NVC data

Leading work within the field,(Smirl et al., 2016, Smirl et al., 2020, Burma et al., 2022, Wright et al., 2017a) typically presents data as: 1) baseline CB_v, 2) Absolute and relative Peak CB_v to assess the response magnitude, 2) The area under the curve (AUC) during the first 30s following stimulus onset as a means of evaluating

total activation, 3) time to peak CB_v to provide information about the speed of response and 4) time taken to reach a 1% increase in CB_v above baseline (see figure 2.3). Whilst the latter, attempts to indicate the delay between stimulus onset and initiation of the NVC response, the beat to beat variation in CB_v is >1%. Therefore, given that the error within the measure is greater than the threshold used to detect change, it is not deemed appropriate to use this method to assess the response onset. Furthermore, expressing the speed of response as time to peak CB_v fails to provide information on the progression of the response.

Prior work examining the CB_v response to CO_2 breathing demonstrates that dynamic kinetic based changes pose improved sensitivity to detect differences in aging compared to amplitude based measures (Tallon et al., 2020, Koep et al.). Kinetic modelling also illustrates how the response progresses to peak and details the delay between stimulus onset and the start of the response. Therefore the addition of kinetic modelling outcomes alongside traditional amplitude based metrics may provide added insight into the regulatory response.

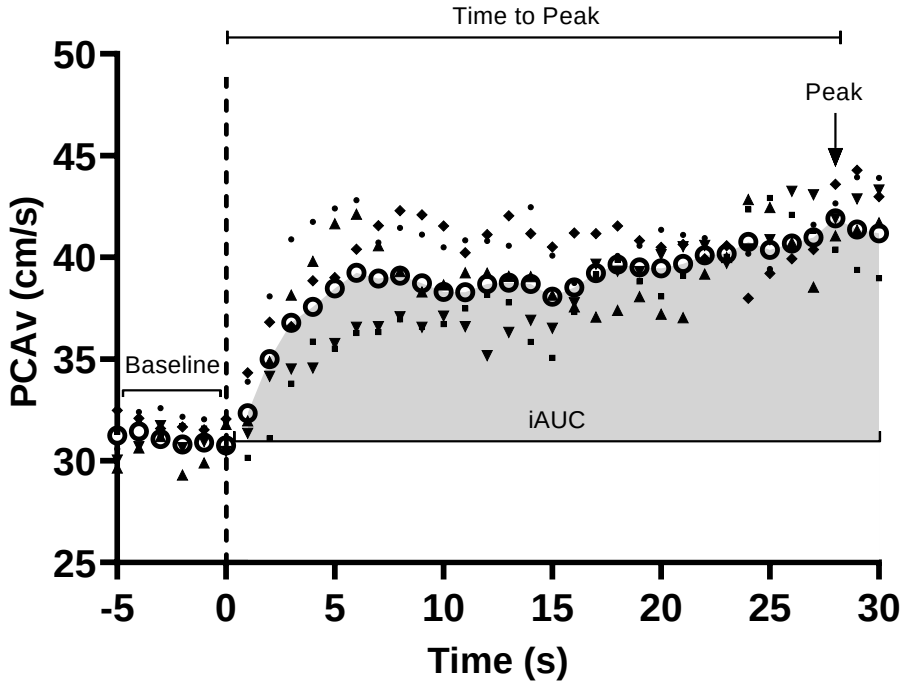

Figure 2.3 - A representative trace of cerebral blood velocity through the posterior cerebral artery from one participant during an NVC test. The dotted vertical line denotes the start of the visual search task. Data is displayed for each of the 5 trials alongside the ensemble average (open circles). Note that time to 1% increase is not displayed, but occurred 1 second into the eyes open response.

2.7. Head Impacts and regulation of cerebral blood flow

2.7.1 Concussive impacts and regulation of cerebral blood flow

In an attempt to better understand the pathophysiological effects of mild traumatic brain injury (mTBI) and concussion recent work has investigated how CBF regulation is impacted post injury. Compared to healthy controls Len et al. (2011) observed reduced CVR_{CO2} within 7 days of a sports related concussion. Purkayastha et al. (2019) attempted to extend this work by tracking the time course of CVR_{CO2} alterations. Results demonstrated an attenuated CVR_{CO2} response at 90 days post injury (final time point), however, authors conceded that an extended follow-up

period is required to fully understand the physiological recovery time course (Purkayastha et al., 2019).

Alterations in CA are also present post mTBI (Junger et al., 1997, Strebel et al., 1997) and concussion (Wright et al., 2018b). These changes are clinically significant as impaired CA is predictive of patient outcome following TBI, with the poorest CA associated with the worst outcome (Czosnyka et al., 1997, Kirkness et al., 2001, Sorrentino et al., 2012).

Data is limited on how NVC is altered after mTBI and concussion. Cross sectional work in retired rugby players reported impaired NVC in athletes with a history of 3 or more concussions compared to controls with no concussion history (Sharma et al., 2020). This finding is supported by recent work demonstrating reduced oxygenation in the left prefrontal cortex during a Where's Wally search task, in male contact sport athletes that reported a history of 3 or more concussions (Sirant et al., 2022). Acutely, work by Mayer et al. (2014) and Wright et al. (2017b) demonstrate altered NVC 2 weeks post traumatic brain injury and sports related concussion, respectively. Interestingly, NVC alterations were seen despite earlier symptom resolution suggesting that physiological impairment extends beyond clinical recovery. Mayer et al. (2014) utilized functional MRI imaging and a simple sensory motor task to assess the haemodynamic response. Results indicated regional specific changes driven by a faster initial rise (2-4 seconds following stimulus onset) and a quicker overall time-to-peak. Visual inspection of activation maps also showed larger activation areas with the mTBI group. Wright et al. (2017b) used visual stimulation and TCD assessment to examine the NVC response. Results revealed an acutely delayed CBF response (56.6% slower) and a greater total response magnitude (31% increase in CBv area under the curve), 72hrs post injury. These results lead authors

to hypothesise that physiological alterations may reflect recruitment of additional neural resources to compensate for cognitive deficits caused by injury. It is important to note that this work was performed in men and currently there is no data available in women post-concussion.

2.7.2 Subconcussive head impacts and regulation of cerebral blood flow

As previously stated, the repeated exposure to subconcussive head impacts (i.e. heading) might partly explain the increased risk of neurodegenerative disease in retired footballers (Mackay et al., 2019, Russell et al., 2021), and therefore require consideration. In the absence of concussion, impairments in CVR_{CO2} have been witnessed in male footballers following a playing career of 18 ± 6 years (Marley et al., 2021) and in female footballers across a season (Svaldi et al., 2017). Additionally Wright et al. (2018a) demonstrated that CA was significantly altered following a season of contact sports participation, again in the absence of concussion. Whilst authors cautiously linked observations to repetitive subconcussive head impact exposure (heading), due to the nature of the study designs it is not possible to fully establish causality.

Studies examining the effect of subconcussive impacts on the NVC response across a season of contact sport participation (Including Ice hockey and American football) report no difference between pre and post seasons measures (Smirl et al., 2017, Wright et al., 2017a).

Interventional work has since attempted to isolate the effect of heading and improve our understanding of the neurophysiological changes associated with repetitive subconcussive impacts. Smirl et al. (2020) evaluated the NVC response and scores following an acute bout of soccer heading, in males. A Where's Wally search task

was used to evoke the NVC response which was assessed using TCD, pre and post control, sham and heading conditions as described in Smirl et al. (2016).The heading condition consisted of a bout of 40 headers in 20 minutes, delivered at 77.5 ± 3.7km/h from a JUGS machine positioned ~25m away from participant. During the sham and control conditions heading was replaced with body contact and quiet rest, respectively. No changes were seen in PCA derived NVC responses across conditions. However following controlled heading authors observed a reduction in total activation through the MCA of 67% compared to pre heading measures. No change was observed following sham or control conditions. This suggests that heading causes alterations in the functional supply to the frontotemporal (MCA supplied) regions of the brain but not the occipital (areas supplied by the PCA).

Whilst the stimulus employed in this study is not representative of typical exposure it may allow inferences to be made regarding the long term pathological effects of heading. Furthermore it suggests that this regulatory mechanism maybe sensitive to damage induced by this type of head trauma. However the issue with this approach is that it risks sensationalising any findings which may result in misconceptions regarding the danger of heading. Additionally this work was performed in males only. Work is therefore needed exploring the effect of heading in females after exposure to a more realistic stimulus.

2.8 Head impacts and sex

Epidemiological work demonstrates higher concussion rates amongst women compared to men across a range of sports, including, football, basketball, lacrosse and softball (Zuckerman et al., 2015). Specifically within football the concussion risk is thought to be almost double for females (Bretzin et al., 2021). Additionally sex

differences have been reported in the type of symptoms experienced, the severity of neurocognitive impairment and recovery time, with these typically being worse in women (Covassin et al., 2018). It should be noted however that female athletes are more likely to report concussion than males which likely contribute to these sex differences (McGroarty et al., 2020).

Despite potentially increased severity , the frequency of concussion in women's football remains low (Putukian et al., 2019) with some studies reporting rates of 0.54 per 1000 athlete exposure (Kerr et al., 2017) Incidences do not typically stem from contact with the ball but rather from contact with another player, the goal post or the ground, (Dick et al., 2007). Heading may court these types of contacts by presenting scenarios more likely to result in head-head or head to ground contacts however a survey based study conducted over a season even demonstrated that no reported concussion was associated with intentionally heading the ball (Boden et al., 1998).

Whilst purposeful heading may not result in concussion it does represent significant sub-concussive impact exposure. Indeed, it has been shown that elite male footballers with no concussion history have widespread differences in cerebral white matter integrity when compared to non-contact athletes (swimmers) (Koerte et al., 2012). Additionally, white matter disruptions in male and female amateur footballers were associated with the number of headers performed in the previous 12 months but not with the number of concussions (Lipton et al., 2013). Later work by the same group identified a heading related sex difference with similar heading exposure being associated with greater white matter disruption in females (Rubin et al., 2018). These findings are in line with work by Tierney et al. (2008) who showed that head accelerations experienced by women are 10% greater than men when heading a ball at the same velocity. This work was later been supported by Bretzin et al. (2017) and

Cassese et al (2018 who respectively observed experience greater rotational and linear head accelerations in female footballers (1416.13 ± 507.63 rad/s^2 vs 774.60 ± 501.13 rad/s^2 and 40.9 ± 13.9g vs 27.6 ± 8.5g, respectively). Bretzin et al. (2017) and Müller and Zentgraf (2021) both observed that female footballers have lower neck flexion strength than males (23.12 ± 5.38kg vs 34.66 ± 8.60kg, P= 0.012 and 48.6 ± 7.9N vs 95.2 ± 36.5N , p = 0.004, respectively). These observations combined with females lower head mass (Yoganandan et al., 2009) are thought to explain much of the proportionally higher head acceleration experienced (Basinas et al., 2022). Indeed, neck strength correlates with linear accelerations and rotational velocities experienced during heading (Bretzin et al., 2017), which, based on impact modelling are thought to be predictive of neuronal injury (Ji et al., 2014).

Recent Interventional work has helped strengthen the evidence of association between heading and neuronal injury. Wallace et al. (2018) demonstrated an increase in neurofilament light chain proteins, a biomarker which is thought to reflect the severity of axonal injury (Ljungqvist et al., 2017), following a bout of 40 headers in 20 minutes. This led authors to conclude that heading is a potential mechanism of axonal injury. A limitation of this work however is that the number of headers used far exceeds that which would reasonably occur during a match or in training (Kenny et al., 2022). However, pilot work by Wirsching et al. (2019) has since shown an increase in neurofilament light chain protein following a more representative 10 headers in males and females, although the study was insufficiently powered to detect sex differences.

2.9 Heading in football

2.9.1 Current heading guidelines

Given the current concern that the repeated act of heading might contribute to the observed increased risk of neurodegenerative disease in former professional, male footballers, the sport's governing bodies have recently revised there guidance on heading. Prior to the 2021-2022 season the FA developed precautionary guidelines (details of which can be found in section 2.8.1) to ensure player welfare whilst additional research into the pathophysiological effects of heading is gathered. These guidelines have been implemented across every level of the footballing pyramid and have are endorsed by the Premier league, English Football League, Professional Footballers Association, and the League Managers Association.

The guidance for players and coaches has been tailored to the level of participation. Heading for professional clubs (those in and above step 4 of the national league system and tier 2 of the women's pyramid) is limited to 10 high force headers, those occurring from long passes (>35 meters), in any training week. Coaches are also encouraged to tailor heading training for each player to reflect the type and frequency of headers typically experienced during matches (League, 2021).

Heading practice for adult amateur footballers, up to and including step 5 of the national league system and tier 3 of the women's pyramid, should be limited to one session per week with a maximum of 10 headers per session. It is recommended that technique is practiced using thrown delivers to reduce the potential load placed on the head (FA, 2021c).

Finally, the FA's youth football guidelines state that heading should not be introduced during the foundation phase (up to U11) and a graduated approach should be used during the development stage (U12 – U16). Coaches are also encouraged to focus on the development of other skills including ball control and passing throughout both stages. Details on heading limits throughout youth football can be found in table 2.1. It should be noted that current guidance does not affect matches, a decision justified by the low occurrence of heading in youth matches (FA, 2021d).

Table 2.1. Youth football heading exposure limits, produced using data from the FA's heading guidelines in youth football (FA, 2021d)

Age Group	Exposure Guidance
Under 7's – Under 11	Heading should not be introduced during training
Under 12	1 session per month (Max of 5 headers)
Under 13	1 session per week (max of 5 headers)
Under 14 – Under 18	1 session per week (max 10 Headers)

Recently the Scottish FA expanded these guidelines, banning heading the day before and the day after matches in an attempt to further reduce the overall exposure (BBC, 2022). This additional change followed recommendation from Professor Willie Stuart, a corresponding author of the work which highlighted then link between football participation and increased neurodegenerative disease risk (Mackay et al., 2019, Russell et al., 2021)

2.9.2 Typical Heading exposure

A recent review of 42 articles that objectively quantified heading exposure attempted to shed light on the number of headers typically experienced during training and matches (McCunn et al., 2021). Whilst differences in how studies reported impact exposure made comparison difficult, the authors concluded that the number of headers experienced during matches ranged from 1 to 9, meanwhile, infrequent

reports on impact exposure during training left authors unable to comment on incidence rates (McCunn et al., 2021). Notably, the majority of studies included (33/42) observed youth/student populations which means findings may not represent the number of headers experienced during adult professional or amateur football. Still, given the colligate population used in the current work these findings likely represent a good estimate of the number of headers they will typically experience. However, the review failed to consider differences in heading frequency between sexes.

Lynall et al. (2016) quantified heading impact exposure in collegiate female footballers across a season. The reported impact exposure was 7.16 per player per 90 minutes. These values are considerably different to recent reports from Kenny et al. (2022) who also examined collegiate female footballers. Mean impact exposure in this study was 0.34 per match and 2.99 per practice. Authors also noted positional differences with defenders experiencing the greatest impact exposure overall and during matches while forwards had the highest exposure during practice. Variations in reported exposure is likely caused by differences in a teams playing style and coaching philosophies. Brooks et al. (2021) demonstrated that 41.2% of purposeful headers occurred following an aerial pass. It would therefore be reasonable to assume that a team whose philosophy centres on paying aerial passes would experience a greater number of heading incidence. The disparity between reported exposures may also be effect by the level of play.

2.10 The effect of heading on cognitive function

Cognitive function is a collective term that refers to several mental abilities including learning, reasoning, remembering, problem solving, decision making and attention

(Fisher et al., 2019). The changes in the ability of an individual to perform these processes are the symptomatic manifestation of dementia. Cognitive abilities are also impaired post-concussion and is therefore assessed as part of pitch side concussion checks such as the SCAT5. There is therefore interest in understanding how heading acutely influences cognitive function.

A recent systematic review identified 12 studies that investigated the relationship between objectively quantified heading exposure and acute alterations in cognitive function (McCunn et al., 2021). Only 3 of the 12 studies observed a decline in cognitive function and deficits where limited to memory and reaction time (McCunn et al., 2021). It should be noted that 2 of these studies examined adolescent populations which limits the generalisability of findings as cognitive function is effected by pubertal maturation. Additionally, there is evidence to suggest that teenager athletes may be more vulnerable to head impacts in comparison to more mature collegiate athletes (Field et al., 2003). The remaining study identified impairments in short and long term memory in a group of adult amateur footballers (22 ± 3 years) following a bout of 20 headers in 10 minutes (Di Virgilio et al., 2016). This work however failed to assess sex differences and implemented a stimulus far above that which could reasonably be expected in a single session. Since this review (Ashton et al., 2021) demonstrated impairments in working memory post heading. However they also implemented an unrealistic heading stimulus (20 headers in 3 minutes) and only examined male footballers. Studies examining the relationship between heading and cognitive impairment in female footballers show no change in high school and college players after exposure to 15 headers (Gutierrez et al., 2014) or post season (Kaminski et al., 2007), respectively.

2.11 Intracranial pressure (ICP)

Intracranial pressure (ICP) refers to the pressure that the cerebrospinal fluid exerts in the subarachnoid space. It is well documented that ICP rises following moderate and severe TBI (Haider et al., 2018). As the brain is situated within a hard, inflexible skull, increased ICP can impair CBF and result in secondary ischemic injury (Czosnyka et al., 2017). ICP is related to patient outcome following severe TBI, with differences being observed up to 168 hours post injury between functional survivors and those with a fatal outcome (Adams et al., 2017). Whilst work is limited, a recent systematic review suggested that ICP is also elevated following mTBI, such as concussion (Haider et al., 2018). Additionally animal studies suggest that ICP recovery following mTBI corresponds with symptomatic and functional recovery, indicating this is an important marker of full recover following injury (Haider et al., 2018).

Chronic exposure to elevated ICP was first hypothesised to contribute to the development of AD in 1994 (Wostyn). This has since been supported by data demonstrating high incidence rates of AD in patients with normal-pressure hydrocephalus (Golomb, 2000), a disease state associated with elevated ICP (Sahuquillo et al., 1991). It is thought that prolonged increases in ICP result in downregulation of cerebral spinal fluid, causing impaired clearance of extracellular fluids, leading to the accumulation of neurotoxic substances in the brain (Silverberg et al., 2002). Wostyn since hypothesised that repetitive intermittent ICP elevations may lead to a similar cascade of CSF circulatory failure, due to the cumulative effect of repeat exposure (Wostyn, 2004). If this proves to be true, activities which result in repetitive increases in ICP may significantly increase neurodegenerative disease risk. This hypothesis can be tested by examining subject's whose occupations and hobbies are associated with repetitive exposure to elevated ICP.

Currently only two studies have attempted to assess changes in ICP following caused by heading (Lee et al., 2020, Sadrameli et al., 2018). Lee et al. (2020) utilised acoustically evoked tympanic membrane displacements (TMD) to estimate of ICP. A greater negative volume displacement was observed following 6 headers of a football kicked from 35 yards away (Lee et al., 2020). Whilst this data suggests that ICP increased post heading Shimbles et al. (2005) demonstrated that TMD could only successfully be applied to 40% of patients with intracranial hypertension. Additionally inter-subject variability was so great that authors concluded that this method is not a reliable indicator of ICP. Sadrameli et al. (2018) on the other hand examined the change in optic nerve sheath diameter (ONSD), a suitable surrogate measure of ICP (see section 2.10.1), in female colligate footballers across a season. The findings revealed a significant increase in ONSD (4.14 ± 0.6mm to 5.02 ± 0.72mm, $P < 0.0001$) in the absence of concussion, which suggests that alterations may have been caused by the repetitive sub-concussive act of heading. Currently no interventional work has explored the acute effect of heading on ONSD. Such work would strengthen the argument that it is in fact the act of heading which resulted in the increase in ONSD seen by Sadrameli et al. (2018).

2.11.1 Optic nerve sheath diameter

Whilst invasive monitoring techniques such as external ventricle drainage are considered the gold standard (Raboel et al., 2012), they require highly specialised neurosurgical expertise and strict neurosurgical settings. Furthermore, insertion of ICP monitors are associated with complications including; infection in 2-27% of cases (Beer et al., 2008) and haemorrhage in 5.7% of cases (Binz et al., 2009).

Therefore there is a need for a non-invasive measure that provides fast accurate assessment of intracranial pressure.

Ultrasound based measurement of the diameter of the optic nerve sheath is plausible alternative methods to invasive ICP monitoring. As the subarachnoid space of the optic nerve is continuous with the subarachnoid space of the brain, elevations in intracranial pressure are transmitted to the optic nerve causing it to swell. Work by Kimberly et al. (2008) demonstrates that the diameter of the optic nerve is positively correlated (0.59) with direct measures of intracranial pressure. Additionally data suggest that assessment of ONSD can be used as a screening tool to identify elevated ICP (>20mmHg), with a measurement sensitivity of 96% and specificity of 94% (Rajajee et al., 2011). Importantly, ONSD measurement is also sensitive to dynamic changes in ICP, as shown by Chen et al. (2019), reporting a significant correlation between ΔONSD and ΔICP (0.451). Ultrasound based assessment of ONSD also possess good inter- and intra-observer reliability (Shah et al., 2009) and this can be achieved without specialised probes (Bäuerle et al., 2013). These studies indicate that measurement of the ONSD can be used as a surrogate measure of ICP that can detect changes in real time. ONSD measurement is considered the best non-invasive tool for dynamic ICP estimation (Richards et al., 2023)

2.12 Literature review summary

The potential link between heading and long term neurodegenerative disease is an area of study gaining extensive media coverage. This review highlights the lack of research into the effects of heading on women despite huge increases in participation levels and evidence that they may be more effected by this type of trauma (Rubin et al., 2018). The review provides details on how two important markers of brain health believed to be effected in the development of

neurodegenerative disease, NVC and ICP, maybe influenced by repetitive head trauma's such as those experienced when heading. Furthermore the reviews critical analysis of current heading literature calls attention to the lack of ecological validity. This provides the rationale for the work current work, presented below, which exclusively recruited female footballer's whist addressing the limitations of previous work by employing a more ecologically valid stimulus.

Chapter 3: Neurovascular coupling and intracranial pressure is not acutely altered following a representative number of headers in women footballers.

3.1 Introduction

With over 265 million active participants worldwide, football is the most popular sport in the word (FIFA, 2007). However, concerns regarding participation have arisen following work which highlights that former professional male footballers are at 3.5 times greater risk of neurodegenerative disease (Mackay et al., 2019). Further analysis by this group revealed that disease risk differed by playing positon, with defenders - a position associated with the greatest heading frequency (Cassoudesalle et al., 2020) - being the most at risk (Russell et al., 2021). Meanwhile, goalkeepers whose heading incidence is extremely low, posed no additional risk when compared to the general population (Russell et al., 2021). This has led to speculation that the repeated act of heading maybe responsible, at least in part, for the increased risk of neurodegenerative disease (Nowinski et al., 2022, Lipton et al., 2013).

It is shown that footballers with no history of concussion have widespread differences in cerebral white matter integrity, compared to non-contact athletes (Koerte et al., 2012), with later work demonstrating that these disruptions were associated with heading exposure (Lipton et al., 2013). Recent interventional work also shows that heading acutely alters the regulation of cerebral blood flow (Smirl et al., 2022, Smirl et al., 2020). Specifically, alterations in the NVC response - the ability to adjust cerebral blood flow in response to changes in local metabolic demand - is present in those with AD (Kisler et al., 2017, Rombouts et al., 2000) and is thought to be a sentinel event in its progression (Cai et al., 2017). Animal models support this hypothesis, with impaired NVC resulting in cognitive decline (Tarantini et al., 2015)

which is significantly improved following restoration of this regulatory mechanism (Tarantini et al., 2018).Therefore it is thought that exposure to repetitive intermittent alterations in this regulatory mechanism may explain at least some of the increased risk of neurodegenerative disease seen in retired footballers.

A limitation of current work examining the NVC response post heading, is the unrealistic stimulus used (40 headers in 20 minutes) (Smirl et al., 2020). In contrast it is reported that collegiate women footballers perform an average of 7 headers per 90 minute game (Lynall et al., 2016). However, recent reports suggest heading exposure could be even lower (0.34 per match and 2.99 per practice) (Kenny et al., 2022). Whilst studies which employ an exaggerated heading stimulus allow valuable inferences to be made regarding the long term pathological effects, they risk sensationalising the true effects. Given the current evidence we believe that a bout of 6 headers in 60 minutes represents a more reasonable maximum exposure that will help address limitations within existing work (Smirl et al., 2020).

Whilst previous NVC work may lack ecological validity work by lee et al 2004 utilised a more representative stimuli to examine how heading effects another clinically relevant marker of brain health, intracranial pressure. Intracranial pressure – the pressure exerted by the cerebral spinal fluid within the subarachnoid space – is shown to be related to patient outcome following brain trauma (Adams et al., 2017) and symptomatic and functional recovery following mild traumatic brain injury (Haider et al., 2018). Work by Lee et al. (2020) demonstrates that ICP pressure, estimated using acoustically evoked TMD, is increased following a bout of 6 headers. Wostyn (2004) hypothesised that exposure to repetitive transient increases in ICP may lead to a cascade of CSF circulatory failure resulting in impaired clearance of extracellular fluids and the accumulation of neurotoxic substances in the brain. Therefore

elevations in ICP witnessed after heading may significantly contribute to the development of neurodegenerative disease. Examining how a representative bout of heading acutely influences NVC and ICP may improve current understanding of the pathophysiological underpinnings that result in the associated increased neurodegenerative disease risk.

Whilst Lee et al. (2020) appears to provide compelling evidence that ICP is raised following heading, Shimbles et al. (2005) suggested that the measurement tool implemented (TMD) could not be used as a reliable indicator of ICP due to poor inter-subject variability. Therefore the effect of heading on ICP warrants further investigation. Measurement of the ONSD is shown to be a valid alternative to invasive ICP monitoring (Kimberly et al., 2008, Rajajee et al., 2011, Shah et al., 2009) with good reliability (Bäuerle et al., 2013). Importantly ONSD is associated with patient outcome following server TBI (Legrand et al., 2013) and is sensitive to acute changes in ICP (Chen et al., 2019) making this a suitable measurement tool to track changes post heading.

A further limitation of the current evidence base linking football, and by extension heading, with an increased neurodegenerative disease risk is that it is solely driven by data generated from former male footballers (Mackay et al., 2019, Russell et al., 2021). This is primarily because no such female evidence bases exists. Additionally, interventional work examining changes in NVC and ICP following heading has only examined male cohorts (Smirl et al., 2020, Lee et al., 2020). Despite this female footballers experience ~10% greater head accelerations during heading (Bretzin et al., 2017, Tierney et al., 2008) and present with greater white matter alterations than males when matched for heading exposure (Rubin et al., 2018). This could suggest that heading poses a greater risk of neurodegenerative disease in women compared

to males. The purpose of this study therefore was to further understanding of the pathophysiological effects of heading by identifying how a representative bout acutely influences NVC and ICP in women footballers. Given that cognitive tests are routinely used to detect any deleterious effect of head impacts in research and practice, we also assessed working memory (McCunn et al., 2021).

3.2 Materials and Methods

3.2.1 Ethical approval

Ethical approval was granted by the University of Exeter Sport and Health Sciences Ethics Committee (2012-A-01). The study was conducted in accordance with the standards set forth in the 1964 Declaration of Helsinki. Participants were given an information sheet which detailed the experimental procedure and highlighted potential benefits and risks of taking part. Written informed consent was then obtained prior to participation.

3.2.2 Participants

This study used purposive sampling; participants had to be women footballers involved in regular practice and competitive matches (~1 practice and ~1 match per week). Exclusion criteria included any diagnosed neurological condition, contraindications to performing headers such as meningitis and a history of seizures, not being an outfield player, and having sustained a concussion within the 2 months prior to participation. Only 3 participants reported any concussion history, each limited to 1 episode. Nineteen female footballers (10 defenders, 5 midfielders and 4 forwards) were recruited (table 3.1).

Table 3.1. Participant characteristics (n=19)

	Mean ± Standard Deviation
Age (y)	21.9 ± 3.1
Height (m)	1.64 ± 0.05
Mass (kg)	64.3 ± 7.9
Body mass index (kg/m^2)	24.0 ± 2.9
Playing Experience (y)	11.7 ± 5.4

3.2.3 Study design

Participants completed both control and heading interventions on separate days, spaced at least 7 days apart. Visit order was counterbalanced for the first 18 participants with the 19th being randomly assigned. Variables of interest (outlined below) were measured before and one hour after the control (time-matched seated rest) or heading protocol (figure 3.1). During the heading intervention participants stood ~15 m from a motorised ball launcher (Ball Launcher Pro Trainer) and performed 6 headers in an hour – one every 10 minutes. The ball used was an official size 5 UEFA Women's Champions League football, inflated to the regulation 12 psi and propelled from the ball launcher at 40 ± 5 km/h which is in line keeping with previous work (Caccese et al., 2018, Di Virgilio et al., 2016). The speed of delivery and the number of headers were selected following work by Barfield et al. (2002) and Lynall et al. (2016) who respectively showed that female players strike the ball at an average ball velocity of 58.32 ± 4.68 km/h when using there instep, and perform 7 headers in a 90 minute match. Ball speed and pressure was confirmed prior to each testing session using a Supido Multi Sports Speed Radar and Forza Ball Pressure Gauge, respectively. In an attempt to mimic a corner kick, an element

of play consistently resulting in heading situations, participants were instructed to direct headers towards a researcher positioned perpendicular to the flight of the ball.

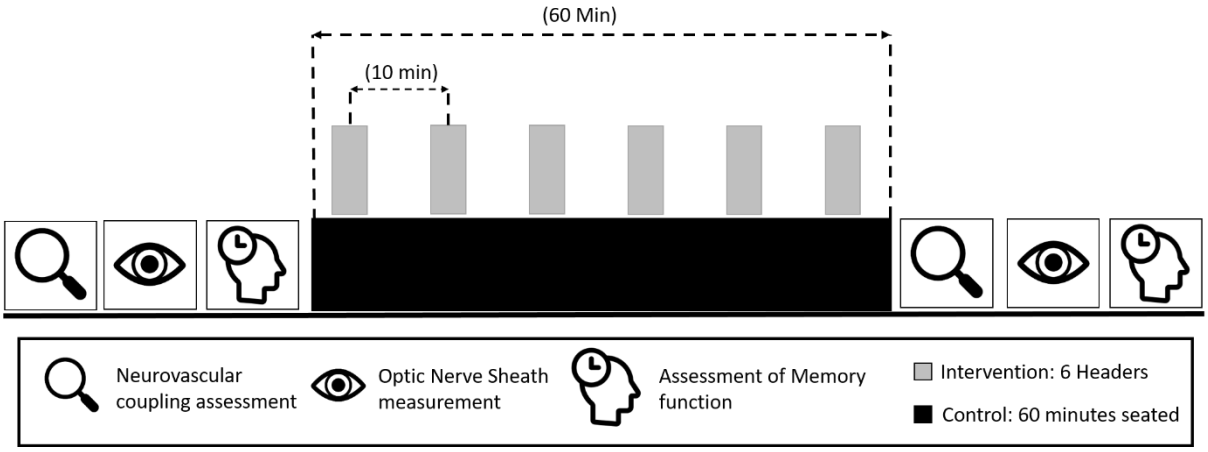

Figure 3.1. Experimental protocol

3.2.4 Instrumentation

For NVC assessment, cerebral blood velocity through the PCA (PCAv) was measured via TCD in accordance with current guidelines (Willie et al., 2011a). Briefly, PCAv was quantified using a 2-MHz ultrasound probe, positioned over the right temporal acoustic window and held in place using a customisable headset (DiaMon, DWL). The p-1 segment of the PCA was insonated and optimised by assessing the waveform, signal depth and blood velocity and confirmed via visual stimulation or carotid compression. The scan depth and probe position was noted and an effort was made to replicate these within and between days for each participant. Beat-by-Beat mean arterial pressure was obtained via finger photoplethysmography (Finometer PRO, Netherlands). All data was time-aligned and sampled continuously at 200Hz using an analogue-to-digital converter (powerlab; model – 8/30, ADInstruments, Colorado Springs, CO, USA) linked to a laptop computer for subsequent offline analysis using commercially available software (LabChart Version 8, AD Instruments).

OSND was quantified via high resolution ocular ultrasonography (Apogee 1000, SIUI, China) using a 13 MHz probe. The probe angle was adjusted to display the entry of the optic nerve into the globe and images were optimised by modifying the gain to provide clearly defined boundaries (Figure 3.3).

3.2.5. Quantification of NVC

NVC was assessed via a visual search task following recent methodological guidelines (Smirl et al., 2016) and in keeping with previous work (Smirl et al., 2020). After a 1 minute baseline (with eyes closed), volunteers performed 5 cycles of 20 seconds eyes closed followed by 40 seconds eyes open, within which a visual search task was presented. Subjects were seated ~60cm from a “Where’s Wally”® book and instructed to ‘find Wally’ whose appearance they were familiarised with prior to initiating the assessment. Upon successful identification a new search task was presented, thus permitting 40s of continuous search.

The PCA_v and MAP response for each trial was scrutinised before ensemble averaging the 5 transitions. Four participants were excluded from analysis as a result of consistently poor PCA_v signal quality across visits. In keeping with previous literature (Smirl et al., 2020, Smirl et al., 2016, Wright et al., 2017a, Burma et al., 2022) data was presented as the mean, absolute peak and percentage increase in PCAv above the last 5 seconds of averaged eyes closed baseline, each within the first 30s of the search trial. The time to peak PCAv was recorded and incremental area under the curve (iAUC) indexed by calculating the baseline adjusted area under the PCAv curve against time (Figure 2.3).

The reliability of traditional measures were assessed in accordance with contemporary guidelines for sports medicine (Hopkins, 2000). When assessed in this

manner, other than iAUC, the NVC outcomes demonstrated within- (CV <21.9%) and between-day reliability (CV <22.0%) that are comparable to previous work (Burma et al., 2022). For transparency, the within-day (i.e. pre and post control condition) and between-day (pre control versus pre heading) reliability for our NVC outcomes are presented in Table 3.2. Whilst both the within and between-day reliability of the iAUC is not as good as previous reports, the mean difference between assessments was small and did not reach statistical significance (MD = 5.8 cm/s/30s, *P* = 0.73; MD = 1.1, *P* = 0.96, respectively). Similarly, whilst significant differences were present for baseline PCAv and mean PCAv within-day, these differences were of small magnitude (<2cm/s) and the increase in PCAv, when expressed as a percentage of baseline, was not different.

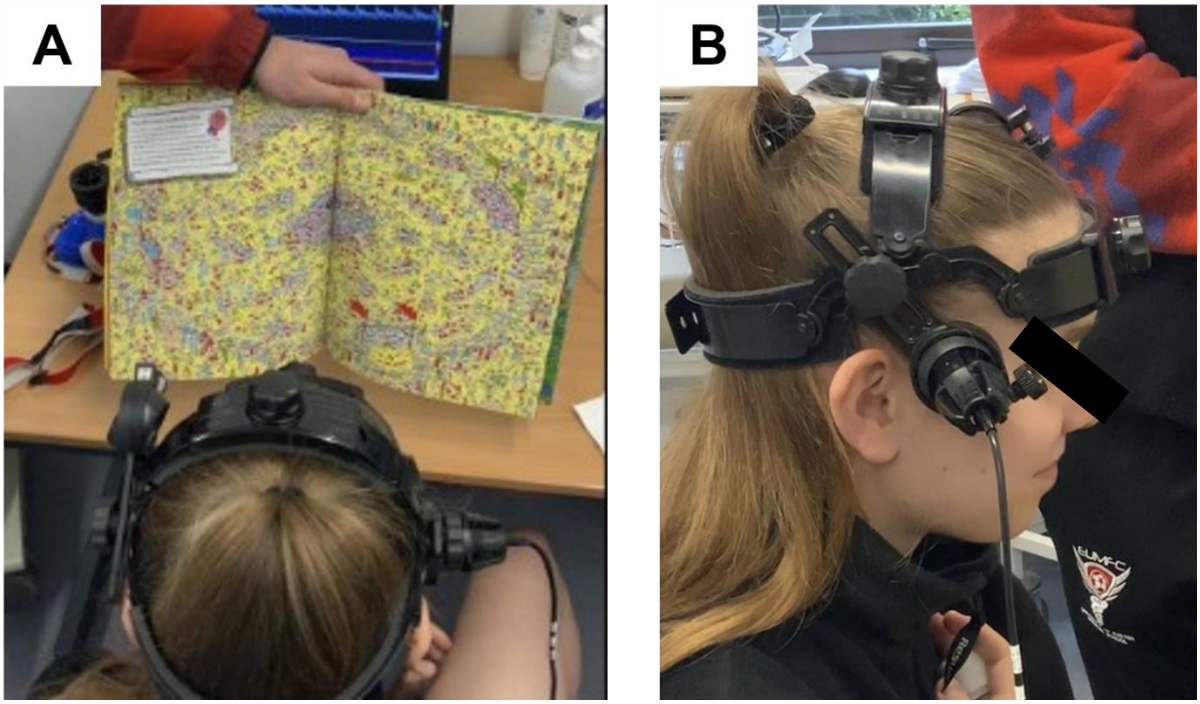

Figure 3.2. Example of experimental setup used to measure posterior cerebral artery blood velocity for neurovascular coupling assessment. A. Where's Wally search task displayed ~60cm in front of participants. **B.** A customisable headset was used to secure a 2-MHz ultrasound probe over the right temporal acoustic window.

3.2.6 Kinetic analysis of the NVC response

To map the time course of the PCAv response, kinetic analysis was performed on the averaged, time aligned PCAv data. Data were baseline corrected using the averaged last 5 seconds of eyes closed baseline and analysed using a mono-exponential model (Equation 3.1) in GraphPad Prism.

Equation 3.1: Mono-exponential model without time delay

$$PCAv\ (t) = \Delta PCAv_A\left(1 - e^{-(t/\tau)}\right)$$

Where PCAv(t) is the PCAv at a given time (t), $\Delta PCAv_A$ is the amplitude of the PCAv change from baseline to its asymptote, and τ is the time constant. Whilst the majority of trials appeared to present no time delay, for some trials (13) the PCA_v response could be mapped more closely with the inclusion of a time delay (equation 3.2). The model selected was driven by the goodness of fit.

Equation 3.2: Mono-exponential model with a time delay

$$PCAv\ (t) = \Delta PCAv_A\left(1 - e^{-(t-TD/\tau)}\right)$$

To optimize the fit of the exponential, PCA_v , data were modelled from the start of the exponential increase until a visible departure from steady state. The start, end, overall fit and model selected (with/without a time delay) was verified by three researchers (BB, AL, JK) who were blinded to the condition (i.e. heading or control). When determining the appropriateness of model fits, consideration was given to the distribution of the residuals and a goodness of fit (R^2) >0.5. The precision of the τ value was determined using the 95% confidence intervals.

The reliability of kinetic variables was also assessed and is presented in table 3.2. The tau shows poorer reliability than traditional metrics used to characterise NVC, however this is typical of time-based responses (Koep et al., 2022).

Table 3.2 Reliability of NVC outcomes and ONSD

				Within-day reliability					
	N	Pre Control (mean ± SD)	Post Control (mean ± SD)	MD	*P* value	*d*	ICC	CV (%)	*r*
Baseline PCAv (cm/s)	15	37.6 ± 5.4	39.3 ± 5.8	1.7	0.03	0.30	0.89	5.3	0.89
Mean PCAv (cm/s)	15	46.8 ± 8.7	48.4 ± 8.1	1.6	0.02	0.19	0.97	3.5	0.97
Peak PCAv (cm/s)	15	50.9 ± 9.7	51.7 ± 8.6	0.9	0.26	0.09	0.96	3.9	0.96
Peak PCAv (%)	15	34.8 ± 12.3	31.6 ± 11.2	3.2	0.15	0.27	0.75	21.9	0.78
PCAv iAUC (cm/s/30s)	15	273.9 ± 129.2	268.1 ± 105.0	5.8	0.73	0.05	0.52	37.6	0.88
PCAv Amplitude (cm/s)	9	14.2 ± 5.5	13.0 ± 3.7	0.8	0.57	0.26	0.85	17.9	0.77
PCAv τ (s)	9	5.4 ± 2.0	5.2 ± 2.0	0.2	0.88	0.10	0.12	47.1	0.10
ONSD (mm)	16	4.8 ± 0.6	4.8 ± 0.6	<0.1	0.70	<0.01	0.82	6.3	0.80
				Between-day reliability					
	N	Pre Control (mean ± SD)	Pre Heading (mean ± SD)	MD	*P* value	*d*	ICC	CV (%)	*r*
Baseline PCAv (cm/s)	15	37.6 ± 5.4	37.3 ± 7.1	0.3	0.83	0.05	0.68	10.6	0.70
Mean PCAv (cm/s)	15	46.8 ± 8.7	46.6 ± 8.9	0.3	0.86	0.02	0.86	7.9	0.81
Peak PCAv (cm/s)	15	50.9 ± 9.7	50.0 ± 9.5	0.9	0.57	0.09	0.88	7.6	0.82
Peak PCAv (%)	15	34.8 ± 12.2	34.4 ± 10.0	0.3	0.91	0.04	0.74	22.0	0.72
PCAv iAUC (cm/s/30s)	15	273.9 ± 129.2	272.9 ± 96.6	1.1	0.96	0.01	0.73	37.8	0.82
PCAv Amplitude (cm/s)	9	14.2 ± 5.5	14.1 ± 4.6	0.1	0.46	0.02	0.71	23.8	0.73
PCAv τ (s)	9	5.4 ± 2.0	5.0 ± 2.3	0.4	0.66	0.19	0.19	48.1	0.17
ONSD (mm)	16	4.8 ± 0.6	4.8 ± 0.6	<0.1	0.70	<0.01	0.82	6.3	0.81

N, participant number; MD, mean difference; *d*, Cohen's standardised effect size; ICC, intraclass correlation coefficient; CV, typical error expressed as the coefficient of variation; *r*, Pearson's correlation coefficient.

3.2.7 Assessment of Optic Nerve Sheath Diameter

Following completion of the NVC test, ultrasound measurements were performed using the 'axial' scanning technique. Briefly, participants rested in the supine position and were asked to 'look forward' whilst the ultrasound probe was applied anteriorly over the closed right eye to attain a scan in the axial plane (figure 3.3). The frame displaying the highest resolution image was frozen and ONSD was assessed 3 mm behind the retina using electronic callipers (figure 3.3), which is in keeping with standard protocols (Chen et al., 2015). Each measurement was verified by a second researcher prior to data entry. The within-day and between-day reliability of the ONSD is presented in Table 3.2.

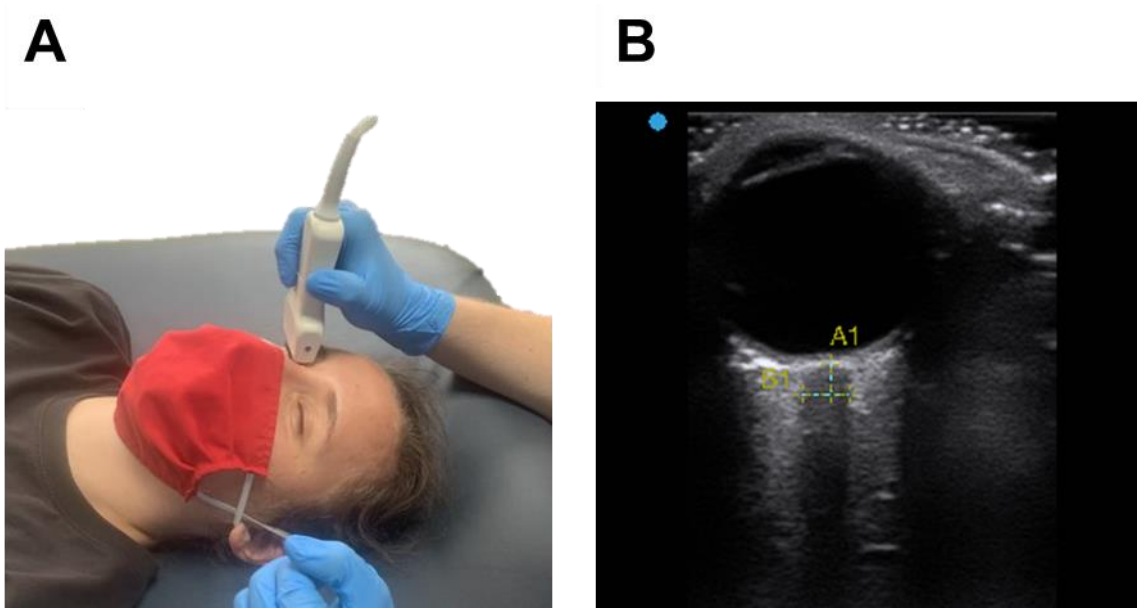

Figure 3.3. Example of the Optic Nerve Sheath Diameter measurement. **A.** Axial scanning technique implemented. **B.** A representative scan for one participant demonstrating measurement of the optic nerve sheath 3mm behind the retina.

3.2.8 Cognitive function

Following previous work which observed that cognitive function deficits after heading exposure were limited to memory function and reaction time, participants performed a modified version of the international shopping list test (Lim et al., 2009). This test is shown

to be highly reliable and sensitive to mild Alzheimer's disease (Thompson et al., 2011). Subjects were instructed to remember a list of items which were clearly read out by a researcher, whom was kept consistent for all tests. Once the list had been read out fully, participants were asked to recall as many items as they could within 1 minute. This process was repeated 3 times. Delayed recall was assessed 5 minutes following the completion of the 3rd trial by asking participants to recall the list, without prior prompt. Following unpublished pilot work from our laboratory, the number of items in each recall list was extended from 12 to 15 in order to provide a greater challenge.

3.2.9 Statistical Analyses

All data are presented as mean ± standard deviation. Statistical analyses were conducted using SPSS, version 26 (IBM). Statistical significance was set *a priori* at an alpha level of <0.05. Mauchly's and Shapiro-Wilk tests were used to assess the assumptions of Sphericity and normality, respectively. The response to heading and control protocols for each parameter of interest was assessed via a series of two way ANOVA's. Outcomes were inspected for a trial by time interaction effect, with effect sizes (partial eta squared, η_p^2) reported to support *P* values generated by each ANOVA. Any significant ANOVA interactions were explored using follow up pairwise comparisons and effect sizes (Cohen's *d*). The effect size was interpreted as small (<0.06), moderate (0.06-0.14) and large (>0.14) (Cohen, 2013).

3.3 Results

3.3.1 Neurovascular Coupling

There was no trial by time interaction effect present for baseline PCAv (p=0.32, η_p^2=0.07). No changes were observed in any of the metrics used to quantify the NVC response

(figure 3.4). Specifically, no significant trial by time interaction effect was apparent for mean PCAv (P=0.94, η_p^2<0.01), peak PCAv (P=0.56, η_p^2=0.03), percentage increase in PCAv (P=0.58, η_p^2=0.02), time to peak PCAv (P=0.59, η_p^2=0.02) or iAUC (P=0.14, η_p^2=0.15).

Data from 7 individuals could not be appropriately modelled using the monoexponential equation and was therefore excluded form kinetic analysis. No significant trial by time interaction for PCAv amplitude (P=0.37, η_p^2= 0.11) or τ (P=0.88, η_p^2=0.003) was observed within the remaining 9 participants (figure 3.4).

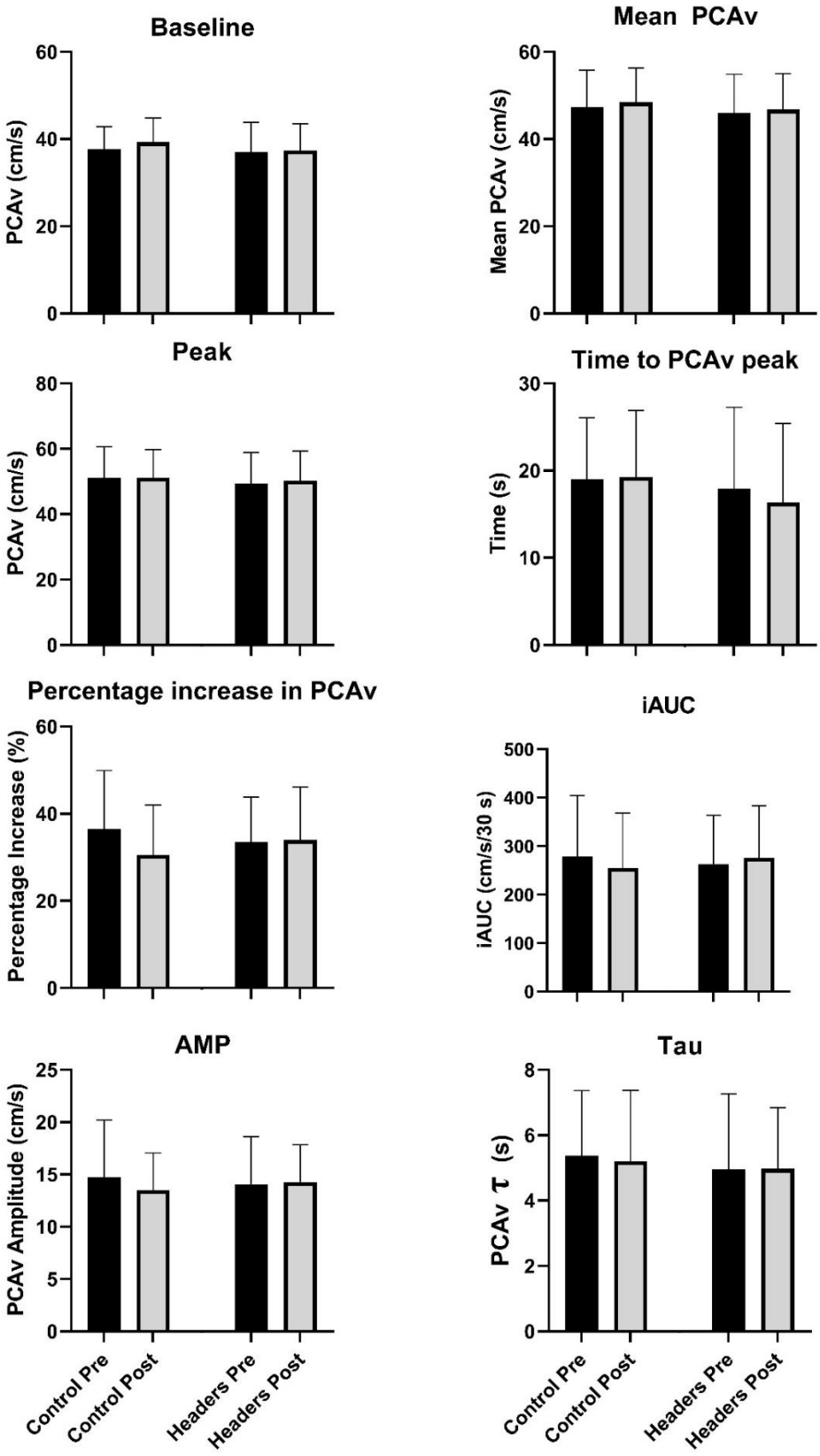

Figure 3.4. No change was observed in any neurovascular coupling metric ($P>0.14$ and $\eta_p^2<0.16$ for the ANOVA time by trial interaction effect across all outcomes). Error bars describe the standard deviation. N=15 (PCAv Amplitude and PCAv τ, N=9). PCAv, cerebral blood velocity in the posterior cerebral artery; iAUC, incremental area under the curve versus time; AMP, Amplitude; τ, Tau.

3.3.1 Optic nerve sheath diameter

Due to insufficient image quality, 3 participants were removed from analysis (n=16). Measurements of optic nerve sheath diameter were not altered by the heading protocol (ANOVA interaction effect; P=0.65, η_p^2 = 0.01).

3.3.2 Cognitive function

No time by trial by attempt interaction effect was observed for each attempt at the international shopping list memory task (P=0.11, η_p^2 =0.10, Table 3.3). Additionally no trial by time interaction effect was present when each attempt was averaged within a trial (P=0.053, η_p^2 = 0.192).

Table 3.3. Performance in the modified international shopping list test

	Attempt 1	Attempt 2	Attempt 3	Delayed Recall
Control Pre	10 ± 2	12 ± 2	13 ± 2	13 ± 1
Control Post	9 ± 2	12 ± 2	13 ± 2	12 ± 2
Heading Pre	9 ± 2	12 ± 2	13 ± 2	13 ± 2
Heading Post	8 ± 2	11 ± 2	13 ± 2	11 ± 3

3.4 Discussion

This was the first study to examine the acute effect of heading on NVC and ONSD as a surrogate measure of ICP in female footballers. Data from the current study demonstrates no significant changes in any NVC, ONSD or cognitive function outcome following the heading trial.

Whilst our data displayed no changes in any metric used to assess the NVC response, previous interventional heading work conducted in men has demonstrated acute alterations in the NVC response in other regions (reduced in total activation assessed via

the MCA) (Smirl et al., 2020). Additionally work by this group also demonstrates acute changes in other cerebral blood flow control mechanisms, namely dynamic cerebral autoregulation (Smirl et al., 2022). Comparison between these studies and the current work is problematic however given the disparity between heading stimuli, 40 headers in 20 minutes compared to 6 headers in 1 hour. It is believed that our protocol reflects the upper limit of heading exposure experienced within a match or training session (Lynall et al., 2016, Press and Rowson, 2017) and therefore provides important information regarding the physiological consequences typically experienced.. Despite this lack of ecological validity, these studies may provide understanding of how the accumulation of heading impacts over a playing career might be linked to neurodegenerative disease development. It is currently unknown if a dose-response relationship exists between heading and acute NVC alterations, or if there is an exposure threshold for such changes. Additionally the importance of recovery time between headers is yet to be explored. For example our findings cannot rule out alterations following 6 headers if they are performed consecutively. Future research should look to address these questions especially given the observation of a threshold effect between headers accrued in a year and abnormal white matter microstructure and cognitive performance (Lipton et al., 2013).

Although the current study replicated the methodology used to quantify NVC from Smirl et al. (2020) to improve cross comparison, the current study only assessed the response via the PCA. This highlights a considerable limitation as the reductions in total activation seen by (Smirl et al., 2020) were only observed via the MCA. Like the current study NVC measured via the PCA was not affected. As dual monitoring was not possible the posterior cerebral artery blood flow velocity response was used to assess NVC in an attempt to capture defects caused by injury to the occipital lobe, accrued via countercoup injury during heading (Babbs, 2017). It should also be noted that this work also forms part of a

larger study which assessed cerebral blood flow regulation through the MCA in response to cerebrovascular reactivity and autoregulatory challenges. This allowed us to capture alterations which may have been caused by damage to the frontal/temporal lobes, providing a broader picture of global cerebral blood flow regulation. Given that the PCAv response is altered in the days following concussion, the results of the current study and those of (Smirl et al., 2020) suggest that heading and subconcussive impacts might not influence NVC in the posterior cerebral circulation. However, as dual monitoring of the MCA and PCA was not possible, we cannot comment on any regional differences in the regulation of cerebral blood flow.

ONSD was unchanged after repeat heading. As measurements of ONSD are shown to be positively correlated with direct measures of ICP and sensitive to dynamic changes in pressure, the current findings suggest that ICP is not acutely altered following a representative bout of headers. These findings contradict previous interventional work (Lee et al., 2020) which displayed increased ICP estimated via acoustically evoked TMD. Although participants in the previous study performed the same number of headers, authors failed to report the recovery time separating each header. Furthermore, ball speed was not measured and due to the delivery method (kicked) could not be standardised. This makes it impossible to comment on whether the heading stimulus prescribed might explain the difference in findings.

Whilst it is difficult to understand if the heading protocol used may have contributed to differing results, the reliability of the method used to assess changes in ICP in the previous work by Lee et al. (2020) (TMD) has been questioned (Shimbles et al., 2005). Given that measurement of the ONSD is shown to be reliable and sensitive to changes in ICP (Shah et al., 2009, Chen et al., 2019), the results of the current study may provide more dependable information on the relationship between heading and ICP.

Longitudinal work in female colligate footballers however demonstrates significantly increased ONSD across a season (Sadrameli et al., 2018). The disparity between those findings and the current work may suggest the existence of a cumulative exposure threshold. In other words a singular bout of heading is insufficient to induce alterations, however, when assessed over a season the cumulative exposure exceeds the body's ability to tolerate such impacts resulting in increased ICP. Future study is required to explore this concept.

Findings of a recent review revealed that only 3 out of 12 studies saw deficits in cognitive function post heading (McCunn et al., 2021). These defects were limited to memory and reaction time. Since this review was published, further work by Ashton et al. (2021) also demonstrated impairments in working memory following heading. Nevertheless, our findings are in agreement with the majority of pervious work and provide supporting evidence that memory function is not altered following a representative bout of headers. Of the 4 studies which reported opposing findings 2 examined adolescent populations, which makes comparison problematic given that cognitive function is effected by pubertal maturation (Steinberg, 2005). The remaining two studies by Di Virgilio et al. (2016) and Ashton et al. (2021) were performed with a similar population to that used in the current work, however, they lack ecological validity due to the heading stimulus used (20 headers in 10 minutes and 20 headers in 3 minutes, respectively). Additionally, Ashton et al. (2021) only examined males, whilst Di Virgilio et al. (2016) did not assess the effect of sex. Consistent with the current findings the only other study to examine the acute effect of heading on memory function in females showed no change following 15 headers. Therefore current literature suggests that memory function is not acutely altered following exposure to a realistic heading stimulus in female college footballers. However, given the findings of Di Virgilio et al. (2016) and Ashton et al. (2021) future work should explore the

possibility of a dose response, especially as females experience greater head accelerations when heading (Bretzin et al., 2017, Tierney et al., 2008).

Whilst the findings of the present study suggest that a representative bout of headers does not affect any measure studied it is important that we consider them in relation to the wider evidence base. This is especially important given the state of concern regarding the cumulative effect of subconcussive impact in sport. Cross sectional work examining other cerebral blood flow control mechanisms demonstrates impaired CVR_{CO2} in male footballers (Marley et al., 2021) and rugby players (Owens et al., 2021), meanwhile CA alterations are observed following a season of contact sport which included footballers (Wright et al., 2018a). Importantly this evidence suggests that a history of concussion may not be a prerequisite for these observations but rather alterations maybe caused by the accumulation of sub concussive impacts (Marley et al., 2021, Wright et al., 2018a). Furthermore, observed alterations in CVR_{CO2} are associated with accumulated head impacts accrued across a season in adolescent females (Svaldi et al., 2017). Therefore the absence of significant changes in the current study should not be used as 'evidence of absence' meaning these findings do not absolve the act of heading regarding any potential future neurodegenerative disease risk. It is entirely possible that important physiological impairments are present that where unrelated to the specific measures examined. This work does however add to the existing evidence (Smirl et al., 2020) to suggest that regulation of posterior cerebral blood flow is likely unaffected following an acute bout of heading.

3.4.1 Limitations

As discussed previously a limitation of the current work is that we did not simultaneously assess the MCAv and PCAv response of the visual search task, making comparison with

existing work more challenging and mean that findings cannot be extrapolated beyond the single cerebral artery insonated (PCA). This work does however further our understanding regarding the effects on posterior cerebral blood flow control which might be sustained as a result of countercoup injury. Additionally, information on physiological and functional consequences to global cerebral health is provided by ONSD and cognitive function data, respectively.

The menstrual cycle was not controlled for in the current study. Whether this should be accounted for when examining vascular control remains a topic of debate within the scientific community (Stanhewicz and Wong, 2020, Wenner and Stachenfeld, 2020). Previous work has attempted to counteract the effect of hormonal variation by testing participants between days 3 and 7 of the follicular cycle when hormones levels are at the most stable (Shechter and Boivin, 2010, Burma et al., 2021). However any effect on NVC has recently been called into question (Leacy et al., 2022), with pilot data showing no change in NVC magnitude between the early follicular and mid luteal phase (Davenport et al., 2015).

A common limitation of work utilising TCD is that it does not directly measure CBF, but rather CBv. TCD therefore only remains a valid surrogated measure of CBF if the diameter of the vessel remains unchanged (Ainslie and Hoiland, 2014). MRI work has revealed that when $P_{ET}CO_2$ is within 7.5 mmHg of eucapnia the diameter of cerebral arteries remains constant (Verbree et al., 2014). A further limitation of the current work is that $P_{ET}CO_2$ data was not collected due to covid-19 restrictions requiring participants to where a face mask , therefore we cannot say with certainty that eucapnia was maintained throughout. However, given that previous work utilising the same visual search paradigm saw no change in $P_{ET}CO_2$ (Smirl et al., 2020), we do not expect this to have influenced our results and are confident in the assumption that vessel diameter remained consistent.

Finally it was beyond this studies scope to quantify the magnitude of head accelerations that players experienced during the heading protocol. Previous interventional (Smirl et al., 2020, Smirl et al., 2022) and observational (Wright et al., 2018a) has utilised accelerometers to quantify both mean and cumulative exposure. Capturing these metrics would have helped place this work in the context of existing literature and may have provided additional insight into the cause of opposing findings.

3.5 Conclusion

With growing concerns linking headers accrued over a footballing career with increased risk of future degenerative disease, this study aimed to explore whether exposure to a realistic bout of headers alters important physiological and functional markers of cerebral health, in women. No acute changes were observed NVC, ONSD or cognitive function following six headers in one hour in collegiate female footballers. This suggests that the stimulus experienced did not negatively impact these markers of cerebral health. Future work should look to examine the potential threshold effects and explore how these outcomes may be effected following repeat bouts across a season or career.

Chapter 4: Future Directions

4.1 Recommendations for future studies

The current work is one of only two interventional studies to consider the acute effects of heading on NVC or ICP and the first to examine women, who might be more vulnerable to detrimental change post heading (Rubin et al., 2018, Bretzin et al., 2017). Additionally, and unlike previous NVC work, these effects were assessed in response to an ecologically valid stimulus. These considerable strengths add to current understanding, however, given the infancy of this field there remains much for future work to explore. This will provide additional insight needed to understand the increased risk of neurodegenerative disease observed in retired footballers (Mackay et al., 2019, Russell et al., 2021).

As previously discussed (Chapter 3.4: Discussion) the lack of observed changes to the NVC response in this study contradicts previous interventional work which utilised an exaggerated heading stimulus in men (Smirl et al., 2020). Additionally ONSD has been shown to increase following a season of football participation (Sadrameli et al., 2018), an observation which was not witnessed in this study following a singular exposure. When considered together, these findings suggest either that chronic exposure is required, or that a threshold effect for acute changes may exist. This concept is supported by observational data which indicates a nonlinear relationship between heading exposure over a year and white matter abnormalities (Lipton et al., 2013). Considering competitive female footballers may experience up to 26 headers in a 2 week period (Stewart et al., 2018), work should examine how outcomes of interest are influenced when a realistic heading stimulus is repeated over multiple days. This work would highlight the importance of the recent change to heading guidelines made by the Scottish FA, which bans heading the day before and the day after a match (BBC, 2022). In addition to examining the

repetitive effect acutely, future work could consider how these outcomes are effected at regular time points throughout a season compared to measures taken at the end of the off season, prior to resuming training. This would be particularly pertinent to our understanding of the effects on NVC which, to our knowledge, has not been examined across a season in any contact sport. Work of this nature will provide information on the cumulative effects of repetitive heading and may help uncover whether there exists an exposure limit, i.e. a point at which the cerebrovasculature fails to cope with repetitive insult. Such research is important as establishing the threshold at which heading results in detrimental effects will help shape policies and guidelines that aim to protect players against future neurodegenerative concerns.

To support the aforementioned work it would be helpful to capture head acceleration data. Such data would make comparison between studies easier and, compared to simply reporting heading frequency, provide a more sensitive measure of exposure when considering any dose response on markers of interest. Additionally measuring head kinematics provides linear and angular accelerations, enabling researchers to assess how the type of force experienced may play a role in any potential decline. Previous work in football has utilised accelerometers; mounted behind the ear, in custom mouth-guards and in headbands (Basinas et al., 2022). The premier league is currently using 'PROTECHT' mouth-guards to assess exposure during training, in twelve clubs across the premier league, English football League and FA Women's Super League (Premier-League, 2022). However, they are not currently used during matches, possibly due to the associated participant burden that might impact performance. A potential development within this field would be to embed the match ball with the accelerometer. Initial investigations using an 'Adidas micoach' ball suggest this is a promising alternative, however authors concede that a sensor capable of detecting a greater range of magnitudes is required for effective

assessment (Stone et al., 2016). Additionally, video analysis is require to identify impacts resulting from head contact, although, recent studies indicate that computer vision algorithms can effectively streamline this process (Rezaei and Wu, 2022).

The current literature which examines the acute effects of heading on indices thought to play an important role in the development on neurodegenerative disease is limited to young adults. Whilst this provides valuable insight, future interventional work should consider measuring these outcomes in retiring professional players, to provide information on the influence of life time exposure. Additionally work is needed which examines these effects throughout adolescence. This is particularly important considering the understanding that weaker neck strength and smaller head masses are associated with larger head accelerations (Tierney et al., 2008), and that this might result in greater neuronal injury (Ji et al., 2014). Given the population, careful consideration needs to be given to ethical implication of proposed study designs. Instead of interventional work it maybe more ethical to observe alterations following heading organically performed in a match. This work is needed to support current guidelines limiting the number of headers during the development phase and the decision to ban heading during footballs foundation phase (FA, 2021d).

4.2 Impact on current guidelines

Although our study does not provide evidence that 6 headers has a detrimental effect, we caution the use of these results to absolve heading as ‘safe’. Absence of evidence should not be interpreted as evidence of absence, and as such adjustments to the current policies and guidelines currently in place (Chapter 2.8.1) should continue to be carefully considered.

Current policies limit the number of headers that should be performed amid remaining concern that these repetitive impacts may play an important role in the increased risk of neurodegenerative disease seen in retired players. With the findings of the current study and no knowledge of the upper exposure limit which prevents the manifestation of detrimental effects, it may be reasonable to lower guidance on the number of high force headers in a training week from 10 to 6. It is important to remember however that our findings do not mean that a singular bout of 6 headers have no negative effects as they maybe unrelated to outcomes measured. Furthermore there is no evidence to indicate that 10 headers is above this upper exposure limit.

In addition to limiting exposure in adults current policies have banned heading during the foundation phase (FA, 2021d). An argument could be made against this decision as developing good technique and conditioning the muscles in the neck may help mitigate unfavourable physiological outcomes by reducing the head accelerations experienced (Peek et al., 2019). However, neck strength can be improved in the absence of head impact (Peek et al., 2022). Coaches therefore should look to increase neck strength prior to the introduction of heading into matches and games. Moreover, coaches should seek inventive ways of practicing technique that minimises the forces experienced.

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Appendices

Appendix 1: Certificate of Ethical approval

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

St. Luke's Campus
University of Exeter
Heavitree Road
Exeter
EX1 2LU
United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 201209-A-01

Title: **A multidisciplinary approach to examine heading in football**

Applicants: **Dr Bert Bond** Oliver Smail,Jacob Jack,Alex Woodgates,Harry Johnson,Kaela Townson,Dr Genevieve Williams

The proposal was reviewed by a Representative on the Committee.

Decision: This proposal has been approved until 31/10/2021

Signature:  Date: 21/11/2020

Name of Ethics Committee Reviewer: Richard Pulsford

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Appendix 2: Notification of Substantial Amendments

Sport and Health Sciences

Ethics Committee

shs-ethics-admin@exeter.ac.uk

Notification of Substantial Amendment(s)

This template **must only** be used to notify of amendments which are **NOT** categorised as Minor Amendments.

If you need to notify a Minor Amendment to your study then you MUST use the appropriate form.

For guidance on amendments refer to https://www.hra.nhs.uk/approvals-amendments/amending-approval/examples-of-substantial-and-non-substantial-amendments/

1. Study Information

Details of lead applicant: Name: e-mail	 Bert Bond B.Bond@exeter.ac.uk
Full title of study:	**A multidisciplinary approach to examine heading in football**
Date study commenced: **Date study due to end/ended:**	01/01/21 31/10/21* * we want to extend this
SHS ethics application reference number:	201209-A-01
SHS admin amendment number:	AM 21-12-08-03
HTA application to store in date?	n/a

2. Type of amendment

(a) Amendment to information previously given on the SHS ethics application form?

Yes No

(b) Amendment to the protocol/proposal or participant pathway?

Yes No

*If yes, please submit either the revised protocol with a new version number and date, highlighting changes **in bold,** or a document listing the changes and giving both the previous and the revised text.*

(c) Amendment to the information sheet(s) and consent form(s) for participants, or to any other supporting documentation for the study (any amendment to the protocol will require changes to the PIS and consent form.

Yes No

*If yes, please submit all revised documents with new version numbers and dates, highlighting the new text **in bold**.*

3. Summary of the amendment(s)

No.	**Brief description of amendment** *(please enter each separate amendment in a new row)*	**List relevant supporting document(s), including version numbers** *(please ensure all referenced supporting documents are submitted with this form)*	
		Document	**Version and date**
1	The end date of data collection was previously listed as 31/10/21.We are seeking to extend this to 31/05/22	Amended ethics form	Version 2, dated 09/11/2021
2	We are altering our original 4 day protocol to 2 days.	Amended ethics form, PIS and informed consent sheet	All now Version 2 and dated 09/11/2021

	Originally, we invited participants into the lab to repeat the heading trial or control trial within 48 hours of their initial heading/control visit (thus, 4 days in total). We now want to drop this additional "repeat" visit. All procedures remain the same, but there is no "extra" follow up visit. Thus, we are left with just 2 visits – heading and control. This change is best described in the figure overleaf		
3	We would like to add Felix Brown to the study, as an undergraduate dissertation student	Amended ethics form	

4. Scientific update

If the amendment significantly alters the research design or methodology, or could otherwise affect the scientific value of the study, supporting scientific information should be given (or enclosed separately). Indicate whether or not additional scientific critique has been obtained.

This amendment will not change our primary question – what is the influence of heading on cerebrovascular and cognitive function, and neuromotor control. However, it will mean that we can no longer look to see if a subsequent exposure to the heading stimulus (on a consecutive day) has an

5. Human Tissue storage

Additional information for studies requesting an extension to the study end date for the storage of human tissue

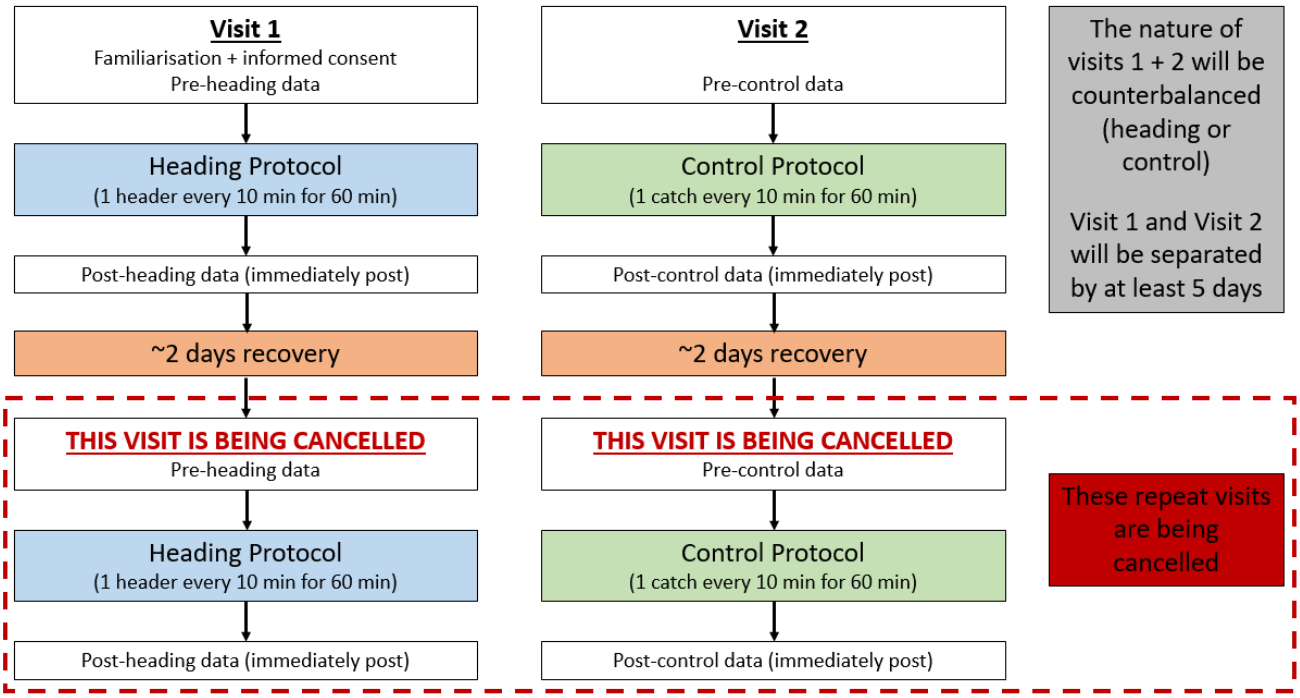
1. State whether or not there have been any previous requests for an extension to the study end date and for what purpose**. NO – we are not storing any biological samples, so this is not applicable to our request**
2. Describe why it was not possible to undertake the proposed analysis in the period specified and approved in the original application.
3. Make a clear statement that NO analysis will be undertaken during the extension period that was not specified in the original application.
4. Submit the original application form, PIS and consent form with the amendment request.
5. Provide a rationale for the requested period of the extension up to a maximum of 12 months

6. Declaration by lead applicant (Chief Investigator)

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendment(s) to be implemented.

Signature of Chief Investigator: … ……

Here is our protocol schematic, which quickly identifies the changes we want to make:



Appendix 3: Approved substantial amendment

SHS Ethics Mail <shs-ethics-admin@exeter.ac.uk> | Bond, Bert; SHS Ethics Mail

AM21-12-08-03 Amendment to 201209-A-01 accepted

Dear Bert

***TITLE* A multidisciplinary approach to examine heading in football**

I am pleased to advise that your application for an amendment to the above study has been approved. Please keep this email with the original approval certificate.

***Comments**: You will need to carefully amend the revised ethics application to remove all mention of the '2 days post' analysis (e.g., in the lay summary).*

Kind regards

Rosy Armstrong

Ethics Committee Administration
Sport and Health Sciences

University of Exeter 01392 72 **4371**

G14 Lafrowda House, St Germans Road, Exeter, Devon, EX4 6TL

Appendix 4: Informed consent form

Participant Identification Number:

CONSENT FORM

Title of Project: A multidisciplinary approach to examine heading in football

Name of Researcher (s): Dr Bert Bond, Ollie Smail, Jacob Jack, Alex Woodgates, Kaela-Mei Townson, Harry Johnson, Dr Genevieve Williams

	Please initial box
1. I confirm that I have read the information sheet dated 09/11/2021 (version no 2) for the above project and the Covid-19 information supplement dated 09/11/2021 (version no2) I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my legal rights being affected.	☐
3. I understand that relevant sections of the data collected during the study, may be looked at by members of the research team, individuals from the University of Exeter, or where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4. I understand that taking part involves two visits to the laboratory; a heading trial, and a control trial (sitting, no headers). The “heading” days involve performing 6 headers in an hour.	☐
I understand that ultrasound will be used at the side of my head and to scan through the eye. The purpose of these measures has been made clear to me.	☐
I understand that I will be asked to squeeze a device between my thumb and finger in accordance with the direction provided, as well as complete some cognitive tasks and an assessment of balance	☐

5. I understand that my data will be stored in pseudonymised form until 01/01/31, and may be: ☐

   shared with other researchers for use in future research projects ☐

   published in an academic publication ☐

   included in teaching or training materials for use in University activities ☐

6. For Covid 19 security measures, I understand that taking part involves me:
   a. Considering the risk to me, my household, or my support bubble before attending for each study visit.
   b. Self-screening for new and worsening signs or symptoms of possible COVID-19 before each visit to the campus/research site
   c. Maintaining social distancing wherever possible or wearing a mask/face covering when social distancing is not possible.
   d. Maintaining good personal hygiene, including proper hand washing, cough/sneeze etiquette, not touching my face, eyes nose and mouth.
   e. 'Checking in' using the **NHS QR** code or providing my details for contact tracing if necessary ☐

7. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of researcher taking consent	Date	Signature

When completed: 1 copy for participant; 1 copy for researcher/project file

Appendix 5: Participant Information sheet

VERSION 1 28/10/2020

Participant Information Sheet

Title of Project: A multidisciplinary approach to examine heading in football.

Researcher name: Dr Bert Bond

Invitation and brief summary:

You have been invited to take part in this research study as you are a footballer. This project is interested in the acute responses to heading. This information sheet has been provided to help you decide whether to take part. Please take time to read this information carefully and discuss with whomever you wish. Please ask a member of the team listed below if you have any questions. Thank you for expressing an interest in partaking in the study.

Purpose of the research:

Heading a football is common practice in training, and for some players in game play. We are interested in whether these headers have any immediate effect on the ability to perform simple tasks or markers of brain health. The purpose of this study is to further understand the immediate effects, if any, of heading a football.

Why have I been approached?

To participate in this study, you must identify as someone who plays football (at any level), is over the age of 18, have never been diagnosed with epilepsy, seizures, encephalitis, psychoses or meningitis or taken recreational drugs, and have no other known neurological conditions. You must not have received a recent head injury or concussion.

What would taking part involve?

You will take part in four testing sessions at St Luke's campus. Upon arrival you will be given a demonstration of each test and the heading protocol. The tests are: 1) A pinch grip test where you will squeeze a small piece of plastic between your thumb and forefinger to make a target reach a line on a screen 2) The balance error scoring system (BESS) which involves balancing 4 balance tasks. 3) A symptom severity scale where you will rate your feelings of concussion symptoms, such as dizziness. 4) Cognitive function tests (verbal recall of a list of household items). 5) A battery of tests which measure how your brain regulates blood flow (See image below) when challenged, and 6) an ultrasound scan of the eyeball to determine pressure in the skull (See image). The tests for brain blood flow control are non-invasive and require simple tasks such as alternating between squatting and standing for 5-minutes, holding your breath

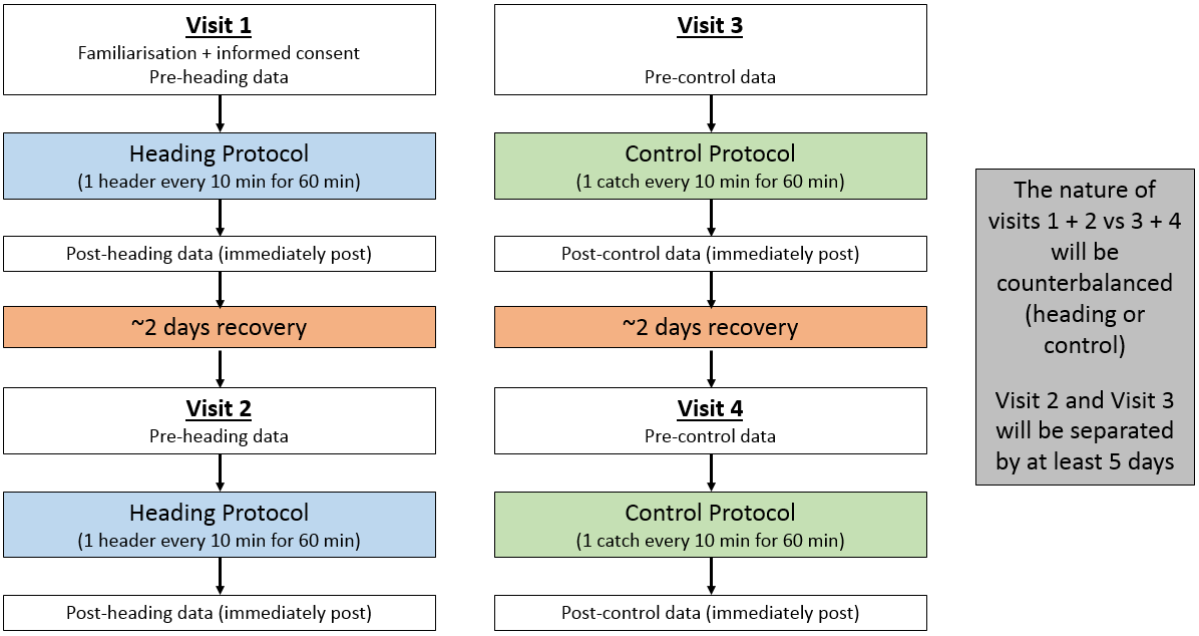
for 20 seconds, hyperventilating for 20 seconds and performing a visual search task such as 'where's wally?'. Once these baseline tests have been done, you will perform the heading protocol.

The heading protocol involves 6 headers with standard footballs over 60 minutes at 40km/h (typical of a corner kick). You will then repeat this 1 day later (visit 2). On the third and fourth visits you will undergo the same protocols, except heading will be replaced with seated rest to act as our control condition. Note that half of the participants will complete this in the reverse order (i.e. heading in visit 3 and 4).

Following completion of the headers or control, you will then repeat each of the concussion tests again. Please see the figure overleaf which describes the testing protocol. The testing will last up to 3.5 hours for each visit. You will be required to wear sports clothing that allows you to jog and head a football in the sports hall or on a field. Please wear trainers.

Ultra sound scan to determine pressure in the head (non-invasive)

Set up for brain blood flow measures (non-invasive)



What are the possible benefits of taking part?

You may find the experience interesting and educational, but there are no direct or financial benefits from taking part in this study.

What are the possible disadvantages and risks of taking part?

There are some risks, but these are considered to be minor and short-lived. Therefore, we want to make you aware of them so we can help to prevent them from happening. The risks are:
Experiencing dizziness and/or a headache as a result of heading the football.
Experiencing shortness of breath as you will be jogging and heading a football.
There is a risk that you may trip over in the sports hall, leading to a risk of musculoskeletal and superficial injuries associated with tripping.

We aim to minimise the likelihood of these risks. For example, the area will be cleared of trip hazards, and the research group will know the process for reporting injuries and calling a first aider. Your wellbeing will be closely monitored throughout, and we have also avoided the more extreme heading protocols used elsewhere (20 headers in 10 minutes) so that our heading protocol is more reflective of what might happen in training or a game (6 headers in 60 minutes). Therefore, by design, you will not be exposed to more head impacts than might reasonably be expected to occur. Please contact Dr Bert Bond for more information if required.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any point, without having to give a reason. Your data will be destroyed and not included in the write up. There will be no negative consequence of this.

How will my information be kept confidential?

The University of Exeter processes personal data for the purposes of carrying out research in the public interest. The University will endeavour to be transparent about its processing of your personal data and this information sheet should provide a clear explanation of this. If you do have any queries about the University's processing of your personal data that cannot be resolved by the research team, further information may be obtained from the University's Data Protection Officer by emailing informationgovernance@exeter.ac.uk. or at http://www.exeter.ac.uk/ig/

When enrolled in the study you will be given a unique participant ID number. This will be used to store and analyse your data anonymously during the study. This data will be stored in a password protected computer which will only be accessible by the researchers of this project. The data will be stored until 01/06/2030 for possible further analysis.

What will happen to the results of this study?

The data collected will be written up in academic journal articles, and as pilot data for grant applications.

The data collected will be used to write up MSc dissertations which participants are welcome to read.

Who is organising and funding this study?

This study was designed by Dr. Bert Bond and Dr. Genevieve Williams, and funding granted by UEFA.

Who has reviewed this study?

This project has been reviewed by the Research Ethics Committee at the University of Exeter.

Further information and contact details

If you have any questions or would like more information about the procedure our contact details are below:

Gail Seymour, Research Ethics and Governance Manager
g.m.seymour@exeter.ac.uk, 01392 726621

Jacob Jack
Jdij201@exeter.ac.uk

Dr. Bert Bond
b.bond@exeter.ac.uk, 01392 724903

Thank you for your interest in this project.

Working safely during coronavirus (COVID-19)

Supplementary information for research participants

during COVID-19 restrictions.

Title of Project: A multidisciplinary approach to examine heading in football

(Dr Bert Bond, Oliver Smail, Jacob Jack, Alex Woodgates, Kaela-Mei Townson, Harry Johnson, Dr Genevieve Williams)

Researcher name: Dr Bert Bond

This information is in addition to the Participant Information (version 1, dated 28/10/20) you have already considered for this project. If you no longer wish to continue, remember that your participation is entirely voluntary and you are free to withdraw at any time without giving a reason. Withdrawal will not affect any access to services or care either now or in the future.

During the current coronavirus pandemic, we are taking measures to ensure that everyone can work safely and that our research projects can begin or continue where appropriate. To minimise risk of infection to you as a participant and to the research team and others we have taken all of the following measures based on the UK government guidance https://www.gov.uk/guidance/working-safely-during-coronavirus-covid-19:

We will ask you to self-screen for new or worsening signs or symptoms of possible COVID-19 before each visit to the campus/research site.

We will ask you to consider the risk to yourself, your household, or support bubble before attending for each study visit.

We will:

- Provide 'HALO' coronavirus testing for any University of Exeter staff and students who are acting as researchers and/or participants where it is considered necessary (for example in close contact activities). https://www.haloverify.com/who-halo-is-for.html

  Asymptomatic individuals who test **positive** will need to remain isolated for at least **10** days from the day of the test and anybody who lives in their support bubble or close contacts must also self-isolate for **14** days.

- Ask you to self-screen for new or worsening signs or symptoms of possible COVID-19 before each visit to the campus/research site
- Maintain social distancing wherever possible
- Ask you to wear a face mask or face covering where social distancing is not possible
- Maintain good personal hygiene, including proper hand washing, cough/sneeze etiquette, avoid touching the face, eyes, nose and mouth
- Have hand sanitizer available at all access points
- Use appropriate Personal Protective Equipment to protect ourselves and others from the spread of the virus while within the research environment
- Regularly and frequently clean/disinfect high-touch locations in all shared spaces and

Specifically for this research project we will:

- Mark areas with tape, paint or other visible signs to help people keep to a 2m [or *as indicated by current guidance*] distance and remind workers and visitors of social distancing guidance.
- Where possible arranging one-way traffic through the workplace.
- Arrange visits and activities by appointment only or stagger arrival and departure times.
- Keep the activity time involved as short as possible.
- Use an alternative face covering (i.e. visors) where clear communication may be compromised with a face mask in place.
- Clean all door and cabinet handles, bench surfaces, keyboards, instrument control panels, etc. at the beginning and end of the day, or, if researchers are working in shifts, at the beginning and end of every shift.

Appendix 6: Modified versions of the International shopping list used for assessment of memory recall

Participant Con, 1, Pre

Item	Set1	Set2	Set3	Delayed
Yoghurt				
Grapes				
Biscuits				
Spaghetti				
Margarine				
Lettuce				
Peanuts				
Coleslaw				
Sultanas				
Toilet roll				
Aspirin				
Cola				
Coffee				
Flour				
Bananas				
Total				

Participant Con, 1, Post

Item	Set1	Set2	Set3	Delayed
Bread				
Marmalade				
Cabbage				
Cheese				
Salmon				
Strawberries				
Crisps				
Sausages				
Deodorant				
Batteries				
Rice				
Washing Powder				
Oranges				
Wine				
Onions				
Total				

Participant Int, 1, Pre

Item	Set1	Set2	Set3	Delayed
Pepper				
Haddock				
Tin Foil				
Squash				
Avocado				
Bagels				
Candles				
Ice Cream				
Walnuts				
Shower Gel				
Bleach				
Sweets				
Hot Chocolate				
Plums				
Wet Wipes				
Total				

Participant Int, 1, Post

Item	Set1	Set2	Set3	Delayed
Bin Bags				
Muffins				
Prawns				
Vitamin Tablets				
Toothpaste				
Bacon				
Chips				
Broccoli				
Muesli				
Mayonnaise				
Peaches				
Rubber Gloves				
Hot Dogs				
Cashews				
Basil				
Total				