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Investigating the Impacts of Omega-3 Fatty Acid Supplementation on Cognitive and Neurophysiological Measures in Former Athletes

By

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Abstract

Background: The long-term implications of sport-related concussion is a topic of growing concern. Although cognitive and motor impairments have been demonstrated in former athletes, an effective treatment for long-term concussion impairments is still unknown. Due to the key roles they play within the brain, omega-3 fatty acids (ω-3 FAs) have been suggested as a possible solution however, no studies involving a human population have taken place.

Objectives: This study seeks to determine the effects of 8-week ω-3 FA supplementation on measures of cognition and neurophysiological control in former athletes who have suffered concussion compared to control.

Methods: Six healthy former contact-sport athletes (who had suffered at least one concussion during their career), and 10 age-matched controls were recruited for the study. Participants first attended a familiarisation session to combat possible learning effects. Measures of corticomotor inhibition and corticospinal excitability (using transcranial magnetic stimulation) in the rectus femoris and vastus lateralis were obtained at baseline and at 4- and 8-weeks post ω-3 FA supplementation. Cognitive function was assessed at the same timepoints using the Cambridge Neuropsychological Test Automated Battery (CANTAB), tests used were; Paired Associates Learning (PAL), Verbal Recognition Memory (VRM), Reaction Time (RTI), Cambridge Gambling Test (CGT), Multitasking Test (MTT), Spatial Working Memory (SWM) and Stockings of Cambridge (SOC).

Results: No significant effects were detected in corticomotor inhibition post-supplementation. Significant group effects were observed in corticospinal excitability in the vastus lateralis but no time or interaction effects. Significant group effects were also detected in the PAL and RTI tests.

Conclusion: This study failed to demonstrate any significant impacts of ω-3 fatty acid supplementation on cognition and neurophysiological control in former athletes who had suffered concussion.

Key Words: concussion, omega-3 fatty acid, transcranial magnetic stimulation, CANTAB

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1. Introduction and Literature Review

In recent years, a considerable amount of attention has been focussed on sport-related concussions (SRCs). In what is rapidly becoming a major public health concern, academics have sought to understand the relationship between concussions sustained during a playing career and long-term brain health. Several studies have reported neurophysiological and/or cognitive impairments in former contact sport athletes who had suffered from as little as three concussions [1–4]. Concussion is increasingly becoming a concern for many contact-sport athletes, especially at the collegiate level [5]. This is compiled by Feudtner and Miles [6] who report an almost 5% decrease in high school American Football participation rates between 2008 and 2016. They suggest this is because of the ever-increasing awareness surrounding football related health implications such as chronic traumatic encephalopathy (CTE), a degenerative brain disease which can lead to early on set dementia, resulting in premature death in former athletes [7,8].

Despite evidence demonstrating the possible negative impacts of multiple concussions, there remains no effective treatment for the injury. Omega-3 (ω-3) polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA), have recently been purported as a possible therapeutic aid in recovery from or as a prophylactic nutritional supplement to guard against concussion and other mild traumatic brain injuries (mTBIs). Omega-3 PUFA's have key structural and functional roles within the brain with already recognised clinical benefits in maintaining the development of the brain and cognitive function throughout life [9–11]. However, the literature surrounding ω-3 PUFA's and brain injury, while promising, comes almost entirely from rat models at the acute stage following concussion with data

from human trials being very scarce. Therefore, the purported therapeutic and protective effects of ω-3 PUFA's must be investigated further in a human population.

In the following literature review, the evidence for long-term brain health complications and concussion will be explored. Methodologies to quantify motor and cognitive deficits and the evidence surrounding the therapeutic and protective impacts of omega-3 polyunsaturated fatty acids against mTBI will also be scrutinised and discussed.

1.1 Concussion

Concussion, often referred to as mild traumatic brain injury (mTBI), is common amongst athletes and is especially prevalent within contact sports [12]. Concussion is the result of a sudden, large change in the acceleration of the brain, for example following a blow to the head, which then leads to vestibular, ocular, psychological (mood and anxiety) or cervical dysfunctions. Regardless of the type of concussion suffered, they all result in a multifaceted cascade of molecular events [13,14](*Figure 1*). Following the initial mechanical trauma, disturbances to the cell membrane leads to alterations in sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) ion gradients. Disrupted neuronal cell membranes will, in turn, release large amounts of neurotransmitters, (such as glutamate), resulting in an excitotoxic environment and random initiation of action potentials. These metabolic instabilities are responsible for the initial changes in neural functioning seen in concussed patients[15,16]. ATP-powered pumps are required to work at a rapid rate and therefore demand large amounts of ATP produced through glycolytic pathways. These pathways result in excess lactic acid and free radicals which may damage crucial cellular components [15]. Glucose stores eventually

deplete as a consequence of the hypermetabolic state the brain finds itself in, resulting in a hypometabolic state due to low glucose utilisation. During this time, the brain is considered to be particularly vulnerable if further trauma is suffered with potentially catastrophic consequences [14,17].

Depending on the severity of the concussion, types of symptoms and recovery time may vary. Common symptoms experienced in the short-term are dizziness, headache, irritability and loss of consciousness [18]. Typically, these symptoms will resolve within 10 days for adults [18], however, indicators of neurometabolic dysfunction have been known to persist for up to 4-weeks [19].

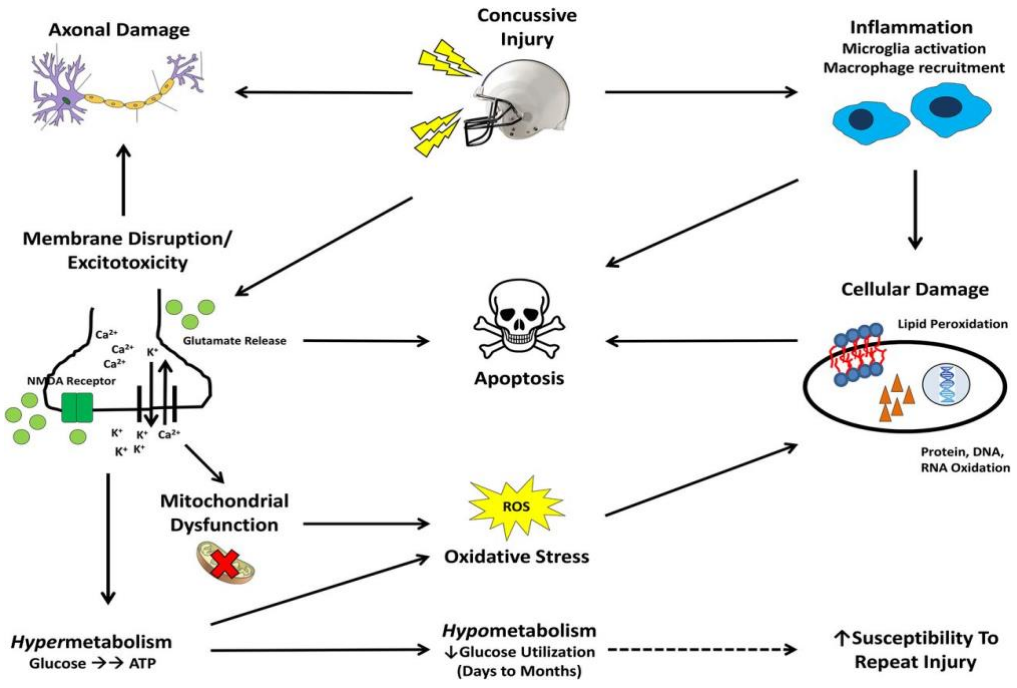

Figure 1. Metabolic Cascade of Events Following Head Trauma Causing Concussion. (Barrett et al., 2014).

1.2 Long-term Deficits in Cognitive and Motor Function

The impacts of concussion on brain function at the acute stage have been well documented [20–23] with the majority of studies reporting cognitive impairments following SRC. Matser et al [24] observed detrimental effects on visual/verbal memory and attention in professional soccer players who had suffered from concussion. They also demonstrated that the level of cognitive impairment was correlated with cumulative concussions. A later meta-analysis [25] on neuropsychological deficits seen after SRC reinforced the above claims and concluded that most symptoms dissipate within 14 days. It should also be noted that electrophysiological dysfunction has been known to persist beyond cognitive recovery, suggesting long-term underlying impairments [26,27].

Montenigro et al [28] conducted a cross-sectional study including 93 ex-collegiate and high school American Football players and demonstrated a link between later-life neuropsychological impairments and previous instances of SRC. More specifically, they found that exposure to repeated head impacts leads to an increased risk of cognitive deficits, depression, self-reported executive dysfunction, apathy, and behavioural dysregulation. Similarly, a study [3] found that retired professional football players who suffered multiple concussions in their playing career had an increased likelihood of developing mild cognitive impairments (MCIs). Further, players who sustained 3 or more concussions were 5 times more likely to have developed MCIs and 3 times more likely to present significant memory deficits compared to players who had not suffered a concussion. The paper also states that no link between recurrent concussion and Alzheimer's disease was found. However, there was an earlier on set within the retiree cohort compared to the

general American male population. The authors suggest that dementia-related syndromes may be initiated by repetitive mTBI's.

In a more recent study, Pearce et al. [29] once more observed poorer cognitive performance within a cohort of 25 former professional rugby players compared to healthy, age-matched controls. This study also observed impairments in motor and neurophysiological measures of dexterity, reaction time and corticospinal silent period (cSP), providing evidence of long-term sequelae in retired athletes with history of multiple concussions. A recent meta-analysis conducted by Zhang et al [30] concluded that, across 11 studies, significant deficits in verbal memory, delayed recall and attention existed within former contact sport athletes compared with populations that had not suffered concussion.

Conversely, several studies have failed to find links between SRC and declines in cognitive functioning. Fields et al [31] recruited 35 former National Football League (NFL) players and carried out neuropsychological measures focussing on attention/processing speed, language and memory. Following correlational analyses between variables such as total number of concussions, concussions involving a loss of consciousness and number of years played, they found no significant links existed between cognitive measures and the exposure variables. Casson et al [32] found that after a battery of neurological, neuropsychological and anatomical (MRI, DTI and SWI) examinations, the majority of retired NFL players had no clinical signs of chronic brain damage. Further, a recent systematic review and meta-analysis [33] investigating the long-term influences on brain health following a career playing Rugby reported mixed results. The authors state that methodological biases reduce the quality of the literature sufficiently to cast doubt over and limit the conclusions that can be made. It was argued that studies demonstrating deficits in fine motor control in retired athletes may

be swayed over the failure to control for upper limb musculoskeletal injuries. The authors also concluded that although cognitive impairments are reported within cohorts of former athletes with and without history of SRC, results do not differ significantly from average population norms.

The conflicting nature of the data presented above highlights the need for further study into the long-term manifestations of sport related concussion. Future studies should seek to implement robust methodological designs and include cohorts from various contact sports and include control groups from both an average population and an athletic population to maximise our understanding of mTBI and brain health.

1.3 Cambridge Automated Neuropsychological Test Automated Battery (CANTAB)

The prevalence of automated neuropsychological test batteries within clinical and research neurology has increased dramatically over the last 40 years [34]. The increased usage of these batteries is likely a result of logistical advantages they have over more traditional neuropsychological tests such as lower costs, automatic scoring and the assessment of multiple facets of cognition all within one programme [35]. The Cambridge Automated Neuropsychological Test Battery (CANTAB) is one of the most used automated programmes currently employed within the literature and has been found able to discriminate between normal adults and clinical populations, including Alzheimer's Disease and even mild cognitive impairment [36]. Learning and memory are thought to be reliant on a delicate balance of γ-aminobutyric acid (GABA) mediated inhibition, with increasing levels of GABA activity potentially resulting in impaired neural plasticity and consequently, significantly

impacted memory processes [37]. Given that an increase in inhibitory activity (in the form of an elongated cortical silent period (see section 1.4)) is seen in populations who have concussion history, it is logical that measures of learning and memory function are investigated. With these physiological processes in mind, this study employed several tests of learning and memory available within the CANTAB software including Paired Associates Learning which measures long-term memory, Verbal Recognition Memory which measures ability to encode and retrieve verbal information and, Spatial Working Memory which assesses ability to retain spatial information and working memory.

Excitotoxicity as a result of altered neurotransmission is an important aspect of the 'concussion cascade' (as discussed in section 1.1) and is thought to impact reaction time [14], we therefore included a reaction time test to investigate motor and cognitive response rates, as well as accuracy and impulsivity. In this test, the participant press and hold a button at the bottom of the screen and, at random intervals, a yellow dot will appear above the box which must then be pressed as quickly as possible.

In summary, the CANTAB is a popular choice for automated neuropsychological testing with good ability to discriminate between normal and clinical populations. Its use and the tests selected for the current study reflect the physiological processes that are observed at the acute and long-term stages following concussion and the subsequent impacts they incur on cognition. These impacts are well established in the literature (section 1.2) and include but are not limited to; dulled reaction times, impaired memory and learning deficiencies.

1.4 Transcranial Magnetic Stimulation

In the pursuit of understanding the neurophysiological deficits seen in former contact sports players, studies have utilised combinations of electrophysiological techniques such as electromyography (EMG), electroencephalography (EEG) and transcranial magnetic stimulation (TMS) [2,38,39]. TMS is a non-invasive technique which allows for the assessment of the structures and integrity of the neuromuscular system [40]. It involves a stimulatory coil generating magnetic pulses over a targeted area of the scalp, these pulses induce a secondary ionic current in the brain [41]. TMS applied over the motor cortex (M1) induces descending volleys in the pyramidal tract which affects the spinal neurons (corticospinal tracts) [42]. The activation of motor-neurons (marshalled by neurotransmitters such as GABA, dopamine, norepinephrine, acetylcholine, and serotonin) in response to the corticospinal volleys results in the generation of a Motor Evoked Potential (MEP) in the muscle, which can be measured through EMG [43]. More specifically, studies involving pharmacologic interventions have determined the physiological underpinnings of MEP inhibition-excitation [44]. Administration of substances that block sodium channels and reduce excitatory I-waves resulted in diminished action potential firing rate and reduced calcium entry into the presynaptic terminal and ultimately affected synaptic transmission [45]. Further, it has been demonstrated that MEP amplitude is depressed following addition of $GABA_A$ mediators and increased after administering dopamine and norepinephrine agonists [43].

It is primarily in tandem with EMG that measures of corticospinal excitability (more specifically MEP amplitude) have been quantified in the literature following a concussion, however, corticomotor inhibition (the ability to quash unwanted actions and movements) is generally regarded as the most robust TMS measure of concussion [46,47]. This inhibitory

parameter manifests as a lengthened corticospinal silent period (cSP) and can give us information regarding cortical control of the motor system [48]. When a single TMS pulse is applied over M1 and contralaterally to the target muscle whilst a person is maintaining a contraction, EMG activity is temporarily suspended (*Figure 2*). It is the length of time that EMG activity is delayed which is known as the cSP [41]. It is thought that an increase in cSP reflects an increase in GABAb receptor pathway activity, this is hypothesised from studies which have utilised GABAb agonists and reuptake inhibitors which increased cSP [49,50].

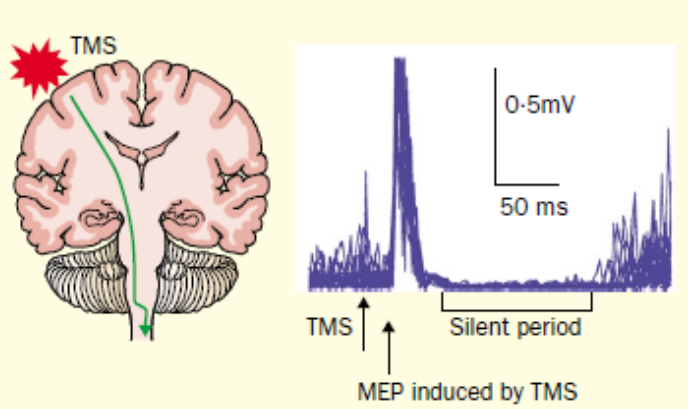

Figure 2. Example demonstrating the delay in EMG activity when assessing cSP following a TMS pulse. Adapted from Kobayashi and Pasqual-Leone (2003).

Despite inhibition being regarded as a reliable measure of neurophysiological abnormalities within previously concussed populaces, the literature exhibits contrasting data. Pearce et al [38] showed reduced short- (GABAa mediated) and long-interval (GABAb mediated) intracortical inhibition in 40 retired Australian Rules football players who had suffered their last concussion ~22 years prior. In a later study, Pearce et al [29] again observed decreased cSP within a cohort of 25 retired professional Rugby League players who had previously suffered concussion vs. 25 age-matched controls. Conversely, De Beaumont et al [2] assessed a population of 19 former contact sport athletes (mixed sports), who had suffered

their last concussion >30 years prior, and found increased intracortical inhibition when compared to former athletes who had no history of concussion. The disparities between the data within these three studies may be due to the sports the cohorts took part in or perhaps the timeframe in which their last concussions were suffered or perhaps through methodological differences. However, the presence of alterations does suggest further study is needed to clarify whether impaired GABAb receptor pathway is indeed a chronic manifestation of concussion. Furthermore, future studies covering a broad range of contact sports should be carried out to increase our understanding of the long-term neurophysiological manifestations of concussion. Methodological differences and robustness between studies also likely contributes to the contrasting data seen, many factors can influence the cSP such as background muscle activity, stimulation intensity, size of MEP generated pre-cSP and indeed analysis of cSP in terms of defining the onset of cSP [51].

Despite the many advantages of TMS and its general reliability, certain limitations with the technique should be noted. Most studies utilising TMS only examine one to two muscles, meaning that the results cannot be generalised to whole body inhibition-excitability. Positioning of the coil (which can be difficult to maintain), the potentially distracting audible 'clicks' and sensations felt on the scalp, can result in the extent or spread of current in the brain to vary with each pulse. The size of the MEP generated can also be influenced due to the factors mentioned above but also through stimulation intensity and the type of coil being used. Due to these methodological limitations which can lead to variability with results garnered, comparisons between studies utilising the technique are difficult to make.

More recently, the differences which may be seen between sexes have been investigated with interesting findings; it has been demonstrated that oestrogen increases excitability as it binds to GABA-mediated neurons and consequently blocks its synthesis and release [52]. Oestrogen has also been shown to increase the effects of glutamatergic receptors, again increasing excitability. Conversely, it has been demonstrated that progesterone has inhibitory effects [53] as it stimulates the impacts of GABA leading to decreased neuronal activity [54].

1.5 Blood Serum Biomarkers

Research within the last decade into the area of sport-related concussion and serum biomarkers has brought a greater understanding of the possible impacts from brain injury within sport [55,56]. When an athlete suffers from a concussive trauma, there are two overarching phases the body goes through [57]; the initial phase consists of the immediate consequences relating to the impact, namely the disruption of the structural integrity of axons, neurons and cell membranes [58]. The secondary phase is then the attempt at repairing and restoring these structures within the brain [58]. The subsequent changes within the axons, neurons and membranes, along with inflammation [59–62], results in distinct biomarker profiles which have the potential to provide information about the severity of the injury within the central nervous system [63].

One serum biomarker frequently investigated within the literature is glial fibrillary acidic protein (GFAP). The benefits of this biomarker from a diagnostics standpoint are that it is released into the serum one hour post-concussion and levels can remain elevated for

several days post the initial trauma [64–66]. The link between GFAP and brain injury has been studied for the last 30 years, however, it is not until recently that serum GFAP and brain trauma has been explored within a sporting context [67]. Despite the reported promise of GFAP in detecting injury, most of the success has come in a clinical setting rather than in a sporting context. Meier et al [68] found that GFAP levels in American Football players did not differ significantly between pre-season baseline measurements and non-concussed athlete controls. The contrast between results is most likely rooted in the fact that samples are drawn within the first 6 hours following trauma in a clinical setting [64–66]. Conversely, sport-related concussion samples tend to be drawn much later, from days to weeks, after concussion [69].

Another biomarker which has shown promise within a sporting context is neurofilament light (NF-L) protein. Following brain injury, the initial influx of calcium into the cell membranes activates calcineurin, a calcium-dependent phosphatase, which then dephosphorylates the NFL-medium or NFL-heavy side arms from the NFL subunit. This process is believed to be what contributes to axonal injury. Oliver et al. (2016) [70] recruited 116 NCAA Division 1 American Football players separated into starting players and non-starting players, and 19 NCAA Division 1 swimming athletes as controls. Blood serum samples were taken during pre-season (following 9-weeks of no contact-sport) and then at 7 more intervals covering the entire season. Serum NFL concentrations significantly increased in starters throughout training camp, with further marked increases observed during periods of higher instances of head impacts. Specifically, the initial spike in levels was observed post-training camp followed by a considerably larger increase during the competitive season which remained elevated until the season end. The conclusion from this

study was that starters within this cohort of Football players may have been suffering from some form of axonal injury. A conclusion reinforced by a later study by Oliver et al. (2018) [71] which employed a similar methodological approach to the previous study [70]. Once more, elevations in starters serum NF-L levels post-training compared to baseline and correlations between the number of head impacts was observed and starters had significantly elevated serum NF-L over the course of the season compared to non-starters. Recently, serum NF-L has also shown potential to demonstrate neuronal degeneration and predict white matter loss over time in former contact-sport players who had suffered concussion. Taghdiri et al. (2019)[72] observed that although serum NF-L concentrations in ex-contact sport players were not significantly different from healthy controls, they were positively correlated with mean diffusivity in the corpus callosum and fornix and total ventricular volume. This study, while promising, does have limitations in that it was observed in a small sample size (n=52), also, co-morbidities such as hypertension and obesity were not considered and could have impacted their results.

Ubiquitin C-terminal Hydrolase-L1 (UCH-L1) is a promising biomarker under investigation as a potential indicator of brain injury. Serum UCH-L1 levels have been shown to be elevated after as little as 1-hour post-concussion and appear to be able to discriminate between concussed and non-concussed trauma patients with high accuracy [65]. Joseph et al. (2018)[73] undertook a series of neuropsychological tests coupled with analyses of multiple biomarkers in 16 High School Varsity Football Players. Results showed significantly increased serum Tau and UCH-L1 levels following matches with HHI compared to non-HHI matches. Post-season Tau and UCH-L1 concentrations were also observed to be significantly elevated compared to baseline, suggesting neuronal and axonal injury. Interestingly, there were no

differences in serum NF-L, a normally reliable indicator of axonal injury, or GFAP between groups which demonstrates the unpredictability of biomarker analysis. This may also suggest an increased sensitivity of both Tau and UCH-L1 to concussive trauma compared to NF-L and GFAP.

S100-β, a protein found within astrocytes that aids in calcium regulation and is linked with astrocyte injury, is one the most studied biomarkers relating to concussion. Several studies have investigated the relationship between soccer players heading a football and S100-β levels [74–77]. Otto et al (2000) [74] found no elevations in serum S100-β levels, within a cohort of 12 soccer players performing 20 controlled headers. This finding was corroborated in later studies by Mussack [77] and Zetterberg [76] who also found no significant increases in S100-β levels. One study which measured S100-β levels pre- and post- a competitive soccer match [78] found significant increases after the match compared to baseline and the increase correlated to the amount of headers performed. In a more recent study, Kawata et al [79] also found increased levels of S100-β in 22 Division 1 American Football athletes post-training practice. They fitted the players with Vector mouth guards to analyse both the magnitude and frequency of head impacts during training, with their results suggesting that athletes with higher total head collisions were significantly correlated with larger pre- to post-practice S100-β levels.

It should also be noted that S100-β levels have been known to be influenced by various physical activities in adults who had not suffered mTBI and so its usefulness in assessing concussion is debatable [74,80,81].

In summation, the use of serum biomarkers to detect concussion in the short-term and predict neuronal degeneration in the long-term is in its infancy. Further understanding

around this topic could provide professionals with vital diagnostic and prognostic information for optimum recovery. Contact-sport athletes are at risk of brain bleeds, axonal damage, and long-term cognitive and motor deficits. The growing prevalence of CTE cases mean diagnostic tests, such as serum biomarkers, that detect brain injury rapidly and with accuracy must be investigated thoroughly. Current disadvantages to this technique include; the specificity of the biomarkers to the brain tissue, the amount of access that can be gained to a relatively damaged blood brain barrier and lack of sensitivity to early injury.

1.4 Potential Protective and Therapeutic Benefits of Omega-3 Fatty Acids

Omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) are a family of essential fatty acids which must be consumed in the diet [82] (primarily from oily fish and fish oil supplements). Alpha-linolenic acid (ALA) [18:3 (n-3)], synthesised from linolenic acid [18:2 (n-6)] via desaturation and catalysed via Δ-15-desaturase, is the simplest form of ω-3 fatty acid (FA) [83]. Humans do not possess the enzyme required to synthesise ALA however, they can metabolise it further into longer chain, unsaturated ω-3 fatty acids by a series of elongation and desaturation reactions (*Figure 3*). The product of these reactions is docosahexaenoic acid (DHA) [22:5 (n-3)] and eicosapentaenoic acid (EPA) [20:5 (n-3)] [84]. These ω-3s have many physiological properties which allow them to influence the structure, function and responses of cells within the brain and throughout the body [85]. Consequently, these fatty acids can play a vital role in treating and preventing many conditions linked to poor health and wellbeing [86–88]. Namely, they have been shown to reduce the risk of cardiovascular disease through influencing endothelial function, inhibiting pro-inflammatory mediators and reducing platelet aggregation [89–91].

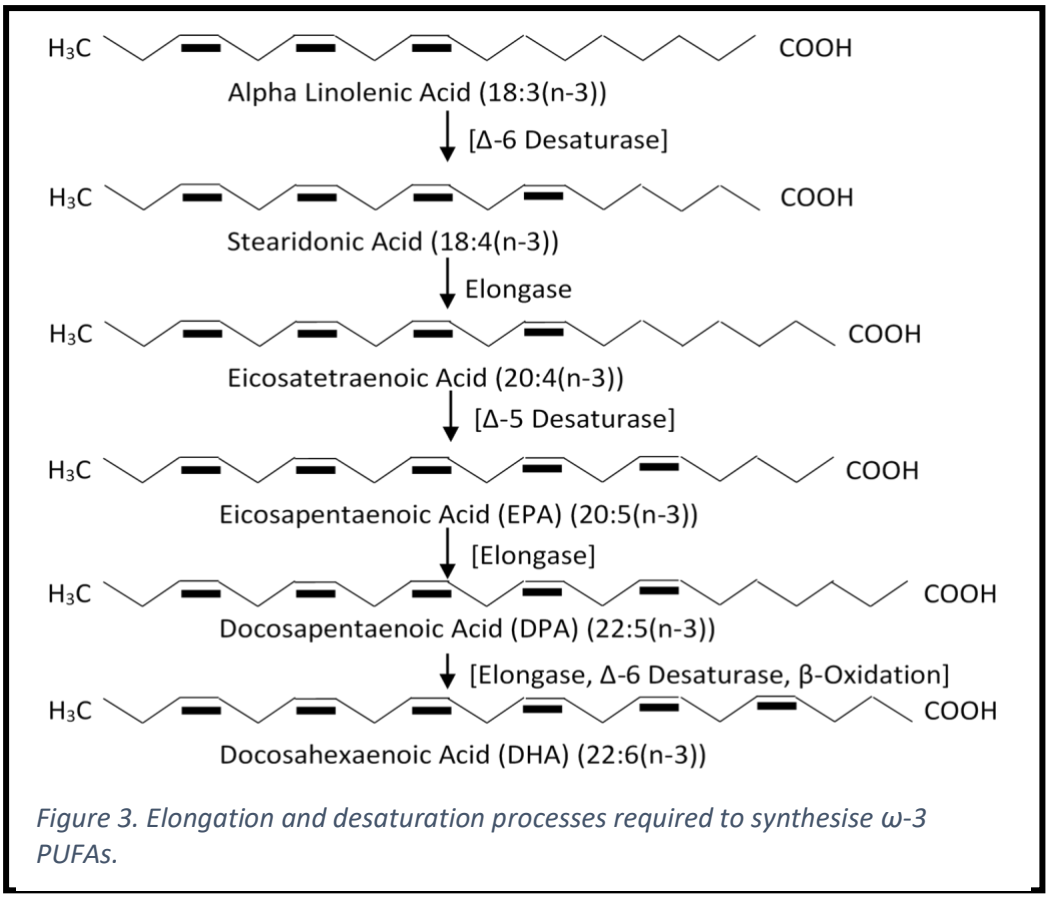

Figure 3. Elongation and desaturation processes required to synthesise ω-3 PUFAs.

DHA constitutes approximately 97% of the ω-3 PUFAs found within the brain and is a vital component of neural membrane phospholipids. Early in life, DHA accumulates in the frontal cortex and hippocampus, regions of the brain associated with memory, learning and executive function. Along with its important structural role, DHA is known to influence several aspects of brain functionality such as fluidity of membranes, receptor affinity and control over signal transduction pathways [92]. Several studies have highlighted the potential neurological benefits of omega-3 PUFA intake later in life. Increased DHA intake and plasma DHA levels are associated with decreased risk of neurodegenerative disease and slower cognitive decline [93,94]. Likewise, multiple epidemiological studies have shown a

positive correlation between increased DHA intake and markers of aging brains such as brain volume and cognition [95–98]. It is purported that ω-3 PUFAs protect the brain following mTBI by limiting axonal damage, inhibiting processes such as cell apoptosis and increasing the expression of protective mediators. It is thought that these protective mechanisms lead to a reduction in cognitive dysfunction caused by trauma [99–102].

One of the primary detrimental outcomes of mTBI is damage to neuronal structures, especially axons, which ultimately leads to apoptosis [14,15]. Prevalence of DHA within the brain following trauma decreases [103], which implies that there may be a greater need for DHA post-concussive injury due to its important role within the structure and function of the brain. Interventions exploring both the prophylactic and therapeutic potential of ω-3 PUFAs have shown inhibition of multiple components of the concussion cascade (*Figure 1*). Using a rodent model, Bailes and Mills [104] found that, following supplementation of DHA (40 mg · kg^{-1} · d^{-1}) post-injury, the amount of amyloid precursor protein-positive axons and apoptotic axons reduced to levels seen in uninjured animals, suggestive of a therapeutic impact of DHA. Supplementation with both DHA and EPA post-injury yielded similar results in a follow-up study by the same authors [105] and, in a later study [99], it was shown that supplementation prior to injury resulted in decreases of axonal injury and markers of cellular apoptosis compared to control fed animals.

Following concussion, oxidative stress occurs due to disruptions in energy metabolism and mitochondrial dysfunction. This leads to cellular damage and dulled expression of membrane homeostasis regulators. Therefore, restoring energy metabolism and membrane functionality may reduce oxidative stress following concussion and, ultimately, aid in recovery [92]. Accordingly, omega-3 PUFA supplementation post-injury has been shown to

reduce oxidative stress as a result of reducing markers of lipid peroxidation [103,106]. Additionally, post-injury DHA supplementation has been shown to inhibit the reduction of scavengers of oxidative stress and molecular biomarkers of membrane homeostasis such as manganese superoxide dismutase, syntaxin-3 and Ca-independent phospholipase A2 [107]. Furthermore, ω-3 FA supplementation led to a reversal of reductions in energy metabolism markers (AMP-activated protein kinase and phosphorylated AMP- activated protein kinase) following head-trauma which indicates a return to normal energy metabolism.

The potential therapeutic and protective effects of ω-3 PUFAs on alleviating concussion symptoms is obviously a complex matter and the examples of purported mechanisms laid out above most likely work in tandem rather than separately. The fact that it has been demonstrated in the literature that ω-3 PUFAs can significantly affect multiple areas of the concussion cascade individually bodes well for their potential success given the multifaceted nature of concussion [92].

1.5 Summary

In summation, the growing body of literature surrounding the potential negative implications of concussion sustained during sport has resulted in a great deal of interest regarding the subject. It is already well-established that multiple concussions sustained during a sporting career can result in long-term cognitive and motor impairments with a number of techniques having been developed and employed to assess these impairments. Thus far, no treatment to prevent or alleviate the symptoms of concussion exists however, omega-3 fatty acids (fish oils) have been touted as a possible solution. The key roles that

DHA and EPA play in the development of the brain, and natural therapeutic benefits they provide, may be able to help protect the brain prior to and following concussion by addressing specific processes we see unfold in the neurometabolic cascade of concussion.

1.7 Aim and Hypothesis

The primary aim of this study is to assess the efficacy of an omega-3 PUFA supplement containing 250mg of DHA and 750mg of EPA on brain-health in former contact sport players. The effectiveness of the 8-week supplementation period will be based on the primary outcome measures of corticospinal excitability (%mMax), corticomotor inhibition (cSP) and total error score from the Cambridge Neuropsychological Test Automated Battery (CANTAB).

We hypothesise that former contact sport players will see an increase in corticospinal excitability and a decrease in corticomotor inhibition post-supplementation. We also hypothesise that there will be a marked improvement in cognitive function highlighted by a reduction in total error scores within the CANTAB tests.

2. Methods

2.1 Approvals and Recruitment

Six healthy ex-contact sport players (all male), who had previously suffered a mTBI, (age 64 ± 9 years, height 179.48 ± 10.56 cm, mass 98.16 ± 19.92 kg, BMI 30.16 ± 4.19 kg/m²) and 10 age-matched controls (6 males and 4 females) with no history of brain injury (age 54 ± 7 years, height 171.05 ± 4.88 cm, mass 72.21 ± 11.24 kg, BMI 24.59 ± 3.79 kg/m²) were recruited for the study. Participants were recruited from physical activity clubs and societies aimed at older adults in Stirling and the surrounding area. Participants completed a TMS pre-screening questionnaire and were excluded for selection if they presented with any of the following risk factors: 1) history of any neurological conditions; 2) previous instances of seizures or syncope; 3) family history of epilepsy or seizures; 4) use of psychoactive recreational or prescribes narcotics; 5) any electrical devices attached to their body (pacemakers, cochlear implants, insulin pumps, neurostimulators etc.); or 6) metal implants in the skull. The NHS, Invasive and Clinical Research (NICR) ethics committee at the University of Stirling approved the study and all procedures carried out conformed to the guidelines laid out in the Declaration of Helsinki. All participants gave written, informed consent prior to taking part in the study.

2.2 Study Design

All participants attended a session prior to the first experimental trial to reduce the likelihood of learning effects in the neurophysiological measurement tests. No familiarisation was given for CANTAB protocols. Participants were instructed to abstain from

vigorous physical activity, alcohol, caffeine and smoking for twenty-four hours before each experimental trial. Furthermore, participants were required to arrive at the laboratory in a fasted state (no food or drink except water from 10pm the previous night). Measures of corticospinal excitability, corticomotor inhibition and cognitive function were assessed at baseline (Pre) and following four (T1) and eight (T2) weeks of omega-3 fatty acid supplementation *(Figure 4)*. All participants completed the National Adult Reading Test (NART) to obtain a predicted IQ based on the Wechsler Adult Intelligence Scale (WAIS) [108] on the day of T1 only. Predicted IQ was obtained using the formulae $Predicted\ WAIS\ Full\ Scale\ IQ = 127.7 - 0.826\ x\ NART\ error\ score$. Omega-3 fatty acid (ω-3 FA) supplements (Organic Technologies, Cochton, OH, United States) in the form of sealed capsules were given to participants following baseline testing. Participants were required to take 5 ω-3 FA capsules per day for the duration of the study. Each capsule contained a minimum of 750mg EPA and 250mg DHA, respectively.

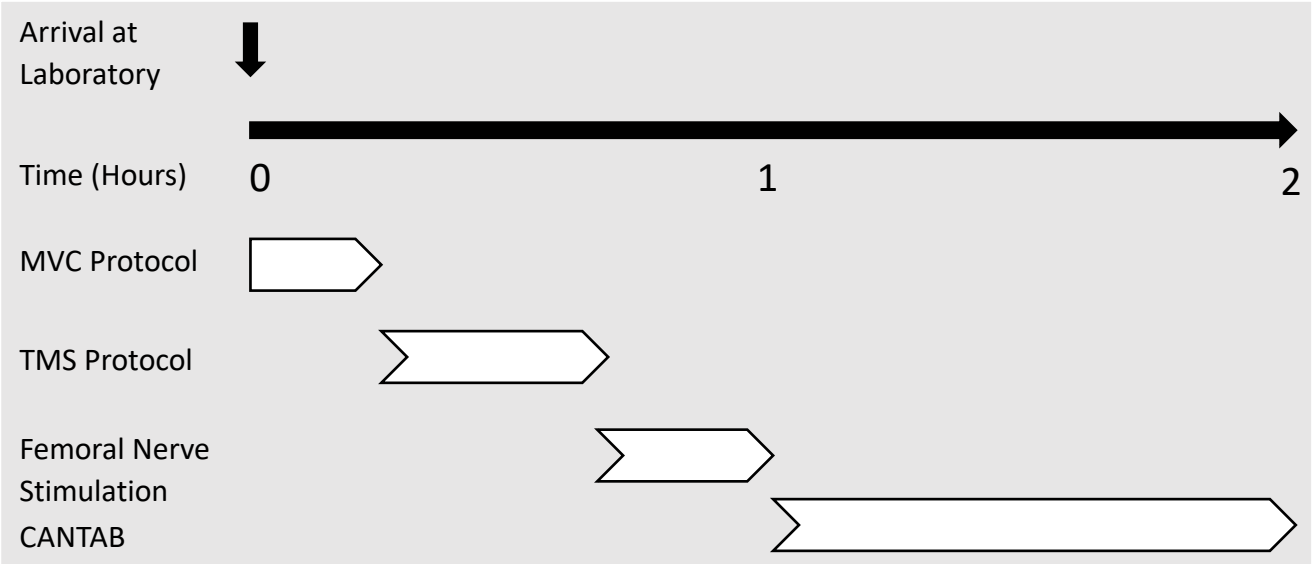

Figure 4. Trial day protocol outlining measurements taken and approximate times. Protocol was identical for both Athlete and Control cohorts across all trials. Supplements for full 8-weeks were given at culmination of Baseline (Pre) testing.

2.3 Electromyography

All EMG parameters and motor evoked potentials (MEPs) were taken with participants sitting on an isokinetic dynamometer (Biodex System 4, New York, NY, United States) and their dominant leg secured to the ankle pad of a calibrated load cell via the strap provided. Knee angle was set to 60^{o} (0^{o} equating a full leg extension) with the axis of rotation of the load cell aligned with their lateral femoral condyle [47]. Participants were further secured to the dynamometer via shoulder, thigh, and waist straps.

Electromyographic activity was recorded using a wireless system (Biopac Systems, Inc. Goleta, CA, USA). Data were sampled at 2 kHz and filtered using 500 Hz low- and 1 kHz high-band filters, respectively. All EMG signals were analysed offline (Acqknowledge, v3.9.1.6, Biopac Systems, Inc., Goleta, CA, United States). EMG activity of the vastus lateralis (VL) and rectus femoris (RF) was obtained using Ag/AgCl ECG surface electrodes (Vermed, Devon, United Kingdom) with an intra-electrode distance of 2cm positioned over both muscles. Prior to electrode placement, the areas of skin over the RF and VL were shaved and abraded as per the guidelines laid out in Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) [109].

Once participants were secured to the dynamometer, and following electrode placement, they were asked to perform 3 isometric contractions at what they perceived to be 50% of maximal effort separated by 5 seconds. Three more contractions were performed at 75% of perceived maximal effort followed by three maximal voluntary contractions (MVC). Each MVC lasted 5 seconds with one minute separating each contraction. Verbal encouragement was given throughout.

2.4 Transcranial Magnetic Stimulation

Motor evoked potentials in the VL and RF of the dominant leg were elicited via single pulse TMS and evaluated through EMG readings. Single electro-magnetic stimuli, 1 ms in duration, were delivered over the contralateral primary motor cortex (M1) through a magnetic stimulator (Magstim 2002 unit, The Magstim Company Ltd., Whitland, United Kingdom) and a 110mm double-cone coil. Optimal coil location was determined by placing the coil over M1, laterally to the vertex; the area in which the largest peak-to-peak MEP was observed was marked on the scalp with semi-permanent ink. The active motor threshold (aMT) was based on the stimulator output scale of 0-100%. Therefore, aMT was determined by increasing the stimulator intensity in 5% increments starting at 10% and whilst participants were contracting at 20% of their MVC (see section *2.3*) until discernible MEPs were apparent [110]. All subsequent stimulations were delivered at 130% of aMT. For example, an aMT of 30% would result in stimulations being administered at 39% (=1.3 * 30).

To assess corticospinal excitability, participants maintained a contraction equal to 20% of their MVC whilst 20 TMS pulses were delivered over M1. The 20 pulses were divided into 4 sets of five, with 30s between each set and 5s separating each pulse. Excitability was defined as the average peak-to-peak MEP amplitude normalised to the maximal muscle output observed via peripheral nerve stimulation (%Mmax) (section *2.5*). Corticomotor inhibition was determined by participants performing 3 MVCs (5s in duration, separated by 60s) and delivering a single TMS pulse over M1. Verbal encouragement was given throughout, and a single stimulus was delivered ~3s into the contraction. Participants were instructed to push through the transient loss in power observed when stimuli are delivered at maximal effort. Corticomotor inhibition was defined as the length (in milliseconds) of the

cortical silent period (cSP), taken from the stimulation artefact to the continuation of clear, uninterrupted EMG activity [111,112]. The average cSP duration from the 3 contractions was used for further analysis.

2.5 Femoral Nerve Stimulation

Stimulation of the femoral nerve was carried out using an electrical stimulator (Biopac Systems, Inc.). The femoral nerve was identified by first locating the femoral artery and applying a self-adhering surface electrode (cathode) laterally to the artery and high above the femoral triangle. The anode was placed on the gluteus maximus. Single stimuli were then administered in increasing intensity to the muscle with the participant at rest. Stimulations increased in intensity until a plateau in the force response and discernible M-waves were apparent in the RF and VL. Three supramaximal stimulations were then delivered at 130% of the stimulator output at which the plateau was observed.

2.6 Cognitive Function

Cognitive function was assessed by using the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition, Cambridge, United Kingdom). The following tests were used: total adjusted error score on Paired Associates Learning (PAL) (a measure of long-term memory); total words recalled and distractor words correctly discounted at the immediate and delayed phases of the Verbal Recognition Memory (VRM); Reaction Time (RTI) simple and 5-choice median movement time; decision making quality during the Cambridge Gambling Test (CGT); total incorrect on the Multitasking Test (MTT);

Spatial Working Memory (SWM) between errors (short-term memory assessment); number of problems solved in minimum amount of moves in the Stockings of Cambridge (SOC). Cognitive function testing was carried out using an electronic tablet, (iPad Air 2, Model A1566) (Apple Inc., Cupertino, CA, United States) with participants seated in a quiet room.

2.7 Statistical Analysis

Statistical analysis was carried out on Jamovi (version 1.6.23). Graphing software used was GraphPad Prism 9 (v.9.2.0; GraphPad Software, Inc.). Two-way analysis of variances (ANOVAs) were used on corticomotor inhibition, corticospinal excitability and cognitive function. Tukey's post hoc test was used to explore any significant differences further. Statistical significance was set at $p \leq 0.05$. All data are presented as mean ± SD unless otherwise stated.

3. Results

3.1 Corticomotor Inhibition

Corticomotor inhibition showed no significant time, group or interaction effects between the athlete and control groups in both the rectus femoris and vastus lateralis *(p > 0.05)*. RF Corticomotor inhibition decreased by 6% from baseline to 8-weeks supplementation in the Control group compared to no change in the Athlete group. VL corticomotor inhibition decreased by 3.5% post-8-week supplementation in the Control group and decreased by 5% in the Athlete group.

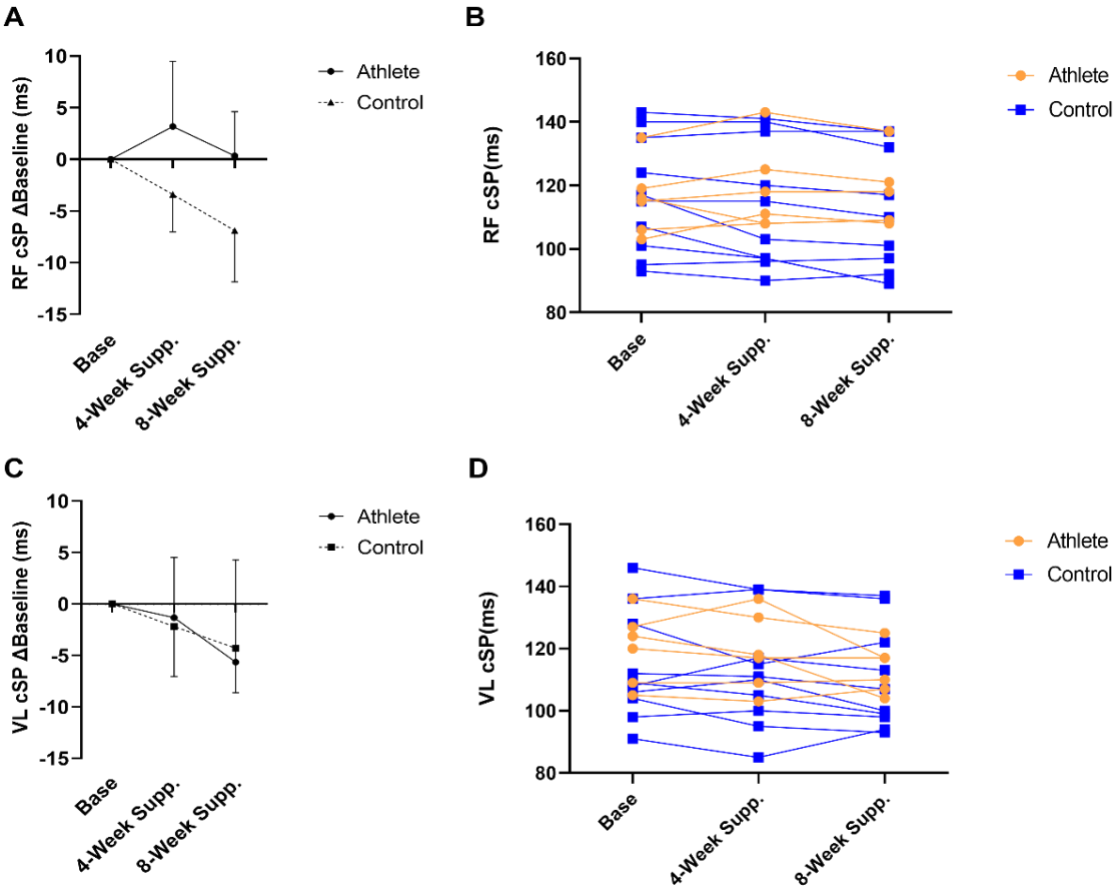

Figure 5. (A) Difference in RF cSP (ms) in Athlete and Control groups following 4- and 8-Week's supplementation, relative to baseline. No significant effects were observed however, inhibition had decreased in the Control group by 6% after 8-Week's supplementation; error bars denote 95% CI. (B) Individual cSP changes for each participant after 4- and 8-weeks supplementation relative to baseline. (C) cSP changes in VL for Athlete and Control groups relative to baseline. No significant effects were observed however, there was a trend of inhibtion declining across trials with a 3.5% and 5% decrease in Control and Athlete groups, respectively. (D) Change in VL cSP duration for each participant from baseline to 4- and 8-weeks supplementation error bars denote 95% CI.

3.2 Corticospinal Excitability

A significant group effect *($p = 0.034$, $F_{(1,42)} = 4.792$)* was observed in corticospinal excitability in the VL following supplementation. No significant group, trial or intervention effects on excitability were observed in the RF between the Athlete and Control groups *($p > 0.05$)*.

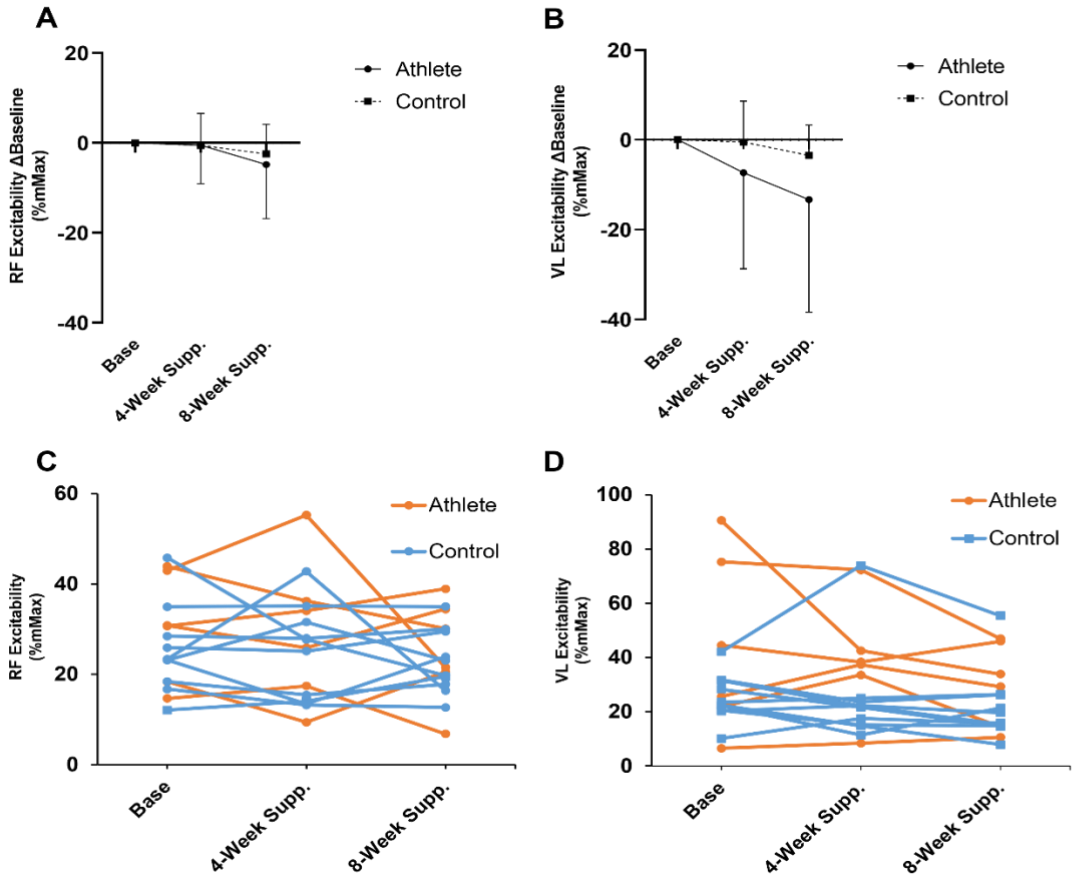

*Figure 6. Difference of corticospinal excitability in RF (A) and VL (B) for Athlete and Control groups following supplementation, relative to baseline. No significant effects were seen in RF however, excitability appears to slightly decrease across the trials. A significant group effect was observed in the VL excitability (p = 0.034), excitability also appears to decrease across trials in both groups although not significantly. *p = <0.05; error bars denote 95% CI. (C) and (D) denote Individual values of RF/VL Corticospinal Excitability for each participant at baseline and following 4- and 8-weeks of supplementation.*

3.3 Cognitive Function

Significant group effects were observed in the RTI test for both the simple *(p = 0.003, $F_{(1,41)}$ = 10.013)* and 5-choice *(p = 0.008, $F_{(1,41)}$ = 7.715)* portions of the test. Athlete reaction time was on average 24% slower across trials than the Control group in the simple section of the task and 21% slower in the 5-choice section. Reaction time remained unchanged in the Control group across trials in both sections of the task but appeared to improve in the Athlete group post-supplementation relative to baseline though not significantly (Figure 7).

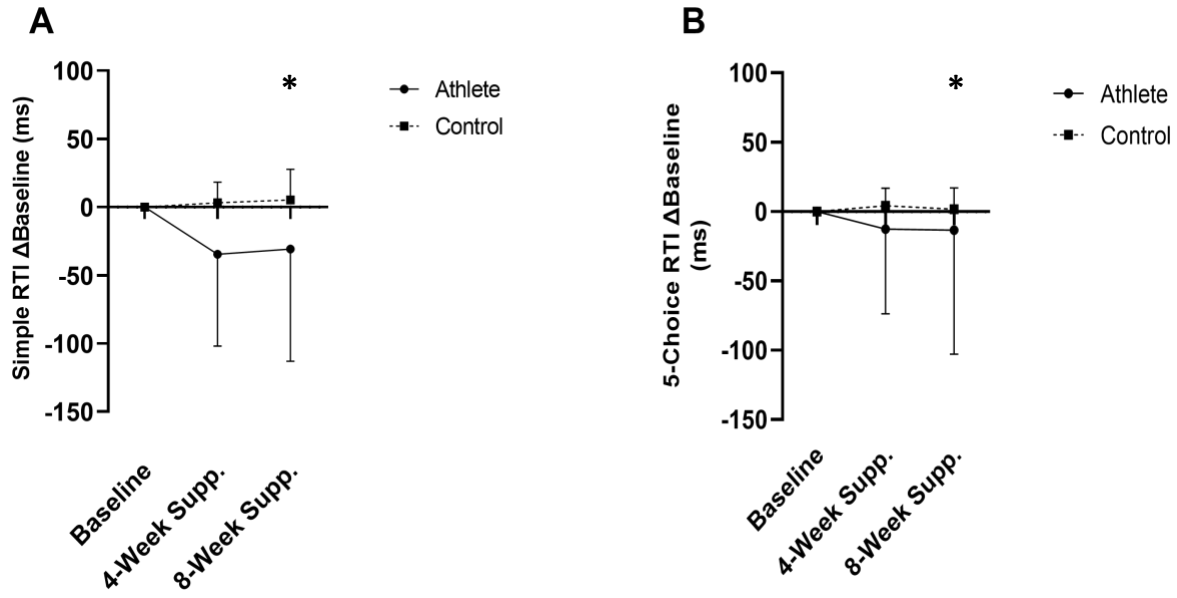

*Figure 7. Difference in reaction time for Athlete and Control groups in the simple (A) and 5-choice (B) sections of the test. Reaction time appeared to improve in the Athlete group in both sections post-supplementation though not significantly. Reaction time remains constant throughout trials in the Control group. *p = < 0.05; error bars denote 95% CI.*

Significant group *(p = < 0.05, $F_{(1,41)}$ = 9.651)* but no time or intervention effects were observed in the PAL task. Errors were on average 133% higher in the Athlete group compared to the Control group. Errors decreased on average 33% across trials in Control

group relative to baseline. Errors also decreased slightly (10%) in the Athlete group but only following 8-weeks supplementation (Figure 8).

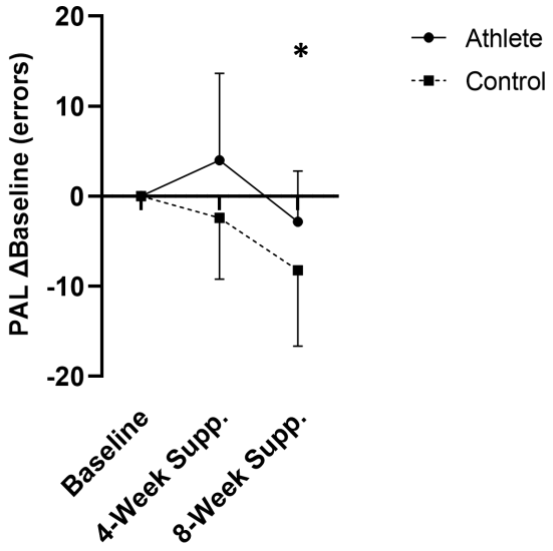

*Figure 8. Difference in total errors during PAL test post-supplementation for Athlete and Control groups relative to baseline. Errors dropped throughout both trials in the Control group though not significantly. Total Errors decreased in the Athlete population compared to baseline only following 8-weeks supplementation and not significantly. *p = < 0.05; error bars denote 95% CI.*

No significant time, group or interaction effects were observed in the SWM, CGT, VRM Immediate, VRM Delayed, SOC or MTT tasks *(p > 0.05)* (Table 1).

CANTAB Test	Group	Baseline	4-Week Supplementation	8-Week Supplementation
SWM (Between Errors)	**Control**	**7.10 ± 9.06**	**9.00 ± 10.3**	**5.11 ± 8.02**
	Athlete	**9.56 ± 8.32**	**7.50 ± 8.76**	**6.00 ± 5.55**
CGT (Decision Making Quality)	**Control**	**0.93 ± 0.12**	**0.94 ± 0.08**	**0.94 ± 0.08**
	Athlete	**0.96 ± 0.04**	**0.94 ± 0.05**	**0.98 ± 0.02**
VRM Immediate (Total Words Recalled)	**Control**	**31.7 ± 3.09**	**31.2 ± 3.33**	**32.3 ± 2.83**
	Athlete	**31.8 ± 1.83**	**33.5 ± 1.05**	**33.0 ± 2.19**
VRM Delayed (Total Words Recalled)	**Control**	**32.2 ± 3.08**	**31.7 ± 3.23**	**32.8 ± 2.99**
	Athlete	**34.0 ± 1.41**	**33.2 ± 1.83**	**32.0 ± 1.26**
SOC (Trials Complete in min. moves)	**Control**	**8.90 ± 2.08**	**9.60 ± 1.26**	**9.11 ± 2.15**
	Athlete	**9.00 ± 2.10**	**8.17 ± 2.79**	**9.17 ± 2.64**
MTT (Total Errors)	**Control**	**4.10 ± 4.48**	**3.00 ± 1.94**	**1.44 ± 1.51**
	Athlete	**2.40 ± 2.07**	**3.33 ± 3.83**	**1.83 ± 0.75**

Table 1. Mean performance scores ± SD of Athlete and Control groups in CANTAB tasks. No significant effects were observed within SWM, CGT, VRM Immediate and Delayed, SOC or MTT tasks.

4. Discussion

The aim of this study was to examine the efficacy of 8-week omega-3 fatty acid supplementation on measures of cognition and neurophysiological control in former athletes who had suffered concussion. We hypothesised that marked improvements in cognitive performance as well as increased corticospinal excitability and a dampened corticomotor inhibitory response would be observed post-supplementation. This hypothesis was based on previous data almost entirely from animal models which had shown improved cognitive performance in rats [106,107] and provided insight into potential mechanisms within the brain driven by ω-3 PUFA supplementation following mTBI.

The current study failed to demonstrate any significant impacts of ω-3 FAs on measures of corticomotor inhibition and corticospinal excitability. It is thought that supplementation could provide a therapeutic effect following mTBI at the acute stage through reducing the amount of structural damage on axons, inhibiting cell apoptosis and increasing the concentration of mediators involved in repair [99–102]. To our knowledge, no study has looked at the effects of ω-3 FA supplementation on long-term impairments following concussion and so the mechanisms behind their potential success remain unclear. Due to the available data being from rodent models, limited mechanistic understandings and small sample size, firm conclusions about the relationship between omega-3 supplementation and mTBI cannot be made.

We also indirectly assessed the chronic changes in GABA related activity in previously concussed versus non-concussed cohorts through analysis of cSP. We found no significant differences between groups which is in contrast to the current available literature [2,26,38,113,114] which generally reports a significantly lengthened cSP in formerly

concussed cohorts compared to control. Tremblay et al. [114] found alterations in the GABA mediated inhibition-excitability relationship in American Footballers who had suffered concussion at least 3 years prior. This finding was investigated through analysis of cSP as well as spectroscopy and suggests that concussion does indeed result in an altered GABA response and should, as a by-product, affect downstream outcomes of GABA activity. The fact that no differences were observed in this study is likely due to the small sample size.

Memory and learning are dependent upon balanced GABA moderated inhibition. When an increase in GABAergic activity occurs, it can result in diminished neural plasticity with the potential to significantly impact memory processes [115]. Studies have found impaired cognitive performance, specifically memory and learning, in populations who had suffered concussion 22 years prior [38] and also transient deficits following repeated sub-concussive impacts [37,47]. We observed significant group differences in paired associated learning error scores, this could be interpreted as a consequence of altered GABA mediated inhibition in line with the current research. However, given we saw no differences in cSP this finding is more likely due to a learning effect as familiarisation sessions were not given for CANTAB test protocols.

We found significant differences between groups at baseline in the Reaction Time and Paired Associates Learning tests (Figure 7 & 8) which are in agreement with much of the literature [28–30]. Conversely, no differences were subsequently observed in either SWM or VRM (Table 1), the inconsistency in detecting impairments in learning and memory between groups are likely due to the small sample sizes used in this study. However, another explanation could be due to the lack of familiarisation tests prior to testing. Lowe and Rabbitt [116] found that the CANTAB was vulnerable to significant practice effects (which

could render comparisons between tests invalid) and had very low test-retest reliability. In addition to this, Smith et al. [34] concluded that although the CANTAB provided an adequate estimation of general cognition, results garnered on specific facets of cognition (such as learning and memory) should be analysed carefully.

A perhaps surprising aspect of our results was the lack of change in the cognitive outcomes measured following supplementation. Several clinical studies had already demonstrated the benefits of ω-3 FA on cognitive outcomes in several populations. Yurko-Mauro et al. [117] found significantly decreased PAL errors and improved immediate and delayed VRM following 900mg/d DHA supplementation versus placebo in adults with self-reported memory issues. This could suggest that a higher dosage of DHA would have been required in this study to see similar effects. It should also be noted that this finding was observed following 24 weeks of supplementation indicating longer-term supplementation may be crucial, a hypothesis corroborated by several other studies with long-term supplementation periods between 3- and 12-months [118–120].

Appropriately, an interesting area for further research would be investigating the optimal dosage and supplementation period of omega-3 FA when treating mTBI. Currently, estimating this dosage is tricky as very little data exists from human trials, a general dosage used in animal models of 40mg · kg^{-1} · d^{-1} DHA [99,104,104] would equate to an approximate 400mg/d DHA in humans following conversion to account for increased body surface area [92]. This total would be in line with recommendations made from previous studies investigating other health benefits of ω-3 FA however, until further studies are carried out specifically addressing mTBI, an accurate recommendation cannot be made.

Another avenue for possible future studies would be to discern the impacts of lone EPA supplementation. Typically, studies use either DHA or a combination of DHA and EPA. Due to the vast majority of ω-3 in the brain being DHA, it is presumed that EPA would play a bit-part in mediating the potential therapeutic benefits of supplementation. This statement is backed up by numerous studies demonstrating diminished levels of DHA post-concussion, implying a heightened need for optimal recuperation. However, Chiu et al. observed, following 24-weeks of ω-3 FA supplementation (1080mg/d EPA and 720mg/d DHA), reduced cognitive impairment in subjects with mild Alzheimer's Disease but also linked higher plasma EPA concentrations to better cognitive outcomes. This suggests that EPA could perhaps be more important than first thought in alleviating mTBI symptoms.

5. Strengths, Limitations and Future Directions

The main limitation of the present study is of course the small sample sizes of both the control (n=10) and athlete (n=6) groups respectively, which made accurate interpretation of the data difficult. Power calculations carried out pre-study suggested sample sizes of at least n=20 per group would be needed to minimise the chances of type I and/or type II errors.

Another limitation would be the lack of familiarisation with the CANTAB protocols which may have resulted in a learning effect as the trials progressed and type I errors. This was especially apparent during data analysis in which several outliers had to be removed from the data sets. Despite this, large standard deviations remained in several CANTAB measures (Table 1) although this was also likely compounded by the small sample size. To date, there have been little studies examining the test-retest reliability of the CANTAB however, one

study [121] demonstrated a possible learning effect specifically within the SWM test. More recently another study [122] found low test-retest reliability and evidence of practice effects in several other CANTAB measures (PAL, RTI and SOC) but demonstrated no practice effect in SWM which the authors contributed to being a consequence of previous literature using participants with mild cognitive impairment.

Outwith the small sample size, perhaps the most limiting factor of this study (and indeed within this area of research as a whole) would be the overall study design. It can be argued that it is inappropriate to employ a cross-sectional design and compare one group of former athletes to results gathered in a control group - baseline measurements of the individual may be drastically different from the beginning. A more longitudinal study design with multiple baseline measurements in which each participant becomes their own control may yield a more accurate representation of the impacts of ω-3 FA supplementation on brain health.

Further, the sporting backgrounds and the time since the last concussion of the former athlete population used in the present study would have been useful information to give added context to the results however, this information was not gathered during testing.

Despite the limitations, it would be remiss not to acknowledge the novelty of the current study in that, to the authors knowledge, for the first time a therapeutic aid has been investigated for the treatment of motor and cognitive impairment in a population of former contact sport athletes with concussion history. The present study demonstrated the feasibility of investigating this area which has gained increasing amounts of public and media attention in recent years. Not only this, but we have also shown preliminary, albeit inconclusive, evidence of the effects that ω-3 FA supplementation may have on measures of

cognitive and motor impairment in a human population. This is in contrast to the current data available which are taken almost entirely from rodent models.

6. Conclusion

In conclusion, our findings demonstrate no changes in corticomotor inhibition, corticospinal excitability, and outcome measures within CANATB testing post-supplementation. The findings within the current study may suggest that ω-3 PUFA supplementation does not have a therapeutic effect on cognitive and motor deficits seen in former contact sport athletes who have suffered mTBI during their careers. However, the results should be interpreted cautiously due to the small sample size within this study. Future studies should therefore seek to replicate these findings with a larger cohort and more outcome measures (such as blood biomarker concentrations) in order to fully understand the efficacy of omega-3 PUFA supplementation on cognition and neurophysiological control following concussion.

Bibliography

1 Daneshvar DH, Nowinski CJ, McKee A, *et al.* The Epidemiology of Sport-Related Concussion. *Clin Sports Med* 2011;**30**:1–17. doi:10.1016/j.csm.2010.08.006

2 De Beaumont L, Théoret H, Mongeon D, *et al.* Brain function decline in healthy retired athletes who sustained their last sports concussion in early adulthood. *Brain* 2009;**132**:695–708. doi:10.1093/brain/awn347

3 Guskiewicz KM, Marshall SW, Bailes J, *et al.* Association between recurrent concussion and late-life cognitive impairment in retired professional football players. *Neurosurgery* 2005;**57**:719–26; discussion 719-726. doi:10.1093/neurosurgery/57.4.719

4 Jordan B, Bailes JE. Concussion history and current neurological symptoms among retired professional football players. *Neurology* 2000;**54**:A410–1.

5 Johnston W, Coughlan GF, Caulfield B. Challenging concussed athletes: the future of balance assessment in concussion. *QJM* 2017;**110**:779–83. doi:10.1093/qjmed/hcw228

6 Feudtner C, Miles SH. Traumatic Brain Injury News Reports and Participation in High School Tackle Football. *JAMA Pediatr* 2018;**172**:492–4. doi:10.1001/jamapediatrics.2017.5242

7 Mannix R, Meehan WP, Pascual-Leone A. Sports-related concussions — media, science and policy. *Nat Rev Neurol* 2016;**12**:486–90. doi:10.1038/nrneurol.2016.99

8 Stein TD, Alvarez VE, McKee AC. Concussion in Chronic Traumatic Encephalopathy. *Curr Pain Headache Rep* 2015;**19**:47. doi:10.1007/s11916-015-0522-z

9 Salem N, Litman B, Kim HY, *et al.* Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids* 2001;**36**:945–59. doi:10.1007/s11745-001-0805-6

10 Lauritzen, L, Hansen, H, Jørgensen, M, *et al.* The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipid Res* 2001;**40**:1–94. doi:10.1016/s0163-7827(00)00017-5

11 Hashimoto M, Hossain S, Shimada T, *et al.* Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. *J Neurochem* 2002;**81**:1084–91. doi:10.1046/j.1471-4159.2002.00905.x

12 Tommasone BA, Valovich McLeod TC. Contact Sport Concussion Incidence. *J Athl Train* 2006;**41**:470–2.

13 Stillman A, Alexander M, Mannix R, *et al.* Concussion: Evaluation and management. *CCJM* 2017;**84**:623–30. doi:10.3949/ccjm.84a.16013

14 Giza C, A. Hovda D. The New Neurometabolic Cascade of Concussion. *Journal of athletic training* 2001;**36**:228–35. doi:10.1227/NEU.0000000000000505

15 Barkhoudarian G, Hovda DA, Giza CC. The molecular pathophysiology of concussive brain injury. *Clin Sports Med* 2011;**30**:33–48, vii–iii. doi:10.1016/j.csm.2010.09.001

16 Dashnaw ML, Petraglia AL, Bailes JE. An overview of the basic science of concussion and subconcussion: where we are and where we are going. *Neurosurg Focus* 2012;**33**:E5: 1-9. doi:10.3171/2012.10.FOCUS12284

17 Bey T, Ostick B. Second Impact Syndrome. *West J Emerg Med* 2009;**10**:6–10.

18 McCrory P, Meeuwisse W, Aubry M, *et al.* Consensus statement on Concussion in Sport--the 4th International Conference on Concussion in Sport held in Zurich, November 2012. *J Sci Med Sport* 2013;**16**:178–89. doi:10.1016/j.jsams.2013.02.009

19 Shrey DW, Griesbach GS, Giza CC. The pathophysiology of concussions in youth. *Phys Med Rehabil Clin N Am* 2011;**22**:577–602, vii. doi:10.1016/j.pmr.2011.08.002

20 Broglio SP, Puetz TW. The Effect of Sport Concussion on Neurocognitive Function, Self-Report Symptoms and Postural Control. *Sports Med* 2008;**38**:53–67. doi:10.2165/00007256-200838010-00005

21 McCrea M, Guskiewicz KM, Marshall SW, *et al.* Acute Effects and Recovery Time Following Concussion in Collegiate Football Players: The NCAA Concussion Study. *JAMA* 2003;**290**:2556–63. doi:10.1001/jama.290.19.2556

22 Henry LC, Tremblay S, Boulanger Y, *et al.* Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. *J Neurotrauma* 2010;**27**:65–76. doi:10.1089/neu.2009.0962

23 Iverson G, Gaetz M, Lovell M, *et al.* Cumulative effects of concussion in amateur athletes. *Brain injury : [BI]* 2004;**18**:433–43. doi:10.1080/02699050310001617352

24 Matser JT, Kessels AG, Lezak MD, *et al.* A dose-response relation of headers and concussions with cognitive impairment in professional soccer players. *J Clin Exp Neuropsychol* 2001;**23**:770–4. doi:10.1076/jcen.23.6.770.1029

25 Belanger HG, Vanderploeg RD. The neuropsychological impact of sports-related concussion: a meta-analysis. *J Int Neuropsychol Soc* 2005;**11**:345–57. doi:10.1017/s1355617705050411

26 Pearce AJ, Hoy K, Rogers MA, *et al.* Acute motor, neurocognitive and neurophysiological change following concussion injury in Australian amateur football. A prospective multimodal investigation. *Journal of Science and Medicine in Sport* 2015;**18**:500–6. doi:10.1016/j.jsams.2014.07.010

27 Livingston SC, Goodkin HP, Hertel JN, *et al.* Differential Rates of Recovery After Acute Sport-Related Concussion: Electrophysiologic, Symptomatic, and Neurocognitive Indices. *Journal of Clinical Neurophysiology* 2012;**29**:23–32. doi:10.1097/WNP.0b013e318246ae46

28 Montenigro PH, Alosco ML, Martin BM, *et al.* Cumulative Head Impact Exposure Predicts Later-Life Depression, Apathy, Executive Dysfunction, and Cognitive Impairment in Former High School and College Football Players. *Journal of Neurotrauma* 2016;**34**:328–40. doi:10.1089/neu.2016.4413

29 Pearce A, Rist B, Fraser C, *et al.* Neurophysiological and cognitive impairment following repeated sports concussion injuries in retired professional rugby league players. *Brain Injury* 2018;**32**:498–505. doi:10.1080/02699052.2018.1430376

30 Zhang Y, Ma Y, Chen S, *et al.* Long-Term Cognitive Performance of Retired Athletes with Sport-Related Concussion: A Systematic Review and Meta-Analysis. *Brain Sciences* 2019;**9**:199. doi:10.3390/brainsci9080199

31 Fields L, Didehbani N, Hart J, *et al.* No Linear Association Between Number of Concussions or Years Played and Cognitive Outcomes in Retired NFL Players. *Arch Clin Neuropsychol* 2020;**35**:233–9. doi:10.1093/arclin/acz008

32 Casson IR, Viano DC, Haacke EM, *et al.* Is There Chronic Brain Damage in Retired NFL Players? Neuroradiology, Neuropsychology, and Neurology Examinations of 45 Retired Players: *Sports Health* Published Online First: 25 June 2014. doi:10.1177/1941738114540270

33 Cunningham J, Broglio S, Wilson F. Influence of playing rugby on long-term brain health following retirement: a systematic review and narrative synthesis. *BMJ Open Sport & Exercise Medicine* 2018;**4**:e000356. doi:10.1136/bmjsem-2018-000356

34 Smith PJ, Need AC, Cirulli ET, *et al.* A comparison of the Cambridge Automated Neuropsychological Test Battery (CANTAB) with "traditional" neuropsychological testing instruments. *Journal of Clinical and Experimental Neuropsychology* 2013;**35**:319–28. doi:10.1080/13803395.2013.771618

35 Schatz P, Browndyke J. Applications of computer-based neuropsychological assessment. *J Head Trauma Rehabil* 2002;**17**:395–410. doi:10.1097/00001199-200210000-00003

36 Saunders NLJ, Summers MJ. Attention and working memory deficits in mild cognitive impairment. *Journal of Clinical and Experimental Neuropsychology* 2010;**32**:350–7. doi:10.1080/13803390903042379

37 Di Virgilio TG, Ietswaart M, Wilson L, *et al.* Understanding the Consequences of Repetitive Subconcussive Head Impacts in Sport: Brain Changes and Dampened Motor Control Are Seen After Boxing Practice. *Front Hum Neurosci* 2019;**13**. doi:10.3389/fnhum.2019.00294

38 Pearce AJ, Hoy K, Rogers MA, *et al.* The long-term effects of sports concussion on retired Australian football players: a study using transcranial magnetic stimulation. *J Neurotrauma* 2014;**31**:1139–45. doi:10.1089/neu.2013.3219

39 Powers KC, Cinelli ME, Kalmar JM. Cortical hypoexcitability persists beyond the symptomatic phase of a concussion. *Brain Injury* 2014;**28**:465–71. doi:10.3109/02699052.2014.888759

40 Cavaleri R, Schabrun SM, Chipchase LS. The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations using transcranial magnetic stimulation (TMS): a systematic review and meta-analysis. *Systematic Reviews* 2017;**6**:48. doi:10.1186/s13643-017-0440-8

41 Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. *Lancet Neurol* 2003;**2**:145–56.

42 Klomjai W, Katz R, Lackmy-Vallée A. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Annals of Physical and Rehabilitation Medicine* 2015;**58**:208–13. doi:10.1016/j.rehab.2015.05.005

43 Ziemann U. TMS and drugs. *Clinical Neurophysiology* 2004;**115**:1717–29. doi:10.1016/j.clinph.2004.03.006

44 Schmid UD, Boll J, Liechti S, *et al.* Influence of Some Anesthetic Agents on Muscle Responses to Transcranial Magnetic Cortex Stimulation: A Pilot Study in Humans. *Neurosurgery* 1992;**30**:85–92.

45 Ziemann U, Lönnecker S, Steinhoff BJ, *et al.* Effects of antiepileptic drugs on motor cortex excitability in humans: A transcranial magnetic stimulation study. *Annals of Neurology* 1996;**40**:367–78. doi:10.1002/ana.410400306

46 Major BP, Rogers MA, Pearce AJ. Using transcranial magnetic stimulation to quantify electrophysiological changes following concussive brain injury: a systematic review. *Clin Exp Pharmacol Physiol* 2015;**42**:394–405. doi:10.1111/1440-1681.12363

47 Di Virgilio TG, Hunter A, Wilson L, *et al.* Evidence for Acute Electrophysiological and Cognitive Changes Following Routine Soccer Heading. *EBioMedicine* 2016;**13**:66–71. doi:10.1016/j.ebiom.2016.10.029

48 Hupfeld KE, Swanson CW, Fling BW, *et al.* TMS-induced silent periods: A review of methods and call for consistency. *Journal of Neuroscience Methods* 2020;**346**:108950. doi:10.1016/j.jneumeth.2020.108950

49 McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res* 2006;**173**:86–93. doi:10.1007/s00221-006-0365-2

50 Tremblay S, de Beaumont L, Lassonde M, *et al.* Evidence for the specificity of intracortical inhibitory dysfunction in asymptomatic concussed athletes. *J Neurotrauma* 2011;**28**:493–502. doi:10.1089/neu.2010.1615

51 Škarabot J, Mesquita RNO, Brownstein CG, *et al.* Myths and Methodologies: How loud is the story told by the transcranial magnetic stimulation-evoked silent period? *Experimental Physiology* 2019;**104**:635–42. doi:10.1113/EP087557

52 Ss S, Cs W. Cellular and molecular effects of steroid hormones on CNS excitability. *Cleveland Clinic journal of medicine* 2004;**71 Suppl 2**. doi:10.3949/ccjm.71.suppl_2.s4

53 Smith SS, Woodward DJ, Chapin JK. Sex steroids modulate motor-correlated increases in cerebellar discharge. *Brain Res* 1989;**476**:307–16. doi:10.1016/0006-8993(89)91251-1

54 Ansdell P, Brownstein CG, Škarabot J, *et al.* Menstrual cycle-associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic women. *J Appl Physiol (1985)* 2019;**126**:1701–12. doi:10.1152/japplphysiol.01041.2018

55 Papa L, Ramia MM, Edwards D, *et al.* Systematic review of clinical studies examining biomarkers of brain injury in athletes after sports-related concussion. *J Neurotrauma* 2015;**32**:661–73. doi:10.1089/neu.2014.3655

56 Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol* 2013;**9**:201–10. doi:10.1038/nrneurol.2013.9

57 Agoston DV, Shutes-David A, Peskind ER. Biofluid biomarkers of traumatic brain injury. *Brain Injury* 2017;**31**:1195–203. doi:10.1080/02699052.2017.1357836

58 Büki A, Povlishock JT. All roads lead to disconnection? – Traumatic axonal injury revisited. *Acta Neurochir (Wien)* 2006;**148**:181–94. doi:10.1007/s00701-005-0674-4

59 Hill CS, Coleman MP, Menon DK. Traumatic Axonal Injury: Mechanisms and Translational Opportunities. *Trends Neurosci* 2016;**39**:311–24. doi:10.1016/j.tins.2016.03.002

60 DiSabato D, Quan N, Godbout JP. Neuroinflammation: The Devil is in the Details. *J Neurochem* 2016;**139**:136–53. doi:10.1111/jnc.13607

61 Finnie JW. Neuroinflammation: beneficial and detrimental effects after traumatic brain injury. *Inflammopharmacology* 2013;**21**:309–20. doi:10.1007/s10787-012-0164-2

62 Chen X-H, Johnson VE, Uryu K, *et al.* A lack of amyloid beta plaques despite persistent accumulation of amyloid beta in axons of long-term survivors of traumatic brain injury. *Brain Pathol* 2009;**19**:214–23. doi:10.1111/j.1750-3639.2008.00176.x

63 Sharma R, Laskowitz DT. Biomarkers in Traumatic Brain Injury. *Curr Neurol Neurosci Rep* 2012;**12**:560–9. doi:10.1007/s11910-012-0301-8

64 Papa L, Silvestri S, Brophy GM, *et al.* GFAP out-performs S100β in detecting traumatic intracranial lesions on computed tomography in trauma patients with mild traumatic brain injury and those with extracranial lesions. *J Neurotrauma* 2014;**31**:1815–22. doi:10.1089/neu.2013.3245

65 Papa L, Brophy GM, Welch RD, *et al.* Time Course and Diagnostic Accuracy of Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 in a Large Cohort of Trauma Patients With and Without Mild Traumatic Brain Injury. *JAMA Neurol* 2016;**73**:551–60. doi:10.1001/jamaneurol.2016.0039

66 Papa L, Lewis LM, Falk JL, *et al.* Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. *Ann Emerg Med* 2012;**59**:471–83. doi:10.1016/j.annemergmed.2011.08.021

67 Hasselblatt M, Mooren FC, von Ahsen N, *et al.* Serum S100beta increases in marathon runners reflect extracranial release rather than glial damage. *Neurology* 2004;**62**:1634–6. doi:10.1212/01.wnl.0000123092.97047.b1

68 Meier TB, Nelson LD, Huber DL, *et al.* Prospective Assessment of Acute Blood Markers of Brain Injury in Sport-Related Concussion. *J Neurotrauma* 2017;**34**:3134–42. doi:10.1089/neu.2017.5046

69 Neselius S, Zetterberg H, Blennow K, *et al.* Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma. *Brain Inj* 2013;**27**:425–33. doi:10.3109/02699052.2012.750752

70 Oliver JM, Jones MT, Kirk KM, *et al.* Serum Neurofilament Light in American Football Athletes over the Course of a Season. *J Neurotrauma* 2016;**33**:1784–9. doi:10.1089/neu.2015.4295

71 Oliver JM, Anzalone AJ, Stone JD, *et al.* Fluctuations in blood biomarkers of head trauma in NCAA football athletes over the course of a season. *J Neurosurg* 2018;:1–8. doi:10.3171/2017.12.JNS172035

72 Taghdiri F, Multani N, Ozzoude M, *et al.* Neurofilament-light in former athletes: a potential biomarker of neurodegeneration and progression. *European Journal of Neurology* 2020;**27**:1170–7. doi:10.1111/ene.14251

73 Joseph JR, Swallow JS, Willsey K, *et al.* Elevated markers of brain injury as a result of clinically asymptomatic high-acceleration head impacts in high-school football athletes. *J Neurosurg* 2018;:1–7. doi:10.3171/2017.12.JNS172386

74 Otto M, Holthusen S, Bahn E, *et al.* Boxing and running lead to a rise in serum levels of S-100B protein. *Int J Sports Med* 2000;**21**:551–5. doi:10.1055/s-2000-8480

75 Dorminy M, Hoogeveen A, Tierney RT, *et al.* Effect of soccer heading ball speed on S100B, sideline concussion assessments and head impact kinematics. *Brain Inj* 2015;**29**:1158–64. doi:10.3109/02699052.2015.1035324

76 Zetterberg H, Jonsson M, Rasulzada A, *et al.* No neurochemical evidence for brain injury caused by heading in soccer. *Br J Sports Med* 2007;**41**:574–7. doi:10.1136/bjsm.2007.037143

77 Mussack T, Dvorak J, Graf-Baumann T, *et al.* Serum S-100B protein levels in young amateur soccer players after controlled heading and normal exercise. *Eur J Med Res* 2003;**8**:457–64.

78 Stålnacke B-M, Tegner Y, Sojka P. Playing soccer increases serum concentrations of the biochemical markers of brain damage S-100B and neuron-specific enolase in elite players: a pilot study. *Brain Inj* 2004;**18**:899–909. doi:10.1080/02699050410001671865

79 Kawata K, Rubin LH, Takahagi M, *et al.* Subconcussive Impact-Dependent Increase in Plasma S100β Levels in Collegiate Football Players. *J Neurotrauma* 2017;**34**:2254–60. doi:10.1089/neu.2016.4786

80 Michetti F, Bruschettini M, Frigiola A, *et al.* Saliva S100B in professional sportsmen: High levels at resting conditions and increased after vigorous physical activity. *Clin Biochem* 2011;**44**:245–7. doi:10.1016/j.clinbiochem.2010.10.007

81 Schulte S, Podlog LW, Hamson-Utley JJ, *et al.* A Systematic Review of the Biomarker S100B: Implications for Sport-Related Concussion Management. *J Athl Train* 2014;**49**:830–50. doi:10.4085/1062-6050-49.3.33

82 Hooper L, Thompson RL, Harrison RA, *et al.* Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *BMJ* 2006;**332**:752–60. doi:10.1136/bmj.38755.366331.2F

83 Swanson D, Block R, Mousa SA. Omega-3 Fatty Acids EPA and DHA: Health Benefits Throughout Life. *Adv Nutr* 2012;**3**:1–7. doi:10.3945/an.111.000893

84 Domenichiello AF, Kitson AP, Bazinet RP. Is docosahexaenoic acid synthesis from α-linolenic acid sufficient to supply the adult brain? *Progress in Lipid Research* 2015;**59**:54–66. doi:10.1016/j.plipres.2015.04.002

85 Calder PC. Mechanisms of Action of (n-3) Fatty Acids. *J Nutr* 2012;**142**:592S-599S. doi:10.3945/jn.111.155259

86 Harris WS, Miller M, Tighe AP, *et al.* Omega-3 fatty acids and coronary heart disease risk: Clinical and mechanistic perspectives. *Atherosclerosis* 2008;**197**:12–24. doi:10.1016/j.atherosclerosis.2007.11.008

87 Kris-etherton PM, Harris WS, Biol ATV, *et al.* Omega-3 fatty acids and cardiovascular disease. New recommendations from the American Heart Association. *Arterioscler Thromb Vasc Biol 2003*;:151–2.

88 Mozaffarian D, Wu JHY. Omega-3 Fatty Acids and Cardiovascular Disease: Effects on Risk Factors, Molecular Pathways, and Clinical Events. *Journal of the American College of Cardiology* 2011;**58**:2047–67. doi:10.1016/j.jacc.2011.06.063

89 Hardman WE. (n-3) Fatty Acids and Cancer Therapy. *J Nutr* 2004;**134**:3427S-3430S. doi:10.1093/jn/134.12.3427S

90 Swann PG, Venton DL, Le Breton GC. Eicosapentaenoic acid and docosahexaenoic acid are antagonists at the thromboxane A2/prostaglandin H2 receptor in human platelets. *FEBS Lett* 1989;**243**:244–6.

91 Shimokawa H, Lam JY, Chesebro JH, *et al.* Effects of dietary supplementation with cod-liver oil on endothelium-dependent responses in porcine coronary arteries. *Circulation* 1987;**76**:898–905.

92 Barrett EC, McBurney MI, Ciappio ED. ω-3 Fatty Acid Supplementation as a Potential Therapeutic Aid for the Recovery from Mild Traumatic Brain Injury/Concussion,. *Adv Nutr* 2014;**5**:268–77. doi:10.3945/an.113.005280

93 Lopez LB, Kritz-Silverstein D, Barrett Connor E. High dietary and plasma levels of the omega-3 fatty acid docosahexaenoic acid are associated with decreased dementia risk: the Rancho Bernardo study. *J Nutr Health Aging* 2011;**15**:25–31. doi:10.1007/s12603-011-0009-5

94 Gao Q, Niti M, Feng L, *et al.* Omega-3 polyunsaturated fatty acid supplements and cognitive decline: Singapore Longitudinal Aging Studies. *J Nutr Health Aging* 2011;**15**:32–5. doi:10.1007/s12603-011-0010-z

95 Tan ZS, Harris WS, Beiser AS, *et al.* Red blood cell ω-3 fatty acid levels and markers of accelerated brain aging. *Neurology* 2012;**78**:658–64. doi:10.1212/WNL.0b013e318249f6a9

96 Titova OE, Sjögren P, Brooks SJ, *et al.* Dietary intake of eicosapentaenoic and docosahexaenoic acids is linked to gray matter volume and cognitive function in elderly. *Age (Dordr)* 2013;**35**:1495–505. doi:10.1007/s11357-012-9453-3

97 Kalmijn S, van Boxtel MPJ, Ocké M, *et al.* Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. *Neurology* 2004;**62**:275–80. doi:10.1212/01.wnl.0000103860.75218.a5

98 Gu Y, Schupf N, Cosentino SA, *et al.* Nutrient intake and plasma β-amyloid. *Neurology* 2012;**78**:1832–40. doi:10.1212/WNL.0b013e318258f7c2

99 Mills JD, Hadley K, Bailes JE. Dietary supplementation with the omega-3 fatty acid docosahexaenoic acid in traumatic brain injury. *Neurosurgery* 2011;**68**:474–81; discussion 481. doi:10.1227/NEU.0b013e3181ff692b

100 Wu A, Ying Z, Gomez-Pinilla F. Omega-3 fatty acids supplementation restores mechanisms that maintain brain homeostasis in traumatic brain injury. *J Neurotrauma* 2007;**24**:1587–95. doi:10.1089/neu.2007.0313

101 Wu A, Ying Z, Gomez-Pinilla F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. *J Neurotrauma* 2004;**21**:1457–67. doi:10.1089/neu.2004.21.1457

102 Tao Wang, Ken C Van, Brian J Gavitt, *et al.* Effect of fish oil supplementation in a rat model of multiple mild traumatic brain injuries - PubMed. https://pubmed.ncbi.nlm.nih.gov/23835930/ (accessed 25 Aug 2020).

103 Wu A, Ying Z, Gomez-Pinilla F. Dietary strategy to repair plasma membrane after brain trauma: implications for plasticity and cognition. *Neurorehabil Neural Repair* 2014;**28**:75–84. doi:10.1177/1545968313498650

104 Bailes JE, Mills JD. Docosahexaenoic acid reduces traumatic axonal injury in a rodent head injury model. *J Neurotrauma* 2010;**27**:1617–24. doi:10.1089/neu.2009.1239

105 Mills JD, Bailes JE, Sedney CL, *et al.* Omega-3 fatty acid supplementation and reduction of traumatic axonal injury in a rodent head injury model. *J Neurosurg* 2011;**114**:77–84. doi:10.3171/2010.5.JNS08914

106 Wu A, Ying Z, Gomez-Pinilla F. Exercise facilitates the action of dietary DHA on functional recovery after brain trauma. *Neuroscience* 2013;**248**:655–63. doi:10.1016/j.neuroscience.2013.06.041

107 Wu A, Ying Z, Gomez-Pinilla F. The salutary effects of DHA dietary supplementation on cognition, neuroplasticity, and membrane homeostasis after brain trauma. *J Neurotrauma* 2011;**28**:2113–22. doi:10.1089/neu.2011.1872

108 Wechsler D. *Manual for the Wechsler Adult Intelligence Scale*. Oxford, England: : Psychological Corp. 1955.

109 Hermens HJ, Freriks B, Disselhorst-Klug C, *et al.* Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 2000;**10**:361–74. doi:10.1016/s1050-6411(00)00027-4

110 Wilson SA, Thickbroom GW, Mastaglia FL. Comparison of the magnetically mapped corticomotor representation of a muscle at rest and during low-level voluntary contraction. *Electroencephalogr Clin Neurophysiol* 1995;**97**:246–50. doi:10.1016/0013-4694(95)00052-z

111 Werhahn KJ, Kunesch E, Noachtar S, *et al.* Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol* 1999;**517**:591–7. doi:10.1111/j.1469-7793.1999.0591t.x

112 Wilson SA, Lockwood RJ, Thickbroom GW, *et al.* The muscle silent period following transcranial magnetic cortical stimulation. *J Neurol Sci* 1993;**114**:216–22. doi:10.1016/0022-510x(93)90301-e

113 Kim GH, Kang I, Jeong H, *et al.* Low Prefrontal GABA Levels Are Associated With Poor Cognitive Functions in Professional Boxers. *Frontiers in Human Neuroscience* 2019;**13**:193. doi:10.3389/fnhum.2019.00193

114 Tremblay S, Beaulé V, Proulx S, *et al.* Multimodal assessment of primary motor cortex integrity following sport concussion in asymptomatic athletes. *Clinical Neurophysiology* 2014;**125**:1371–9. doi:10.1016/j.clinph.2013.11.040

115 Stagg CJ, Bachtiar V, Johansen-Berg H. The Role of GABA in Human Motor Learning. *Current Biology* 2011;**21**:480–4. doi:10.1016/j.cub.2011.01.069

116 Lowe C, Rabbitt P. Test\re-test reliability of the CANTAB and ISPOCD neuropsychological batteries: theoretical and practical issues. *Neuropsychologia* 1998;**36**:915–23. doi:10.1016/S0028-3932(98)00036-0

117 Yurko-Mauro K, McCarthy D, Rom D, *et al.* Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. *Alzheimers Dement* 2010;**6**:456–64. doi:10.1016/j.jalz.2010.01.013

118 Johnson EJ, McDonald K, Caldarella SM, *et al.* Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. *Nutr Neurosci* 2008;**11**:75–83. doi:10.1179/147683008X301450

119 Kotani S, Sakaguchi E, Warashina S, *et al.* Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. *Neurosci Res* 2006;**56**:159–64. doi:10.1016/j.neures.2006.06.010

120 Lee LK, Shahar S, Chin A-V, *et al.* Docosahexaenoic acid-concentrated fish oil supplementation in subjects with mild cognitive impairment (MCI): a 12-month randomised, double-blind, placebo-controlled trial. *Psychopharmacology (Berl)* 2013;**225**:605–12. doi:10.1007/s00213-012-2848-0

121 Cacciamani F, Salvadori N, Eusebi P, *et al.* Evidence of practice effect in CANTAB spatial working memory test in a cohort of patients with mild cognitive impairment. *Appl Neuropsychol Adult* 2018;**25**:237–48. doi:10.1080/23279095.2017.1286346

122 Karlsen RH, Karr JE, Saksvik SB, *et al.* Examining 3-month test-retest reliability and reliable change using the Cambridge Neuropsychological Test Automated Battery. *Applied Neuropsychology: Adult* 2022;**29**:146–54. doi:10.1080/23279095.2020.1722126