UNDERSTANDING THE ROLE OF OMEGA-3 FATTY ACID SUPPLEMENTATION FOR HUMAN PERFORMANCE

By

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I dedicate this thesis to my late father. I miss you every day.

Declaration

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself, under the supervision of Dr Oliver Witard and Dr Stuart Galloway. Neither the thesis, nor the original work contained therein have been submitted to this or any other institution for a higher degree.

Jordan David Philpott

Stirling, 30/09/2019

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Abstract

Omega-3 polyunsaturated fatty acids (n-3PUFA) ingestion is associated with multiple health benefits, including improved brain and heart function. Research examining the influence of n-3PUFA supplementation on indices of athletic performance remains limited, with studies administering various dosing strategies in an attempt to improve recovery from muscle damaging exercise, revealing mixed findings. Currently, protein is the most common nutritional strategy to improve recovery from muscle damage however, studies have shown mixed results. As n-3PUFA supplementation is known to exhibit anti-inflammatory properties, combining n-3PUFA with protein in supplement form may promote recovery from muscle damaging exercise. This thesis demonstrates that coingesting n-3PUFA with protein serves to reduce muscle soreness and attenuate the rise in putative blood markers of muscle damage following an intense bout of exercise in soccer players. However, there were no improvements in soccer-skill performance observed with n-3PUFA and protein supplementation. The mechanism(s) underpinning the improvement in recovery with n-3PUFA supplementation is not currently known but may relate to the increase in n-3PUFA composition within skeletal muscle.

Dietary n-3PUFA ingestion has been shown to exhibit protective effects on lean tissue during periods of skeletal muscle catabolism such as leg immobilisation and in disease states. This thesis demonstrates that during short-term (2-wk) weight loss, increased dietary n-3PUFA intake fails to attenuate the decline in lean body mass during energy restriction. However, n-3PUFA supplementation improved muscle strength despite the reduction in muscle mass. The explanation for the improvement in muscle strength with n-3PUFA supplementation may be related to a modification of neuromuscular function.

This thesis also examined the biological role of the antioxidant, curcumin, to facilitate the incorporation of n-3PUFA into whole blood and the phospholipid membrane of skeletal muscle. However, we demonstrated that the addition of curcumin to an n-3PUFA-based supplement did not facilitate tissue incorporation of n-3PUFA into blood or skeletal muscle. The explanation for this null finding unclear, but may relate to the dose of curcumin and/or n-3PUFA administered in the supplementation regimen. Abstracts containing actual data are included within each experimental chapter.

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List of Publications

Jordan D. Philpott , Chris Donnelly , Ian H. Walshe , James Dick , Stuart D.R. Galloway , Kevin D. Tipton and Oliver C. Witard (2018) Adding fish oil to whey protein, leucine and carbohydrate over a 6 week supplementation period attenuates muscle soreness following eccentric exercise in competitive soccer players. *Int J Sport Nutr Exerc Metab.* 28(1): 26-36.

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List of Tables

List of Figures

Table of Contents

Chapter 1 - General Introduction

1.1 Introduction

Long chain n-3 polyunsaturated fatty acids (n-3PUFA) continue to receive considerable research attention as a potential ergogenic aid in the context of enhancing sport performance. Fish oil primarily consists of the n-3PUFA's eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), whilst another n-3PUFA, α-linolenic acid (ALA), is derived from plant oils such as flaxseed and soybean oil. ALA is an 18 carbon chain fatty acid with 3 double bonds (18:3), whereas EPA is a 20 carbon chain fatty acid with 5 double bonds (20:5) and DHA is a 22 carbon chain fatty acid with 6 double bonds (22:6). Fatty acids are comprised of a hydrocarbon chain with a methyl group and carboxyl group at opposing ends. Humans do not possess high activity of an enzyme called omega-3 desaturase that initiates the addition of another double bond to the 15th carbon chain. Therefore, n-3PUFA are classified as essential fatty acids because they must be provided in the diet to elevate EPA and DHA. The conversion to EPA and DHA from ALA occurs via several reaction steps. However, the complete conversion of ALA to DHA is less than 3% in males and 10% in females. This inefficient conversion rate is partly attributed to the amount of omega-6 PUFA in the diet since there is competition for the desaturase and elongase phase and the typical western diet contains a higher intake of omega-6 fatty acids than omega-3 fatty acids (Burdge, Jones, & Wootton, 2002; Burdge & Wootton, 2002).

The most common dietary source of n-3PUFA is oily fish. Mackerel contains approximately 3.2g of n-3PUFA per 100g serving and is considered the fish species most rich in n-3PUFA (Sprague, Dick, & Tocher, 2016) (**Table 1.1**). Other fish species that contain an abundance of n-3PUFA include salmon, sardines and tuna. Alternative fish sources of n-3PUFA include walnuts, chia seeds and egg yolks. Indeed, nuts and seeds are common sources of n-

3PUFA consumed by vegan athletes in order to meet daily n-3PUFA needs (Rogerson, 2017). Currently, the optimal dose of n-3PUFA for athletes or the general population is not definitely known. However, the World Health Organization indicate that individuals should aim to consume 1–2 servings of oily fish per week, equivalent to 200–500mg of n-3PUFA per day (World Health Organisation, 2003). In comparison, the recent (2003–2008) National Health and Nutrition Health Survey revealed an average EPA and DHA intake of ~200 mg/day (Papanikolaou, Brooks, Reider, & Fulgoni, 2014). Taken together, these data suggest that most individuals fail to meet daily n-3PUFA intake guidelines, however it is unclear whether athletes currently meet these guidelines.

It is estimated that 85% of elite athletes use at least one dietary supplement as a potential ergogenic aid (Maughan, Depiesse, & Geyer, 2007). Of these supplements, n-3PUFA is one of the most popular (Shaw, Slater, & Burke, 2016). Dietary n-3PUFA supplementation has been proposed to be advantageous for athletes mainly due to its anti-inflammatory properties (Li et al., 2005). Dietary n-3PUFA supplementation has been shown to inhibit the cyclooxygenase-2 (COX-2) pathway (Lim, Han, Dai, Shen, & Wu, 2009) which is known to stimulate inflammation. The incorporation of n-3PUFA's into cell membranes also alters cell membrane fluidity (Calder, Yaqoob, Harvey, Watts, & Newsholme, 1994), thus modifying protein activities and cell function (Murphy, 1990). Taken together, this mechanistic information suggests that n-3PUFA supplementation has the potential to play a role in improving training adaptation, exercise recovery, and subsequent performance across athlete populations, including strength-, endurance- and team-based sport athletes.

The potential health benefits of n-3PUFA's were originally based on findings from epidemiological studies. The dietary intake of Inuit's living in Greenland was rich in oily fish,

Table 1.1 – n-3PUFA content of various commonly consumed food sources

Food Type	g EPA + DHA per 100g serving	Typical Serving Size	Number of servings to equal 1 mackerel fillet
Mackerel	3.2	81g (1 fillet)	-
Sardines	1.9	130g (1 fillet)	1.1
Farmed Salmon	1.4	94g (1 fillet)	2
Wild Salmon	0.7	94g (1 fillet)	3.9
Canned Tuna	0.2	112g (1 can drained)	11.6
Cod Loin	0.2	140g (1 loin)	9.3
Tuna Steak	0.1	120g (1 steak)	21.6
Macroalgue Fed Lamb	0.05	125g	41.5
Chicken	0.02	145g (1 breast)	89.4
Lamb	0.01	125g	207.4
Pork	0.01	100g (1 loin)	259.2
Beef	0.01	125g	207.4

DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid. Adapted from Sprague, Dick & Tocher (2016)

with ~40% of their diet consisting of fat, and ~20% of PUFA (both n-3 and n-6) (Bang, Dyerberg & Sinclair, 1980). In comparison to a Danish diet, Inuits ingested higher amounts of n-3PUFA and reported a lower incidence rate of cardiovascular disease (CVD). The high fat content of the Inuit's diet and lower incidence of CVD was attributed to the high proportion of n-3PUFA in their diet. However, more recent communications have found that the observation study undertaken by Bang, Dyerberg & Sinclair (1980) was not a true representation of the Inuit health (Fodor et al., 2014). The observational study relied on death certificates and hospital admissions, however, more recent studies have found that only 1 in 7 deaths took place in a hospital(REF) and due to the remote location of Greenland, 20% of death certificates were completed without a doctor or an examination of the body (REF). These findings make it difficult to associate n increase in n-3PUFA association with lower CVD risk. Furthermore, a recent meta-analyses found no association between n-3PUFA supplementation and CVD risk (Aung et al.,). It should be noted that the majority of studies examining CVD have used about 1 g/day of n-3PUFA and limited research is known about the influence of n-3PUFA on CVD risk with a higher dosage of supplementation.

1.2 Search methodology

A systematic search strategy was employed to identify citations for this narrative review. We searched the National Library of Medicine database (PubMed), Google Scholar and Web of Science from their inception through to July 2018. The terms "fish oil/omega-3 supplementation "OR "fish oil/omega-3 athletes" OR "fish oil/omega-3 endurance capacity" OR "fish oil/omega-3 muscle protein synthesis" OR "fish oil/omega-3 resistance training" OR

"fish oil/omega-3 recovery" OR "fish oil/omega-3 muscle damage" OR "fish oil/omega-3 energy restriction" OR "fish oil/omega-3 immobilisation" OR "fish oil/omega-3 concussion"

OR "fish oil/omega-3 neuromuscular" OR "fish oil/omega-3 bleeding" OR "fish oil/omega-3 platelet aggregation" were entered into all databases and filters including "articles" were used to refine the search. After initial screening of title and abstracts, selected papers were examined, including the reference lists of the retrieved articles.

Both animal and human studies were included, however, studied human participants met the eligibility criteria if classified as healthy with no medical contraindications. Studies were excluded if they were case studies or they were descriptive studies whereby no control group was used.

1.3 Strength/Power-based Athletes

Previous research has investigated the influence of n-3PUFA supplementation on acute measurements of muscle protein synthesis (MPS) and chronic measurements of changes in muscle mass and neuromuscular function. This line of research is based on the idea that n-3PUFA ingestion sensitises skeletal muscle to the main anabolic stimuli, namely resistance exercise training and protein ingestion. The primary metabolic driver of muscle hypertrophy is an increased stimulation of MPS in response to exercise and nutrition (Biolo, Tipton, Klein, & Wolfe, 1997). Early proof-of-concept studies demonstrated that dietary n-3PUFA supplementation potentiated the response of MPS to amino acid provision, administered intravenously as a hyperaminoacidemic/hyperinsulinemic clamp (Smith et al., 2011a, 2011b) in young (Smith et al., 2011b) and older (Smith et al., 2011a) adults. Although no changes in basal rates of MPS were observed following 8 weeks of n-3PUFA

supplementation, postprandial rates of MPS and the phosphorylation status of anabolic signalling proteins within the mechanistic target of rapamycin complex (mTORC) pathway were potentiated after n-3PUFA supplementation. Consistent with this observation, research from our laboratory demonstrated an increase in skeletal muscle omega-3 lipid content and stimulation of focal adhesion kinase (FAK) —a key signalling protein that regulates MPS— following four weeks of 5g/ day n-3PUFA supplementation in active males (McGlory et al., 2014). The incorporation of n-3PUFA into a muscle cell membrane has been shown to alter the cell's integrity, disrupting the fluidity of proteins and lipids within the cell membrane (Calder et al., 1994). Such structural changes in membrane composition have been proposed to provide a mechanistic explanation for improvements in cell function with n-3PUFA ingestion (Murphy, 1990).

Our research suggests that a minimum supplementation period of 2 weeks is required to observe an increased incorporation of n-3PUFA into the muscle cell (McGlory et al., 2014). The incorporation of n-3PUFA into the muscle cell continues to increase after 4 weeks of supplementation, with no plateau observed. These data suggest that >4 weeks of n-3PUFA supplementation is required to maximise muscle incorporation of n-3PUFA. However, a systematic study is warranted to confirm this assertion. As a note of caution, it should be highlighted that n-3PUFA muscle cell content, rather than muscle cell membrane composition was measured in our study (McGlory et al., 2014). Therefore, it is assumed that the incorporation of n-3PUFA into the muscle cell also translates to incorporation in the membrane.

There is mechanistic evidence from in vitro studies using muscle cell lines that EPA, rather than DHA, is the primary anabolic component of n-3PUFA (Kamolrat & Gray, 2013). In

this study, the incubation of C2C12 myotubules with EPA resulted in increased rates of MPS and decreased rates of muscle protein breakdown (MPB). In contrast, incubation with DHA elicited no changes in MPS or MPB. Utilising a physiologically relevant research design, we recently investigated the influence of 8 weeks of n-3PUFA supplementation (5g/day) on the response of MPS to ingesting 30 g of whey protein with and without resistance exercise in resistance-trained young men (McGlory et al., 2016). In contrast to previous proof-of-concept studies (Smith et al., 2011a, 2011b), no differences in MPS and anabolic signalling were observed between n-3PUFA and placebo (coconut oil) conditions. Our previous research suggests that ~20g of whey protein stimulates a maximal response of MPS following leg-only resistance exercise (Witard et al., 2014). Thus, it is conceivable that the 30g dose of whey protein administered in McGlory et al. (2016) saturated the muscle protein synthetic machinery, meaning that n-3PUFA supplementation could then not provide an additional stimulus for MPS. Therefore, further research is needed to investigate whether the addition of n-3PUFA to a sub-optimal dose of protein would further stimulate MPS following resistance exercise compared to a protein dose alone.

While MPS is the gold standard acute marker of muscle growth, a handful of chronic intervention studies have directly measured changes in fractional synthetic rate or strength in response to a period of n-3PUFA supplementation. In a recent study, older adults underwent 6 months of either n-3PUFA (3.36 g/day EPA + DHA) or corn oil supplementation (Smith et al., 2015). Thigh muscle volume, handgrip strength and 1-RM strength all increased in the n-3PUFA group, whereas no changes were detected in placebo. However, there were no differences in body mass or body fat between the two conditions. Interestingly, only the thigh was measured for muscle volume. Given this increase in thigh muscle volume it was

assumed that muscle mass was increased at the whole body level. More recently, 12 wks of resistance training improved grip strength in older adults regardless of whether the participants supplemented on n-3PUFA or not (Lee, Jo & Khamoui, 2019). Interestingly, no studies have measured the response of muscle growth or strength in response to n-3PUFA supplementation in young adults or athletic populations. Further studies should be designed to examine the responsiveness of muscle strength and volume to a chronic (12 wk- 6 mo) period of n-3PUFA supplementation in power/strength-based athletes.

The first study to measure muscle strength following a period of n-3PUFA supplementation observed an increase in peak torque with 90 or 150 days of n-3PUFA supplementation at a dose of 2g/day (Rodacki et al., 2012). Training-induced improvements in neuromuscular function, such as muscle activation and electromechanical delay, the time delay between the onset of muscle activation and muscle force production, in various muscles including the bicep femoris and vastus lateralis, were enhanced with n-3PUFA vs the training only group (Rodacki et al., 2012). Since DHA is abundant within brain neurons (Kim, Huang, & Spector, 2014), improvements in neuronal adaptation with n-3PUFA supplementation may indicate that neural pathways are modified. However, given that participants in this study were older females, caution should be applied when interpreting these results for athletes. Taken together, these data support a potential anabolic role for n-3PUFA ingestion in the context of preserving muscle mass in older adult populations. However, based on current information, there is limited information available to support an anabolic role of n-3PUFA for muscle growth in athletes.

1.4 Endurance-based athletes

An important aspect of endurance exercise performance and training adaptation is the capacity to utilize substrates efficiently and maximise available energy from adenosine triphosphate (ATP) stores. The mitochondrial content of the cell aids regulation of ATP resynthesis. A key regulator of mitochondrial biogenesis, defined as the process of increasing mitochondrial volume, is Peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α). Previous studies in rodents have shown dietary n-3PUFA supplementation to increase expression of PGC-1α (Hancock et al., 2008) and increase mitochondrial biogenesis (Turner et al., 2007). However, studies investigating n-3PUFA supplementation and mitochondrial biogenesis in humans are limited. Currently, only one study has examined mitochondrial biogenesis with n-3PUFA supplementation and reported that EPA supplementation stimulated mitochondrial biogenesis in obese individuals (Laiglesia et al., 2016). Based on findings from animal studies, it is possible that n-3PUFA supplementation may increase mitochondrial biogenesis leading to improved endurance performance as mediated via the PGC-1α pathway. However, human studies in athletes are warranted in order to examine this theory.

Dietary intake of n-3PUFA also are known to alter membrane fatty acid composition in skeletal (Andersson, Nälsén, Tengblad, & Vessby, 2002) and myocardial (Charnock, McLennan, & Abeywardena, 1992) muscle tissue. These changes in membrane composition can lead to changes in insulin sensitivity (Borkman et al., 1993) via a yet to be determined mechanism. However, a pre-clinical rodent study demonstrated that the addition of n-3PUFA to a high fat diet increased the protein expression of Glucose transporter type-4 (GLUT4; Lanza et al., 2013). GLUT4 is present only in skeletal muscle and adipose tissue and plays a key role in transporting extracellular glucose into cells that are insulin sensitive (Huang &

Czech, 2007). In humans, n-3PUFA supplementation also has been shown to improve insulin sensitivity in skeletal muscle (Borkman et al., 1989). Thus, in theory, an increase in GLUT4 expression with n-3PUFA supplementation may play a key role in improving tissue insulin sensitivity and thus endurance performance.

Dietary n-3PUFA supplementation also has been shown to reduce oxygen consumption (Kawabata et al., 2014; Peoples, McLennan, Howe, & Groeller, 2008), heart rate (Peoples et al., 2008) and perceived exertion (Kawabata et al., 2014) during endurance exercise. The mechanism that underpins the improved oxygen efficiency with n-3PUFA supplementation is unclear, and paradoxically n-3PUFA supplementation has been shown to initiate an increase in resting metabolic rate (Logan & Spriet, 2015). Although speculative, the increase in resting metabolic rate with n-3PUFA ingestion may primarily be due to the increased incorporation of DHA into the cell membrane that has been shown to lead to an increase in Ca2+ ATPase and Na+/K+ ATPase activity that requires more ATP utilization (Hulbert, Turner, Storlien, & Else, 2005). A potential mechanism that may underpin an alteration in the oxygen cost of exercise is through an increase in insulin sensitivity. Intuitively, an increase in insulin sensitivity leads to greater muscle glycogen resynthesis and the subsequent potential to increase carbohydrate oxidation rates and decrease fat oxidation rates (Watt, Heigenhauser, Dyck, & Spriet, 2002). During endurance exercise, a shift in substrate utilization from fat to carbohydrate would reduce the volume of oxygen used to meet demands for ATP resynthesis, and in turn improve the calculated exercise efficiency (Cole, Coleman, Hopker, & Wiles, 2014).

At present, a limited number of studies have examined the influence of n-3PUFA ingestion on markers of energy metabolism and performance in endurance-trained

individuals (**Table 1.2**). In trained cyclists with low habitual n-3PUFA intake, eight weeks of high or low dose DHA-rich n-3PUFA supplementation resulted in a reduced oxygen cost during a cycling time trial compared to a soy bean placebo condition (Hingley, Macartney, Brown, McLennan, & Peoples, 2017). However, the observed increase in omega-3 index and reduction in oxygen cost did not translate into a performance advantage, with no improvements in time trial completion time, mean power during the time trial and quadriceps strength. Further research in endurance athletes is warranted to examine the impact of n-3PUFA supplementation on oxygen kinetics during exercise when oxygen availability is limited, e.g. competition and training at high altitude.

Previous research also has shown that n-3PUFA supplementation has the potential to lower heart rate and blood pressure during exercise. In elite Australian Rules footballers, 5 weeks of DHA rich n-3PUFA (1.56 g/day DHA and 0.36g/day EPA) supplementation significantly lowered heart rate during steady state submaximal exercise, however peak heart rate did not change compared to a sunflower oil placebo condition (Buckley, Burgess, Murphy, & Howe, 2009). Interestingly, diastolic blood pressure increased after 5 weeks of sunflower oil supplementation but did not change in the n-3PUFA group. Previous research demonstrates that DHA rather than EPA is the active lipid component of n-3PUFA's in reducing blood pressure and heart rate in humans (Mori, Bao, Burke, Puddey, & Beilin, 1999). As a logical follow study, healthy males demonstrated a reduction in heart rate during a bout of steady state cycling with DHA rich n-3PUFA supplementation (Macartney, Hingley, Brown, Peoples, & McLennan, 2014). However, during repeated sprints there were no differences in heart rate between conditions. Taken together, these data suggest that whereas the provision of DHA rich n-3PUFAs results in a decreased heart rate response

Table 1.2 – Summary of studies investigating the influence of n-3 PUFA supplement on endurance capacity and performance

n-3PUFA = omega-3 polyunsaturated fatty acid; DHA = Docosahexanoic acid; EPA = Eicosapentaenoic acid; PLA = Placebo; FO = Fish oil; OO = Olive oil; SO = Sunflower Oil; SBO = Soy Bean Oil.

Authors	Participants	Supplement Dose	Supplementation Period	Exercise	Observations
Bortolotti *et al.* (2007)	Sedentary males (n=8)	7.2g/d FO vs. PLA	14 days prior to exercise	30 min cycling (50% VO_{2max})	→ energy efficiency → VO_{2max}
Buckley *et al.* (2009)	Elite Australian Rules League Footballers (n=25)	1.92g/d n-3PUFA vs. SO	5 weeks prior to exercise	Steady-state submaximal running	↓ heart rate ↓ blood pressure → performance → recovery
Hingley *et al.* (2017)	Trained cyclists + runners (n=26)	700mg/d n-3PUFA vs. SBO	8 weeks prior to exercise	Cycling sprints 5 min time trial	↓ oxygen cost → performance
Kawabata *et al.* (2014)	Recreational team-sport males (n =20)	3.6g/day FO vs. PLA	8 weeks prior to exercise	VO_{2max} test and steady state cycling tests	→ VO_{2max} ↓ Oxygen consumption ↓ Rate of perceived exertion
Macartney et al (2014)	Healthy males (n=39)	700mg/d n-3PUFA vs. SBO	8 weeks prior to exercise	Maximal cycling sprints and 5 min time trial	↓ Submaximal and recovery heart rate → Peak heart rate
Ninio *et al* (2008)	Sedentary overweight adults (n=75)	1.92g/day n-3PUFA vs. SO	12 weeks prior to exercise	Graded submaximal test	↓ Resting and submaximal heart rate ↑ heart rate variability
Oostenbrug *et al* (1997)	Trained cyclists (n=24)	6g/d FO + Vitamin E vs. 6 g/g FO vs. PLA	3 weeks prior to exercise	Wmax and endurance cycling tests	→ time to exhaustion → VO_{2max} → maximal power
Peoples *et al* (2008)	Trained cyclists (n=20)	3.2 g/d n-3PUFA vs. OO	8 weeks prior to exercise	Submaximal exercise tests (55% of peak workload)	↓ Peak and submaximal heart rate ↓ Oxygen consumption
Rontoyanni *et al* (2012)	Healthy males (n=22)	4.7 g/d DHA vs. 4.7 g/d EPA vs. SO	Single dose	12 min multi-stage test	→ cardiac output ↓ Systemic vascular resistance
Zebrowska *et al* (2015)	Trained cyclists (n=13)	1.3g/d n-3PUFA vs. PLA	3 weeks prior to exercise	VO_{2max} test	↑ VO_{2max} ↑ endothelial function

during submaximal exercise, at higher exercise intensities n-3PUFA supplementation has no impact on the heart rate response. The mechanism responsible for the modulation of heart rate by n-3PUFA supplementation is thought to involve multiple physiological processes including the regulation of systolic and diastolic left ventricular function, sympathetic activity and vagal tone (Mozaffarian, Gottdiener, & Siscovick, 2006; O'Keefe, Abuissa, Sastre, Steinhaus, & Harris, 2006). For instance, n-3PUFA supplementation is known to increase stroke volume which results in a decrease in heart rate. However, further research is needed in order to fully understand the mechanisms by which n-3PUFA supplementation lowers heart rate.

Although not universally accepted, endurance athletes are often considered more susceptible to developing an upper respiratory tract infection (URTI) that can disrupt training and competitive performance (Peters & Bateman, 1983). Dietary n-3PUFA supplementation has been shown to upregulate the signalling network between cells involved in immune function, resulting in the stimulation of CD4 and CD8 lymphocyte production, thus improving the ability of immune cells to destroy foreign pathogens (de

Lourdes Nahhas Rodacki et al., 2015). In this regard, a recent study examined the influence of adding n-3PUFA to other nutrients (1.1 g/day of n-3PUFA, 10 µg/day Vitamin D and 8 g/day of whey protein isolate) vs. a carbohydrate placebo control on markers of immune function in young active males and females that continued their habitual training over a 16 week period (Da Boit, Gabriel, Gray, & Gray, 2015). Although no differences in markers of immune function were observed between groups, the frequency and duration of URTI symptoms was reduced in the n-3PUFA group. However, it should be noted that diagnosis of URTIs was self reported and not clinically diagnosed by a doctor. Moreover, based on these findings alone,

it is impossible to differentiate between the effects of n-3PUFA, vitamin D, whey protein or the combination of all the ingredients in observed reduction in symptoms days.

1.5 Team-based athletes

The initial 96 hours following exercise is commonly defined as the acute exercise recovery period (Pereira Panza, Diefenthaeler, & Da Silva, 2015). This period is considered crucial in optimising athlete performance, particularly during situations such as fixture congestion for team sport athletes. Repeated eccentric-based muscle contractions are known to cause damage to skeletal muscle fibres (Nédélec et al., 2012). Muscle damaging exercise has been shown to subsequently impair sport-specific performance (Eston, Finney, Baker, & Baltzopoulos, 1996). There is biological rationale behind the notion that n-3PUFA has the potential to promote recovery from muscle damaging exercise. By increasing the structural integrity of the muscle cell membrane. Alternatively, n-3PUFA have the potential to accelerate the recovery process. In this regard, dietary n-3PUFA exhibit anti-inflammatory properties via several pathways. These pathways include inhibition of the COX-2 pathway (Li et al., 2005), the synthesis of lipoxins and resolvins that both exhibit anti-inflammatory functions (Janakiram, Mohammed, & Rao, 2011) and also by reducing chemotaxis of neutrophils and reduce generation of leukotrienes, a family of inflammatory mediators produced by leukocytes (Lee et al., 1985). Therefore, it is intuitive that n-3PUFA supplementation could improve recovery following muscle damaging exercise either by preserving muscle membrane integrity or reducing other inflammatory agents.

A series of experimental studies have examined the influence of n-3PUFA ingestion on recovery from muscle damaging exercise and have revealed mixed results (Gray, Chappell, Jenkinson, Thies, & Gray, 2014; Tsuchiya, Yanagimoto, Nakazato, Hayamizu, & Ochi, 2016)

(**Table 1.3**). A recent study examined the impact of acute supplementation with a high (15:1 ratio of EPA to DHA) or low (1.5:1 ratio of EPA to DHA) dose of n-3PUFA on exercise recovery (Jakeman, Lambrick, Wooley, Babraj, & Faulkner, 2017). The authors reported that the group consuming the high dose of n-3PUFA observed an attenuated decrement in jump height from a starting squat position. However, no differences in markers of muscle soreness and putative blood markers of muscle damage (e.g. creatine kinase (CK)) and inflammation (interleukin-6) were observed between conditions. These data suggest that the high ratio of EPA to DHA may be the key factor in helping to maintain performance following acute supplementation and muscle damaging exercise. However, it is difficult to interpret these data given that at least 2 weeks is required for incorporation of omega-3 into muscle tissue (McGlory et al., 2014) . Therefore, any physiological effect of n-3PUFA in this study are presumed to have been systemic (Jakeman et al., 2017).

Another recent study measured the impact of medium term (21 days prior to muscle damage) n-3PUFA supplementation on indices of recovery following muscle damaging exercise in females (McKinley-Barnard, Andre, Gann, Hwang, & Willoughby, 2018). Participants consumed either 4.2 g/day of n-3PUFA or a placebo supplement consisting of safflower oil for 21 days before undergoing a bout of intense eccentric exercise.

Table 1.3 – Effects of supplementing with n-3 PUFA on indices of recovery following exercise induced muscle damage

DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid; PLA = Placebo; n-3PUFA = n-3PUFA; OO = Olive oil; FO = Fish oil; CHO = Carbohydrate; PRO = Protein; CO = Corn Oil.

Authors	Participants	Supplement Dose	Supplementation Period	Muscle Function	Muscle Soreness	Muscle Damage Markers (CK, Mb etc.)
Corder *et al.* (2016)	Healthy females (n=27)	3g/d DHA vs. PLA	7 days prior and 2 days after exercise		DHA < PLA	DHA = PLA
DiLorenzo *et al.* (2014)	Untrained males (n=41)	2g/d DHA vs. PLA	28 days prior to exercise	DHA = PLA	DHA = PLA	DHA < PLA
Gray *et al.* (2014)	Males (n=20)	3g/d FO vs. 3g/d OO	6 weeks prior to exercise	FO = OO	FO = OO	FO < OO
Jakeman *et al.* (2017)	Healthy active males (n=27)	800mg/10kg/BM EPA vs. 250mg/10kg/BM EPA vs. PLA	Single dose	High EPA < Low EPA, PLA	High EPA = Low EPA, PLA	High EPA = Low EPA, PLA
Jouris *et al.* (2011)	Healthy males (n =11)	2g/day EPA + 2g/day DHA vs. PLA	7 days prior to exercise		FO < PLA	
Lembke et al (2014)	Healthy males and females (n=63)	2.7g/day FO vs. PLA	30 days prior to exercise			FO < PLA
McKinley-Barnard et al (2018)	Healthy active females (n=22)	4.2 g/day n-3PUFA vs. PLA	21 days prior to exercise		FO = PLA	FO = PLA
Philpott et al (2018)	Male soccer players (n=30)	2.2 g/day FO + PRO + CHO vs. PRO + CHO vs. CHO	42 days prior to exercise and 2 days following exercise	FO = PRO, CHO	FO < PRO, CHO	FO = PRO, CHO
Tartibian *et al* (2011)	Untrained males (n=45)	1.8g/day FO vs. PLA	30 days prior and 2 days following exercise.			FO < PLA
Tsuchiya *et al* (2016)	Healthy males (n=24)	2.4g/day n-3PUFA vs. CO	56 days prior and 5 days following exercise.	n-3PUFA > CO	n-3PUFA < CO	n-3PUFA < CO
Tsuchiya *et al* (2019)	Healthy male (n=16)	860 mg/day n-3PUFA vs. CO	8 weeks prior to exercise and 5 days following exercise	n-3PUFA > CO	n-3PUFA < CO	

Supplementation of n-3PUFA failed to attenuate muscle soreness and inflammation measured 24 hours following exercise compared to the placebo condition. Unfortunately, this study did not collect measurements of exercise recovery 48, 72 or 96 hours post-exercise and therefore may have missed important information regarding the effectiveness of n-3PUFA ingestion in promoting acute exercise recovery.

Whilst this body of work (**Table 1.3**) provides proof-of-concept for the potential role of n-3PUFA ingestion in accelerating recovery from muscle damaging exercise, the direct application of these results to team-based athletes should be considered with caution for several reasons. First, these studies are typically performed in untrained participants in which a high degree of muscle damage is likely after unaccustomed exercise. Hence, it may be argued that the application of results is more appropriate in the context of improving compliance of previously sedentary population to a new exercise routine, rather than the elite team-sport athlete with the goal of complete recovery prior to the next match. Second, the ecological validity of the muscle damage protocol used in this study is not directly relevant to sporting movements. Finally, the sensitivity and specificity of endpoint measurements, such as squat jump performance or force on a isokinetic dynamometer, to team sport athletes is weak.

To address these limitations, we recently recruited competitive soccer players to ingest either a combined n-3PUFA (2.8 g/day), whey protein (30 g/day) and carbohydrate (20 g/day) supplement beverage, a whey protein (30 g/day) and carbohydrate (20 g/day) supplement beverage or a carbohydrate (24 g/day) only beverage over a 6 week period prior to performing an intense exercise bout (Philpott et al., 2018, **Chapter 2**). In the 72 hours following the muscle damaging exercise, the soccer players in the n-3PUFA plus protein group

reported reduced levels of muscle soreness. The n-3PUFA group also experienced a reduction in plasma creatine kinase concentrations as a putative blood marker of muscle damage, compared to the whey protein beverage only, or the carbohydrate placebo beverage. As such, these data imply that n-3PUFA supplementation protected the muscle cell from the muscle damage protocol and therefore soccer players experienced less damage during exercise. However, there was no influence of n-3PUFA ingestion on soccer performance tests such as the yoyo intermittent recovery test, a high intensity aerobic field test to measure soccer players aerobic ability, or the Loughborough soccer passing test, a testing protocol aimed to measure soccer players passing ability, which arguably offer greater application for recovery in the team sport athlete. To better understand the impact of n-3PUFA on recovery this study needs to be replicated in a real life football situation, using a simulated soccer match.

Our recent research also has observed that four weeks of n-3PUFA supplementation in soccer players resulted in improved anaerobic endurance running capacity while maintaining their habitual training schedule (Gravina et al., 2017). Over 4 weeks of training, soccer players experienced an increase of 203m in the Yo-Yo level 1 test following ingestion of 0.1 g/kg/day of n-3PUFA, compared to only a 62 m improvement in the placebo group. However, adaptations in power, speed and maximal knee extensor strength were not influenced by the omega-3 supplementation. Therefore, it is possible that n-3PUFA supplementation may improve high intensity running capacity in soccer players, but further research is needed to investigate different athlete populations.

1.6 Special considerations

1.6.1 Energy restriction

Athletes competing in weight category sports often undergo periods of energy restriction. Periods of sustained energy restriction are often accompanied by the loss of muscle mass (Mettler, Mitchell, & Tipton, 2010; Weinheimer, Sands, & Campbell, 2010) due, primarily, to a reduction in basal rates of MPS (Pasiakos et al., 2010) rather than an increase in MPB (Longland, Oikawa, Mitchell, Devries, & Phillips, 2016). Within clinical studies (e.g. cancer patients), n-3PUFA supplementation has been shown to attenuate the loss of muscle mass (Murphy et al., 2011). Within an athletic setting, a recent study from our laboratory examined the influence of n-3PUFA supplementation during 2 weeks of calorie restriction on lean and fat mass loss in resistance-trained athletes (Philpott et al., 2019, **Chapter 3**). Athletes underwent 2 weeks of 40% calorie restriction with the nutritional composition of 50% carbohydrate, 35% fat and 15% protein. Half of the participants (n = 10) supplemented with an n-3PUFA beverage on a twice daily basis, and the other participants supplemented with a carbohydrate placebo beverage while continuing with their habitual training programme. Following the 2 weeks of supplementation, participants lost similar amounts of body mass, muscle mass and fat mass independent of which supplement beverage was consumed. While these initial data do not support the use of n-3PUFA ingestion during periods of calorie restriction, future studies should examine the effects of n-3PUFA supplementation on the attenuation of muscle mass over a longer period of energy restriction in athletes.

1.6.2 Immobilisation

Serious injury in athletes can result in limb immobilisation. The muscle atrophy associated with periods of immobilisation is due, at least in part, to an attenuated response of MPS to ingested protein (Wall & van Loon, 2013); a concept known as anabolic resistance. Pre-clinical studies have used a rodent model to investigate the influence of n-3PUFA supplementation on muscle mass. This work demonstrated that rats consuming a diet consisting of 2% corn oil and 5% cod liver oil retained myosin heavy chain content and inhibited the COX-2 pathway as an inflammatory marker following a 10 day period hind-limb immobilisation compared to rats consuming a diet consisting of 7% corn oil alone (You, Park, Song, & Lee, 2010a). However, when the hind limbs of the rats were remobilized for 13 days following the 10 day hind-limb immobilization (You, Park, & Lee, 2010b) the fish oil group experienced an impaired recovery of myosin heavy chain content compared to the corn oil group. Moreover, following remobilisation the phosphorylation status of mTORC-associated anabolic signalling proteins were increased with corn oil compared to n-3PUFA during the early stages of remobilisation (3 days). Taken together, these data indicate that n-3PUFA ingestion is effective in retaining muscle mass during the immobilisation period. However, n-3PUFA may not influence, and even possibly inhibits, the recovery process during the remobilisation phase (You et al., 2010b). Recently, a follow up study examined body composition and the muscle protein synthesis response immediately post a 2 wk leg immobilisation and after a 2 wk recovery period in healthy young women whilst supplementing with n-3PUFA (McGlory et al., 2019). Dietary n-3PUFA supplementation was shown to attenuate the loss of muscle mass following the leg immobilisation phase. Integrated rates of muscle protein synthesis were stimulated with n-3PUFA supplementation. These results should be interpreted with caution as the study recruited healthy young

women, further research should examine the influence of n-3PUFA supplementation during periods of immobilisation or injury in male participants.

1.6.3 Concussion

The diagnosis and treatment of concussion is currently a hot topic in Sport Nutrition. DHA is abundant in the plasma membranes of the brain which is involved in neuronal signalling (Fontani et al., 2005). Early research examining the effects of n-3PUFA supplementation on recovery from concussion has been conducted primarily in rat models. One of the earliest studies elicited mild traumatic brain injury to rats before conducting the Morris Water Maze test to assess performance on consecutive days 10–14 after the traumatic brain injury (TBI) (Wang et al., 2013). Rats either consumed a diet consisting of 6% n-3PUFA or a diet of 6% soybean oil before and during the recovery phase of brain injury. Rats that consumed the n-3PUFA diet managed to complete the maze faster than the placebo group during TBI recovery. Due to ethical reasons, studying recovery from concussion in humans is challenging. However, a recent study did examine the effect of n-3PUFA supplementation over a full season in American football players. These data revealed n-3PUFA ingestion (2, 4 or 6 g/day) decreased concentrations of serum neurofilament light, a biomarker of head trauma (Oliver et al., 2016). However, more research is required to determine the effectiveness of n-3PUFA in the treatment of TBI and concussion in contact sport athletes.

1.6.4 Bleeding

EPA is known to replace arachidonic acid in the phospholipid layer of platelet cell membranes following n-3PUFA ingestion (Lorenz, Spengler, Fischer, Duhm, & Weber, 1983). As a consequence, platelet aggregation may be reduced due to a reduction in levels of thromboxane A within the plasma. Platelets mediate the wound healing process via blood clotting. Thus, in theory a decrease in platelet aggregation may increase bleeding time. Consistent with this notion, a recent study also suggests that n-3PUFA supplementation may reduce platelet aggregation in healthy individuals and therefore increase bleeding time following surgery or lacerations (McEwen, Morel-Kopp, Chen, Tofler, & Ward, 2013). However, a recent systematic review found no difference in bleeding risk with n-3PUFA supplementation in different populations, including athletes (Begtrup, Krag, & Hvas, 2017). However, the interpretation of this systematic review may be influenced by variations in the dose and duration of n-3PUFA supplementation between studies. Overall, although n-3PUFA supplementation may reduce platelet aggregation there appears to be no effect on bleeding rates following surgery. Therefore, unless athletes are ingesting a high dose of n-3PUFA, concerns over bruising and bleeding following an injury during sport appear unfounded.

1.7 Current issues in omega-3 and sport performance research

As with all nutritional supplements, more research is needed to examine the effects of n-3PUFA on athletic performance. Current issues regarding n-3PUFA supplementation on sport performance are four-fold. First, the duration of time before which n-3PUFA concentrations in muscle and blood return to baseline after cessation of supplementation is currently unknown. This information would be useful in the design of crossover studies that investigate the impact of n-3PUFA ingestion on a chosen marker of sport performance.

Second, significant variation exists with regards to the dose and duration of n-3PUFA supplementation employed between studies. As discussed previously, at least two weeks of n-3PUFA supplementation is sufficient to detect an increase in n-3PUFA content within the muscle lipid pool, but it is not yet known how many weeks is required to maximise this response. Moreover, the optimum dose of n-3PUFA to reduce inflammation, maximise the incorporation of EPA and DHA into the muscle membrane and improve various aspects of sport performance remains unknown.

Third, research examining the effects of fish oil derived n-3PUFA on athletic performance have utilised a range of placebos, including safflower oil, corn oil, olive oil and coconut oil. Corn oil and safflower oil both contain high amounts of omega-6, so when used as a placebo these oils actually alter the omega 3 to omega 6 ratio. To date, the most appropriate placebo appears to be coconut oil which does not contain any omega- 6 or omega-3. However, there is evidence that the short chain saturated fats in coconut oil may have an impact on metabolism (Eyres, Chisholm, & Brown, 2016). Therefore, the most appropriate fish oil placebo for all research testing has yet to be established.

Finally, the ratio of EPA to DHA present within the n-3PUFA supplement should be considered when interpreting the findings from research into the applications of n-3PUFA ingestion for sport performance. Current research has used multiple different ratios of EPA to DHA whether that is 1:1, EPA-rich or DHA-rich supplementation. Given that EPA and DHA exhibit their own active properties and act independently, caution should be applied when interpreting n-3PUFA supplementation research with different ratios of EPA to DHA.

1.8 Practical applications and conclusion

The applications of n-3PUFA supplementation for sport performance are relevant to athletes from strength-, endurance- and team-based sports, with recommendations tailored to the specific performance goals of the athlete (**Table 1.4**). Based on currently available scientific evidence, there is potential for n-3PUFA supplementation to improve muscle adaptation, energy metabolism, muscle recovery and injury prevention. As such, n-3PUFA supplementation for athletes may yet prove to be effective. At the very least the evidence suggests that increasing n-3PUFA in the diet or via a supplement will not be detrimental, except potentially following immobilisation. However, more research is needed to further investigate these promising applications of n-3PUFA supplementation, particularly on skeletal muscle mass retention, growth, and adaptation, as well as in recovery from concussion in athletic populations.

Table 1.4 - Practical applications of omega-3 polyunsaturated fatty acid supplementation for athletic performance

1.9 Aims of the thesis

Topic	Practical Application
Strength/power-based Athletes	• Supplementation of n-3PUFA stimulates signalling proteins involved in muscle protein synthesis. • However, it is believed that getting the optimal amount of protein negates the effect of n-3 PUFA supplementation on muscle protein synthesis.
Endurance-based Athletes	• Dietary n-3 PUFA appears to reduce oxygen cost and increase oxygen efficiency during endurance exercise. However, whether this transfers to an improvement in performance is unclear. • At this point in time, n-3PUFA supplementation does not appear to improve cases of URTI in endurance athletes. • Important to note that a lot of studies investigating n-3PUFA supplementation on endurance is in rodent models.
Team-based Athletes	• Supplementation with n-3PUFA following muscle damage with or without other nutrients appears to not have a negative effect on indices of recovery. However, whether n-3PUFA supplementation improves subsequent performance following muscle damage is unclear. • It is not yet clear whether n-3PUFA has any effects of muscle adaptation for team-based athletes.
Energy Restriction	• At present, n-3PUFA supplementation does not appear to have any effects during periods of energy restriction on the preservation of lean mass.
Immobilisation	• Limited studies suggest that n-3PUFA supplementation may possibly offset the effects of immobilisation. • However it should be noted that the only studies examining n-3PUFA supplementation during periods of immobilisation are in rodent models.
Concussion	• There is potential for n-3PUFA supplementation to have protective effects on concussion however little research has examined this mainly due to ethical reasons.
Bleeding	• Dietary n-3PUFA supplementation does not seem to influence bleeding rates following cuts or surgery.

This thesis describes a series of studies which investigated the influence of n-3PUFA supplementation on human sporting performance. **Chapter 2** describes a study in which we examined the influence of adding n-3PUFA to a whey protein and carbohydrate supplement beverage on performance recovery from a bout of muscle damage. We measured not only muscle performance measures but also, soccer-specific performance, blood makers of muscle damage and inflammation in an attempt to gain an insight into what extent the incorporation of n-3PUFA into muscle tissue influences the muscle during periods of muscle damage. **Chapter 3** examines the impact of n-3PUFA supplementation during a period of short-term weight loss in resistance trained athletes. We measured both body composition and performance of the athletes before and after the weight loss period in order to determine whether n-3PUFA supplementation played a protective role on muscle cells. **Chapter 4** describes a study in which we add anti-oxidants to an n-3PUFA supplement in an attempt to increase the incorporation rate of n-3PUFA into both whole blood cells and phospholipid membrane of skeletal muscle cells. We also measured muscle and neuromuscular function in order to gain a better understanding into the influence n-3PUFA plays on muscle strength enhancements. The practical implications of the performed research are discussed and subsequent aims for future research are provided.

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Chapter 2 - Adding fish oil to whey protein, leucine and carbohydrate over a 6 week supplementation

period attenuates muscle soreness following eccentric exercise in soccer players

2.1 Abstract

Soccer players often experience eccentric exercise-induced muscle damage given the physical demands of soccer match-play. Since n-3 polyunsaturated fatty acids (n-3PUFA) enhance muscle sensitivity to protein supplementation, dietary supplementation with a combination of fish oil-derived n-3PUFA, protein and carbohydrate may promote exercise

recovery. This study examined the influence of adding n-3PUFA to a whey protein, leucine and carbohydrate containing beverage over a 6 week supplementation period on physiological markers of recovery measured over 3 days following eccentric exercise. Soccer players were assigned to one of three conditions (2 × 200mL): Fish oil (FO) contained n-3PUFA (1100 mg DHA/EPA – approx. 550mg DHA, 550mg EPA), whey protein (15g), leucine (1.8g) and carbohydrate (20g); PRO contained whey protein (15g), leucine (1.8g) and carbohydrate (20g) and CHO contained carbohydrate (24g). Eccentric exercise consisted of unilateral knee extension/flexion contractions on both legs separately. Eccentric exercise impaired maximal force production during the 72 hour recovery period (p<0.05). Muscle soreness, expressed as AUC during 72 hour recovery, was less in FO (1948±1091 mm×72 h) than PRO (4640±2654 mm×72 h, p<0.05) and CHO (4495±1853 mm×72 h p=0.10). Blood concentrations of creatine kinase, expressed as AUC, were ~60% lower in FO compared to CHO and ~39% lower than PRO. No differences in muscle function, soccer performance or blood c-reactive protein concentrations were observed between groups. In conclusion, the addition of n-3PUFA to a beverage containing whey protein, leucine and carbohydrate reduced muscle soreness following intense exercise in soccer players.

2.2 Introduction

Elite soccer players may be required to complete two competitive matches per week, interspersed with intense training sessions (Carling et al., 2015). Such intense scheduling, indicative of fixture congestion, places significant physiological stress on soccer players over

the course of a season, as demonstrated experimentally by decrements in sprint speed, jump performance, and distance covered at maximal intensity when soccer players completed two vs. one match per week over a six-week to full season period (Lago-Penas et al., 2011; Rollo et al., 2014). The typical movement patterns performed by soccer players during match-play and training include sprints, explosive jumps, and repeated changes in direction (Bloomfield et al., 2007). Repeated eccentric-based muscle contractions are required to execute these multi-directional and intermittent movements (Jones et al., 2009), but also are implicated in causing skeletal muscle fiber damage (Nédélec et al., 2012; Russell et al., 2015).

Multiple physiological events underpin the muscle damage process following eccentric-based exercise. Mechanical loading on muscle fibers initially serves to overstretch some myofilaments, resulting in sarcomere disruption and Z-line streaming (Morgan & Allen, 1999). Following exercise, the capacity for damaged muscle to produce force is often impaired (Newham et al., 1983). Following these events, muscle cell membrane integrity is compromised (Proske & Morgan, 2001), resulting in the leakage of myofiber proteins (Clarkson & Hubal, 2002). These metabolic events are associated with delayed onset of muscle soreness (DOMS) and local muscular inflammation 24–48 hours after exercise (Armstrong, 1984; Fridén & Lieber, 2001). With a view to minimizing muscle damage and/or accelerating repair of damaged muscle fibers following eccentric-based exercise, a number of interventions have been explored, including cold water immersion (Paddon-Jones & Quigley, 1997), massage (Hilbert et al., 2003), foam rolling (MacDonald et al., 2014), nonsteroidal anti-inflammatory drugs (Baldwin Lanier, 2003), and various nutritional strategies (Jackman et al., 2010; White et al., 2008) that may have application to recovery in elite soccer.

The most commonly investigated nutritional strategy for promoting muscle recovery after eccentric exercise-induced muscle damage is amino acid ingestion, with or without carbohydrate (Howatson & van Someren, 2008). In terms of efficacy, mixed results have been reported for amino acid–based interventions (Jackman et al., 2010; White et al., 2008). Hence, evidence is equivocal that amino acid supplementation alone provides an effective strategy for promoting recovery from muscle damaging exercise (Pasiakos et al., 2014). Given the anti-inflammatory properties of long chain n-3 polyunsaturated fatty acids (n-3PUFA) (DiLorenzo et al., 2014), an alternative nutritional strategy is dietary supplementation with fish oil–derived n-3PUFA (Gray et al., 2014). Moreover, the propensity for n-3PUFA to be directly incorporated into the phospholipid membrane of skeletal muscle (McGlory et al., 2014) and thus preserve cell membrane integrity provides additional rationale for a role of fish oil ingestion in recovery from eccentric exercise recovery. However, studies investigating the influence of n-3PUFA supplementation per se on recovery from eccentric-based exercise have reported mixed results (Corder et al., 2016; Gray et al., 2014) that are likely attributed to differences in dose, duration and timing of n-3PUFA intervention.

The multifactorial nature of recovery from high intensity exercise includes the refuelling, repair and remodeling of skeletal muscle. It follows that optimizing nutritional strategies for promoting muscle recovery should apply a multi-dimensional approach, encompassing the synergistic role of multiple nutrients. Protein is the key nutritional component to facilitate muscle protein remodeling following exercise (Witard et al., 2016) and carbohydrate ingestion is critical for replenishing muscle glycogen stores depleted following intense exercise (Bergstrom & Hultman, 1966). Therefore, the aim of the present study was to investigate the impact of adding fish oil–derived n-3PUFA to a whey protein,

leucine, and carbohydrate containing supplement over a six week period on acute recovery from eccentric muscle damage in competitive soccer players. The rationale for combining n-3PUFA with whey protein, leucine, and carbohydrate is supported by previous literature that demonstrates n-3PUFA enhances the muscle anabolic sensitivity to an amino acid source (Smith et al., 2011). Therefore, we hypothesized that coingesting n-3PUFA with whey protein, leucine and carbohydrate over a six week supplementation period would reduce muscle soreness, attenuate the inflammatory response to exercise and improve soccer-specific performance during a 72-hour exercise recovery period compared to a protein control condition and a carbohydrate control condition.

2.3 Methods

2.3.1 Participants

Thirty young competitive male soccer players (age: 23 ± 1 yrs; body mass: 73.8 ± 5.9 kg; height: 178.9 ± 6.1 cm; Baseline Yo-Yo test score: 395 ± 158 m) were recruited from local soccer teams. All participants engaged in soccer training and/or match-play ≥3 times per week and were not taking n-3PUFA–containing supplements or any other supplement known to

potentially impact recovery from eccentric-based exercise. The study was approved by University of Stirling School of Sport Research Ethics Committee.

2.3.2 Experimental Design

The study design is summarized in **Figure 2.1**. Participants visited the laboratory on five separate occasions, including familiarization. Based on their performance on the YoYo Intermittent Endurance Test Level 2, participants were assigned to one of three supplementation conditions: fish oil plus whey protein, leucine, and carbohydrate (FO); a whey protein, leucine, carbohydrate placebo (PRO); or a carbohydrate-only placebo (CHO). We previously demonstrated that as little as two weeks of n-3PUFA supplementation (4.5 g/day) significantly increased muscle lipid composition and that this response was further enhanced after four weeks of supplementation (McGlory et al., 2014). Therefore, to elicit a pronounced increase in muscle lipid composition, in the present study we implemented a six-week n-3PUFA supplementation period. Following a six-week supplementation period, participants visited the laboratory on four consecutive mornings to complete experimental trials in the fasted state with no pre-exercise meal provision. On the second laboratory visit, baseline testing was followed by an eccentric-based exercise protocol. At each time-point, measurements of muscle soreness and muscle function, as well as blood markers of muscle damage and inflammation were collected. Soccer-specific performance tasks were completed at baseline, 24 and 72 hours postexercise. All laboratory visits started at the same time of day (07:30) and measurements were always collected in the following order: blood sampling for measurements of serum creatine kinase (CK) and plasma CRP concentrations, muscle

soreness, muscle function (completed in ~20 min), soccer skill tests (completed in ~30 min), and anaerobic endurance (completed in ~15 min).

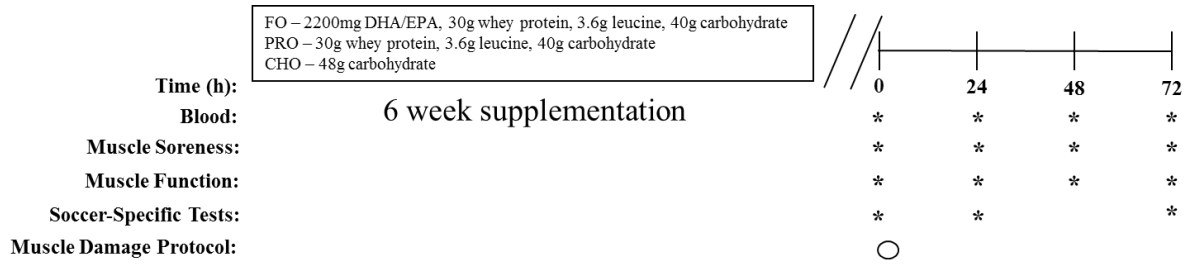

Figure 2.1 — Schematic overview of study design. Abbreviations: FO = Fish Oil supplement beverage; PRO = Protein supplement beverage; CHO = Carbohydrate supplement beverage.

2.3.3 Dietary Supplementation

Within this double-blind, parallel group study, participants consumed 2 × 200 mL juice-based drinks (1 × morning and 1 × evening) daily over the six week supplementation period and on each trial day (Smartfish Sports Nutrition, Ltd). All three supplements were matched for taste. **Table 2.1** details the nutritional composition of each supplement.

Table 2.1 – Supplement Nutrition

	FO	**PRO**	**CHO**
Volume (mL)	200	200	200
Energy Value (kcal)	200	150	200
EPA (mg)	550		

DHA(mg)	550		
Whey Protein (g)	15	15	
Leucine (g)	1.8	1.8	
Carbohydrate (g)	20	20	48
Vitamin D (μg)	3	4	

FO, Fish Oil supplement beverage, PRO, Protein supplement beverage; CHO, Carbohydrate supplement beverage.

2.3.4 Diet and Physical Activity Control

Participants were asked to maintain their exercise training and habitual diet routine throughout the six-week supplementation period. Participants completed a three-day food diary during the first three days of testing. Diet diaries were analyzed for macronutrient and micronutrient content using Microdiet 2 (Downlee Systems Ltd).

2.3.5 Blood Collection and Treatment

On each laboratory visit and following an overnight fast, a blood sample was obtained from a forearm vein. Approximately 1 mL of blood was dispensed onto specialized Whatman 903 blood collection cards (GE Healthcare Ltd, Forest Farm Industrial Estate, Cardiff, CF 14 7YT, UK). The cards were left open and allowed to dry for three hours after which the dried whole blood sample was detached from the collection device using forceps and placed into a screw-cap vial containing 1 mL of methylating solution (1.25M methanol/HCl). The vials were placed in a hot block at 70°C for one hour. The vials were allowed to cool to room temperature before 2 mL of distilled water and 2 mL of saturated KCl solution were added. Fatty acid methyl esters (FAME) were then extracted using 1 × 2 mL of isohexane + BHT followed by a second extraction using 2 mL of isohexane alone. This extraction method has been previously

validated as a reliable measure of whole blood fatty acid composition in our own laboratories (Bell et al., 2011). FAME were then separated and quantified by gas liquid chromatography (ThermoFisher Trace, Hemel Hempstead, England) using a 60 m × 0.32 mm × 0.25 µm film thickness capillary column (ZB Wax, Phenomenex, Macclesfield, UK). Hydrogen was used as carrier gas at a flow rate of 4.0 $mL \cdot min^{-1}$ and the temperature program was from 50 to 150°C at 40°C·min^{-1} then to 195°C at 2°C·min^{-1} and finally to 215°C at 0.5°C·min^{-1}. Individual FAME were identified compared to well characterized in house standards as well as commercial FAME mixtures (Supelco™ 37 FAME mix, SigmaAldrich Ltd., Gillingham, England). Remaining blood was dispensed into vacutainers that were spun at 3,500 revolutions·min^{-1} for 15 min at 4°C in a centrifuge before plasma or serum was extracted and stored at −80°C for further analysis.

2.3.6 Eccentric Exercise Protocol

We utilized a laboratory-controlled eccentric exercise protocol that isolated the hamstring muscles to elicit a physiological state of local muscular stress. Using an isokinetic dynamometer (Biodex Corporation, New York), participants completed 12 sets of an individualized workload on each leg (total of 24 sets), alternating every four sets. In order to calculate an individualized workload, each participant performed three sets of three repetitions of the eccentric exercise protocol, each separated by 1 min. The peak eccentric and concentric forces were determined and the sum was multiplied by an estimated number of total repetitions to complete each set (e.g., 10) as per (Kennedy et al., 2017). This figure was then multiplied by 1.2 to ensure the muscles were maximally worked. Once the workload was reached, the set was completed and the participants had a 1 min rest prior to engaging in the subsequent set. If a participant was unable to complete consecutive sets in less than

30 repetitions, the workload was reduced by 40% to enable the participant to complete the next set in ~20 repetitions. The number of repetitions, the rate of perceived exertion (RPE) and peak force achieved was recorded for each set.

2.3.7 Muscle Soreness

Participants rated muscle soreness with the knee joint flexed at 90°, extended to 0° and in general terms (i.e., on arrival at the laboratory) using a validated 200 mm visual analogue scale (VAS) (Bijur et al., 2001). Participant's marked on a 200 mm Likert scale their perceived soreness in the hamstring muscle from 'no pain' (0 mm anchor point) to 'most pain imaginable' (200 mm anchor point). Soreness was defined by measuring the distance from the 0 mm anchor point.

2.3.8 Muscle Function

A single leg isokinetic/eccentric maximum voluntary contraction (MVC) of the knee flexors was used to assess muscle function. Participants were seated on the dynamometer with their upper body, hips and exercising thigh securely strapped into the seat. The lower leg was attached to the arm of the dynamometer 1 cm above the lateral malleolus ankle joint with the axis of rotation of the dynamometer arm aligned with the lateral femoral condyle. The dynamometer arm was set to start and stop at angles 90° and 0° respectively at the knee joint. Each participant performed 3 × 3 sets/reps of the MVC protocol.

2.3.9 Soccer Skill Test

The Loughborough Soccer Passing Test (LSPT) (Ali et al., 2007) was used to assess soccer skill performance. Participants were required to complete sixteen individual passes to four targets in the quickest time possible. Time started on the participant's first touch of the

ball and stopped on completion of the sixteenth pass. Time was recorded using a standard handheld stopwatch. Time penalties were incurred for the following infringements: the ball touching a cone, passing from outside the box, missing the target area, missing the bench altogether or touching the ball with the hand. Participants were rewarded for a 'perfect pass' in which a pass hit the small target strip in the middle of the bench with time taken away from their final time.

2.3.10 Anaerobic Endurance

The Nike Spark YoYo Intermittent Endurance Test Level 2 (Bangsbo et al., 2008) was used to assess anaerobic endurance. The test involved 40-m shuttle sprints (2 × 20 m) interspersed with 10 seconds of recovery. The test finished when the participant failed to reach the finish line before the cue on two consecutive attempts.

2.3.11 Blood Analysis Plasma

C-reactive protein (CRP) concentrations were measured in duplicate using a double antibody sandwich enzyme immunoassay (Kalon Biological Limited, UK). Creatine kinase (CK) concentrations were measured in duplicate using an iLab Aries automatic biochemical analyzer (Instrumentation Laboratory, USA).

2.3.12 Data Presentation and Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences 21 (IBM SPSS, Chicago, IL). Differences across time for muscle soreness, muscle function, performance, and blood markers of muscle damage and inflammation were analyzed by a mixed-design, twoway

ANOVA with three between-subject (FO, PRO, and PLA) and 3/4 within-subject (0, 24-, 48-, and 72-hour time-points) variables. Where a significant main effect was detected, Tukey posthoc tests were performed to distinguish differences between supplement groups. Muscle soreness, CK and CRP data were expressed as raw values and as area under the curve (AUC) to determine the cumulative response over 72 hours. AUC data were analyzed using one-way ANOVA. Statistical difference was assumed at the level of ≤0.05. Cohen's effect size (d) were calculated to compare differences between conditions. Effect sizes of 0.2 were considered small, 0.5 considered medium and >0.8 were considered large (Cohen 1988). All data are expressed as means ± SEM, with the exception of participant characteristics and the nutritional composition of supplements (M ± SD).

2.4 Results

2.4.1 Dietary Intake

There was no difference in macro- or micro-nutrient dietary intake between the three groups ($p > 0.05$) over the 72-hour recovery period.

2.4.2 Blood n-3PUFA Composition

Baseline (pre) % n-3PUFA/totalPUFA composition in blood was similar between participants in all three groups ($p = 0.25$). Whereas blood n-3PUFA composition increased by 58% following six weeks of supplementation in FO ($p < 0.001$), no changes were observed in PRO ($p = 0.61$) or CHO ($p = 0.93$) (**Figure 2.2A**). The % n-3PUFA/totalPUFA blood composition increased in all 10 participants after supplementation in FO group (**Figure 2.2B**).

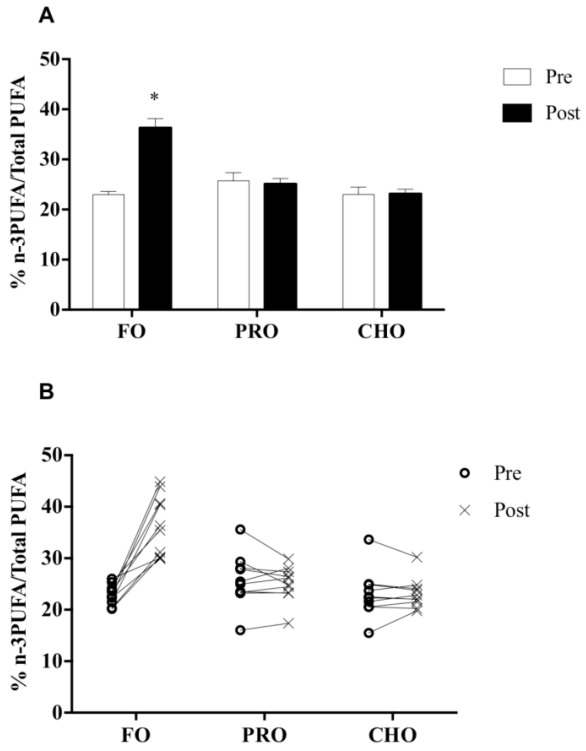

Figure 2.2 – Percentage of n-3PUFA/totalPUFA composition in blood before (Pre) and after (Post) 6 week of supplementation. FO, Fish Oil supplement beverage, PRO, Protein supplement beverage; CHO, Carbohydrate supplement beverage. *A*, group data expressed as means ± SEM. *B*, individual data values. * significant difference from Pre in corresponding supplement condition.

2.4.3 Muscle Soreness

Perceived ratings of general muscle soreness measured at baseline in both dominant and non-dominant legs was similar in all three conditions. Muscle soreness in both legs was elevated above baseline at 24-, 48-, and 72-hour time points. Dominant leg soreness was lower in FO compared with PRO ($p = 0.02$) and CHO ($p = 0.01$) after 24 hours (**Figure 2.3A**). Likewise, soreness was lower in FO compared with PRO ($p = 0.003$) and CHO ($p = 0.03$) after 48 hours and lower in FO than PRO after 72 hours ($p = 0.01$). General soreness of the dominant leg, expressed as AUC over the entire 72-hour recovery period, was 58% lower in FO compared with CHO ($p = 0.02$, $d = 1.7$). Nondominant leg soreness was lower in FO compared with CHO after 24 hours ($p = 0.01$). Likewise, soreness was lower in FO compared with PRO ($p = 0.01$) and CHO ($p = 0.03$) after 48 hours and lower than PRO ($p = 0.02$) after 72 hours (**Figure 2.3B**). General soreness of the nondominant leg, expressed as AUC over the entire 72-hour recovery period, was lower in FO compared with PRO (58%, $d = 1.3$, $p = 0.01$) and CHO (57%, $d = 1.6$, $p = 0.01$). The pattern of muscle soreness scores with the knee joint in a flexed and extended position mimicked that of general soreness.

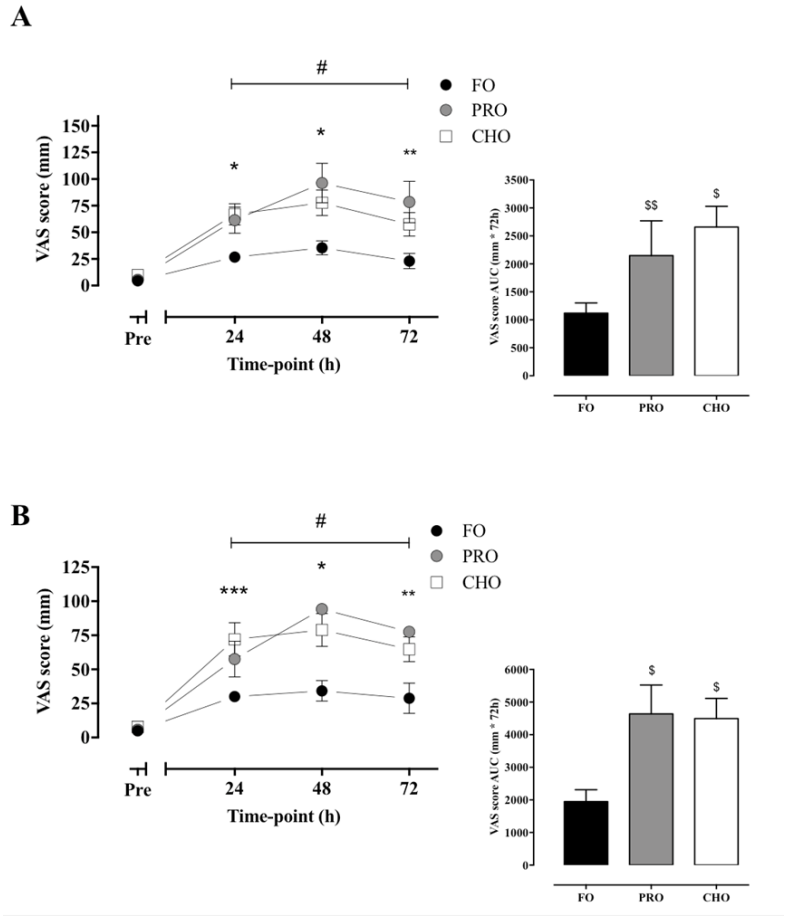

Figure 2.3 – Muscle soreness, expressed as raw values over time and area under the curve during the overall 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means ± SEM. Insert shows area under the curve (AUC). *A and B*, general soreness of the dominant and non-dominant leg respectively. * FO significantly different to PRO and CHO. ** FO significantly different to PRO. *** FO significantly different to CHO. # Significantly different to PRE in corresponding supplement condition. $ Significantly different to FO. $$ Tendency to be different to FO. Abbreviations as in Figure 2.2.

2.4.4 Muscle Function

MVC of both dominant and nondominant legs was reduced below baseline for the entire 72-hour recovery period in all groups ($p < 0.05$; **Figure 2.4**). However, no statistically significant differences in MVC of either leg were observed between groups in either leg ($p > 0.05$).

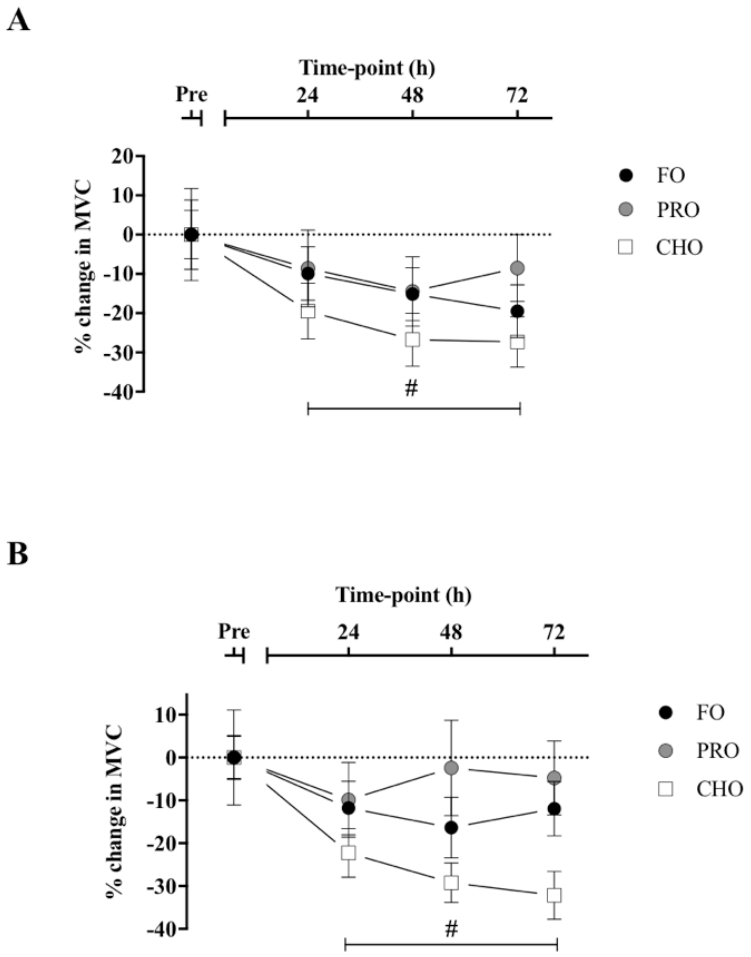

Figure 2.4 - Percentage change in muscle maximum voluntary contraction over the entire 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means ± SEM. *A*, MVC changes in the dominant leg. *B*, MVC changes in the non-dominant leg. [#] Significantly different to Pre. Abbreviations as in Figure 2.2.

2.4.5 Soccer-Specific Performance Tasks

Performance on the LSPT and Nike spark Yoyo intermittent recovery test level 2 did not change statistically from baseline during the 72-hour recovery period in any condition (**Figure 2.5**).

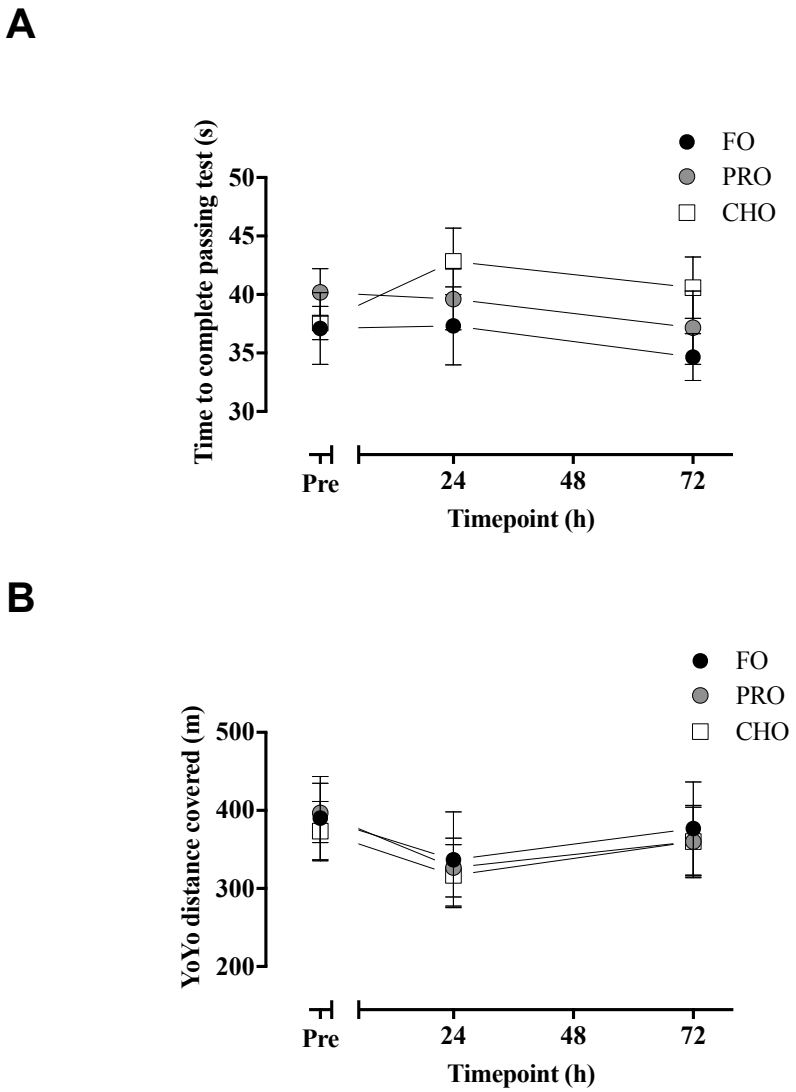

Figure 2.5 – Performance on soccer-specific tasks measured over the entire 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means ± SEM. A, Loughborough Soccer Passing Test; B, Yoyo intermittent recovery Test level 2; Abbreviations as in Figure 2.2.

2.4.6 Blood creatine kinase concentrations

Serum CK concentrations increased from baseline after 24 hours of exercise recovery and remained elevated at 48 and 72 hour timepoints (p>0.05) (**Figure 2.6**). CK concentrations, expressed as AUC over the entire 72 hour recovery period, were lower in FO compared with CHO (p=0.02) and there was a tendency (p=0.07) for CK concentrations were lower in FO compared to PRO.

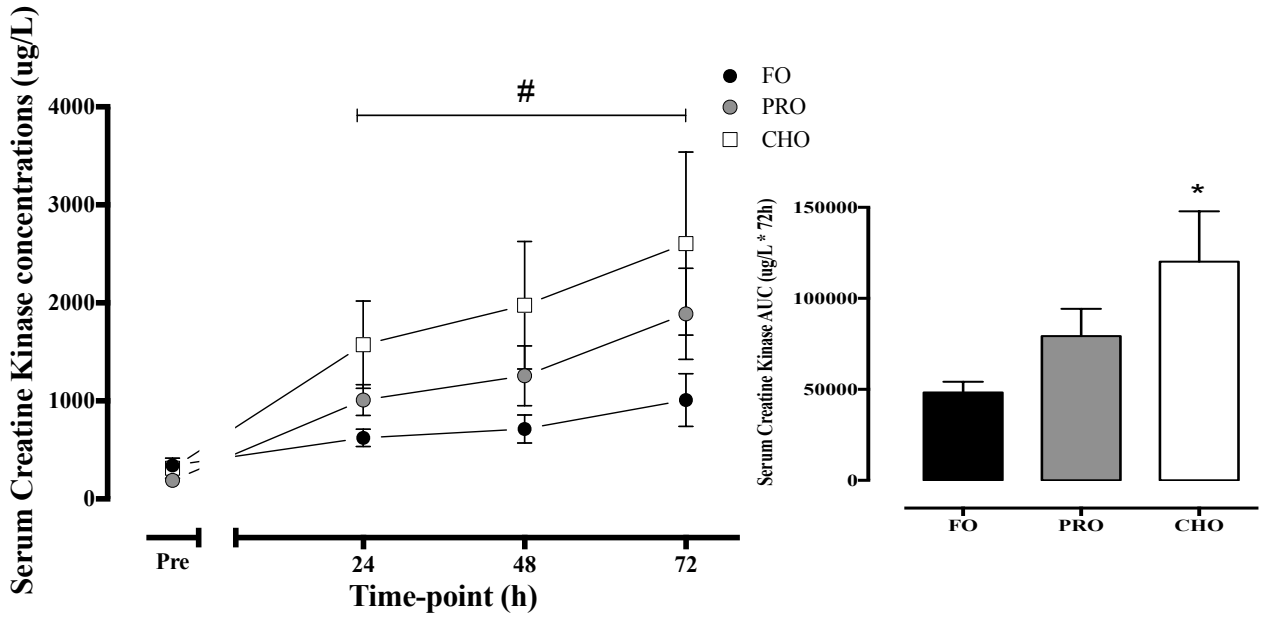

Figure 2.6 – Serum creatine kinase concentrations over the entire 72h recovery period following an intense bout of eccentric exercise. Data are expressed as means ± SEM. Insert shows area under the curve (AUC). * Significantly different to FO. # Significantly different to Pre. Abbreviations as in Figure 2.2.

2.4.7 Blood C-reactive protein concentrations

Serum CRP concentrations increased from baseline after 24 hour of exercise recovery, but returned to baseline at 48 hours and remained at baseline for the remainder of the recovery period (**Figure 2.7**). No statistical differences in CRP concentrations were detected between groups when expressed as time or as AUC over the entire 72 hour recovery period ($p>0.05$).

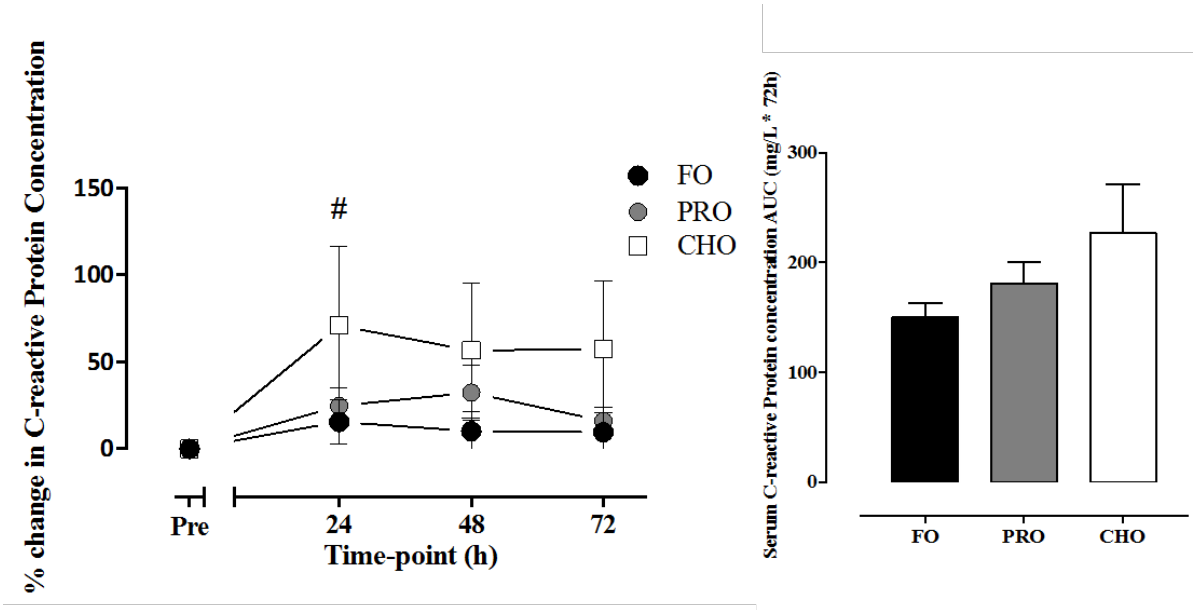

Figure 2.7 – Serum C-reactive protein concentrations over the entire 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means ± SEM. Insert shows area under the curve (AUC). # Significantly different to Pre. Abbreviations as of Figure2.2.

2.5 Discussion

This study demonstrated a decrease in perceived feelings of muscle soreness and serum CK concentrations under fasting conditions in response to eccentric exercise when n-3PUFA were added to whey protein, leucine and carbohydrate over a six-week supplementation period. However, the addition of n-3PUFA to the multi-ingredient beverage did not appear to modulate the systemic inflammatory response or attenuate the decline in muscle function and soccer-specific performance during exercise recovery in competitive soccer players. Taken together, these data suggest that adding n-3PUFA to a whey protein, leucine and carbohydrate containing supplement over a six-week period may elicit a protective role in maintaining the structural integrity of the muscle cell membrane, thereby reducing the severity of muscle soreness experienced following eccentric based muscle damaging exercise.

The repair of damaged muscle fibers following eccentric exercise consists of multiple physiological processes including an inflammatory response (Toumi et al., 2006). The inflammatory response, as typically measured at the systemic level, occurs 24–72 hours following eccentric exercise-induced muscle damage (Pereira Panza et al., 2015) and coincides with peak feelings of muscle soreness (Cheung et al., 2003). In the present study, the reduced perception of muscle soreness following acute exercise when n-3PUFA were added to a mixed ingredient beverage did not appear to be mediated by a reduced systemic inflammatory response to exercise. Consistent with previous studies that utilized a simulated soccer matchplay protocol (Mohr et al., 2016), serum CRP concentrations increased 24 hours postexercise before returning to baseline levels after 48 hours. However, no difference in serum CRP concentrations were reported during acute exercise recovery between test drink conditions. Hence, we detected no apparent difference in the systemic inflammatory

response to eccentric exercise between conditions. One potential explanation for the similar CRP response between conditions may relate to the carbohydrate content of all test drinks. Carbohydrate intake has previously been shown to suppress the production of glucocorticoids, such as cortisol, which is known to reduce inflammation (Yeager et al., 2011). Since all test drinks contained a similar dose of carbohydrate, it is possible that attenuating the cortisol response blunted the systemic inflammatory response in all conditions. Therefore, refuting our original hypothesis, the present data suggest that the addition of n-3PUFA to a mixed ingredient supplement did not influence CRP, our measurement of systemic inflammatory markers in soccer players after eccentric based muscle damaging exefcise.

A local inflammatory response, rather than systemic inflammation, provides a more direct precursor for the onset of muscle soreness following eccentric-based exercise (Malm, 2001). Although speculative, we suggest that the protective effect of n-3PUFA supplementation in reducing muscle soreness may be mediated by a local anti-inflammatory response within the perimysium and epimysium of muscle fascia. Pain receptors called nociceptors are present within the muscle fascia (Mense & Schiltenwolf, 2010). Hence, a dampening of inflammation with n-3PUFA supplementation may stabilize the fascia and thus desensitize nociceptors and reduce pain. Since no muscle biopsies were obtained in the present study, it was not possible to directly measure inflammation of the muscle fascia or z-line streaming as a direct marker of muscle damage and thus future investigation is warranted.

A more likely mechanism for the reduced muscle soreness with the addition of n-3PUFA to a multi-ingredient supplement may relate to a protective effect of n-3PUFA in maintaining the structural integrity of the muscle cell membrane. Previous work

demonstrates that fish oil derived n-3PUFA are incorporated into phospholipid membrane of muscle cells (Smith et al., 2011). The presence of n-3PUFA within the muscle membrane also is thought to improve membrane integrity and thus reduce the leakage of intramyocellular proteins, such as CK (Clarkson & Hubal, 2002). We speculate that the incorporation of n-3PUFA into the muscle phospholipid membrane over six weeks of supplementation enhanced the structural integrity of the muscle cell membrane prior to the eccentric exercise protocol and, as such, protected the muscle fibers of the active muscles from the mechanical stress induced by eccentric exercise. Consistent with this theory, in the present study we report a greater percentage contribution of n-3PUFA in whole blood (Figure 2) and an attenuated increase in serum CK concentrations (Figure 6) during eccentric exercise recovery when fish oil was added to the multi-nutrient supplement compared to our control supplements. Given that the incorporation of n-3PUFA into red blood cells with fish oil supplementation follows a similar, albeit earlier, time course as profiled in skeletal muscle (McGlory et al., 2014), the attenuated CK response reported in the present study implies an enhanced maintenance of cell membrane integrity when n-3PUFA was added to a multi-ingredient beverage.

Muscle soreness following eccentric-based exercise previously has been shown to impair both muscle function (Legault et al., 2015) and sport-specific performance (Eston et al., 1996). In the present study, although eccentric exercise failed to initiate any change in performance of soccer-specific tests, hamstring MVC was impaired over the entire recovery period, indicating that the protocol successfully elicited symptoms of muscle damage. Interestingly, the reduced perceived feeling of muscle soreness reported when adding n-3PUFA to a multi-ingredient beverage did not translate into the better maintenance of muscle function or soccer-specific performance during exercise recovery. This observation, combined

with the attenuated increase in serum CK concentrations following eccentric exercise in FO vs. PRO and CHO suggests that ultrastructural damage occurred in FO, but without disruption to the phospholipid membrane of the muscle cell, although this theory cannot be confirmed without measuring muscle membrane integrity. Whilst there is no definitive reason for this apparent disconnect between measurements of muscle soreness and muscle function/soccer performance obtained in the present study, the most likely explanation may relate to methodological considerations. For logistical reasons, we did not measure soccer-specific performance 48 hours following eccentric exercise when soccer players reported peak muscle soreness scores and when soccer-specific performance scores likely reached their nadir. Thus, we potentially missed any benefit of FO on soccer-specific performance at the peak of muscle soreness. Moreover, the practical application of our study findings are limited to a single muscle damage stimulus over an acute recovery period. In a real-world setting, during periods of fixture congestion, soccer players typically experience several muscle damage stimuli. Rollo et al. (2014) demonstrated that two games vs. one game per week impaired soccer-specific performance in soccer players. Therefore, future studies should examine the impact of adding n-3PUFA to a multi-ingredient supplement on performance indices during recovery from multiple muscle damage stimuli.

To conclude, our results suggest that adding fish oil to a multi-ingredient supplement attenuates muscle soreness following damaging eccentric-based exercise, potentially making n-3PUFA, whey protein and carbohydrate an effective recovery strategy for soccer players.

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Chapter 3 - Influence of fish oil-derived n-3 fatty acid supplementation on changes in body composition and muscle strength during short-term weight loss in resistance-trained men

3.1 Abstract

Background: A common detrimental consequence of diet-induced weight loss, common in athletes who participate in weight cutting sports, is muscle loss. Dietary omega-3 polyunsaturated fatty acids (n-3PUFA) exhibit a protective effect on the loss of muscle tissue during catabolic situations such as injury-simulated leg immobilisation. This study aimed to investigate the influence of dietary n-3PUFA supplementation on changes in body composition and muscle strength following short-term diet-induced weight loss in resistance-trained men.

Methods: Twenty resistance-trained young (23 ± 1 yrs) men were randomly assigned to a fish oil group that supplemented their diet with 4g n-3PUFA, 18g carbohydrate and 5g protein (FO) or placebo group containing an equivalent carbohydrate and protein content (CON) over a 6 wk period. During wks 1-3, participants continued their habitual diet. During wk 4, participants received all food items to control energy balance and a macronutrient composition of 50% carbohydrate, 35% fat and 15% protein. During wks 5 and 6, participants were fed an energy-restricted diet equivalent to 60% habitual energy intake. Body composition and strength were measured during wks 1, 4 and 6.

Results: The decline in total body mass (FO = -3.0 ± 0.3 kg, CON = -2.6 ± 0.3 kg), fat free mass (FO = -1.4 ± 0.3 kg, CON = -1.2 ± 0.3 kg) and fat mass (FO = -1.4 ± 0.2 kg, CON = -1.3 ± 0.3 kg) following energy restriction was similar between groups (all $p > 0.05$; d: 0.16-0.39). Non-dominant leg extension 1RM increased (6.1 ± 3.4 %) following energy restriction in FO ($p < 0.05$, $d = 0.29$), with no changes observed in CON ($p > 0.05$, $d = 0.05$). Dominant leg extension 1RM tended to increase following energy restriction in FO ($p = 0.09$, $d = 0.29$), with no changes in CON ($p > 0.05$, $d = 0.06$). Changes in leg press 1RM, maximum voluntary contraction and muscular endurance following energy restriction were similar between groups ($p > 0.05$, $d = 0.05$).

Conclusion: Any possible improvements in muscle strength during short-term weight loss with n-3PUFA supplementation are not related to the modulation of FFM in resistance-trained men.

3.2 Introduction

The application of diet-induced weight loss extends beyond clinical (overweight and obese) populations. Athletic populations competing in weight-category sports (e.g., boxing), or sports where a high power-to-body mass ratio (sprinting) or aesthetics (gymnastics) are pre-requisites for success also routinely periodize their training programme to include short-term periods of energy restriction (Tipton, 2011). However, a counterproductive feature of diet-induced weight loss in athletes that accompanies the reduction in fat mass includes the decline in fat-free mass (FFM), specifically of skeletal muscle tissue (Mettler et al., 2010; Racette et al., 2017).

Changes in body composition during diet-induced weight loss can be manipulated with nutrition (Witard et al., 2019). Most notably, experimental studies demonstrate that increasing dietary protein intake confers an effective nutritional strategy to promote high-quality weight loss during energy restriction, i.e. loss of fat mass while maintaining muscle mass during short-term weight loss (Mettler et al., 2010; Longland et al., 2016). However, the preservation of muscle mass during energy restriction with a higher protein intake did not translate into the better maintenance of exercise performance in resistance-trained young men (Mettler et al., 2010). The importance of other nutrients for maintaining FFM during diet-induced weight loss has been proposed (Mettler et al., 2010; Longland et al., 2016), but few experimental studies have addressed the effectiveness of these nutrients on changes in body composition during weight loss.

Another potentially effective nutritional intervention to promote high-quality weight loss during energy restriction in athletic populations is the ingestion of omega-3 polyunsaturated fatty acid (n-3PUFA). Both in vitro cell line experiments (Kamolrat & Gray, 2013; Jeromson et al., 2018) and in vivo human studies (McGlory et al., 2019; Smith et al., 2011a; Smith et al., 2011b) support the notion that n-3PUFA exhibit anabolic properties, in particular the omega-3 species eicosapentaenoic acid (EPA). Previous proof-of-principle studies have demonstrated that fish oil-derived n-3PUFA supplementation potentiated the response of muscle protein synthesis (MPS) to the infusion of amino acids and insulin (Smith et al., 2011a; Smith et al., 2011b), and enhanced muscle mass (Smith et al., 15) and strength (Rodacki et al., 2012; Da Boit et al., 2017) in older adults. The mechanism most commonly proposed to underpin the anabolic action of n-3PUFA relates to modifying the lipid profile of the muscle phospholipid membrane. This structural change in integrity of the muscle membrane is understood to activate intracellular signalling proteins (e.g. mTORC1-p70S6k1)

(Smith et al 2011a; McGlory et al., 2014) that upregulate muscle protein synthesis (MPS), thus modulating muscle mass.

Based on current evidence from experimental studies, the metabolic role of n-3PUFA in regulating muscle mass is most evident under catabolic conditions. Consistent with this notion, fish oil-derived n-3PUFA supplementation was shown to exhibit protective roles in preserving muscle mass in a clinical population of cancer cachexia patients (Murphy et al., 2011) and following a short-term period of leg immobilisation in healthy recreationally-active young women (McGlory et al., 2019). Another catabolic situation is diet-induced weight loss, whereby the intracellular activation of AMPK signals an energy deficit within the muscle cell (Pasiakos et al., 2010). Given that MPS is an energetically expensive process, requiring ~4 moles of ATP to bind each amino acid during the elongation process of translation (Browne & Proud, 2004), this activation of AMPK acts to conserve energy during weight loss by down-regulating basal rates of MPS (Areta et al., 2014; Hector et al., 2018; Pasiakos et al., 2010). To our knowledge, all studies to date that have investigated the impact of fish oil supplementation on body composition during weight loss have been conducted in a clinical setting with overweight and/or obese patients (Kunesova et al., 2006; Munro & Garg, 2011). Given the link between dietary n-3PUFA intake, MPS and muscle mass during injury-simulated leg immobilisation in trained young women (McGlory et al., 2019), there is strong rationale to support a protective role of n-3PUFA in maintaining muscle mass and strength during energy-restricted weight loss in athletes.

The primary aim of this study was to investigate the influence of fish oil-derived n-3PUFA supplementation during short-term diet-induced weight loss on changes in body composition and muscle strength in resistance-trained young men. We hypothesized that n-3PUFA supplementation would attenuate the loss of lean body mass and better maintain

lower limb muscle strength following a 2 wk period of an energy-restricted diet compared to placebo.

3.3 Methods

3.3.1 Study Design

Using a parallel research design adapted from Mettler et al. (2010) (**Figure 3.1**), participants were randomly assigned to one of two groups: a fish oil supplement group (FO) or an energy and macronutrient matched control group (CON). Participants consumed their assigned supplement twice daily for the entire 6-week study period. Participants consumed their habitual diet for the first 3 weeks of the study, with week 1 used to assess energy intake and energy expenditure. During week 4, all food items and fluids were supplied by researchers, providing 100% of habitual energy intake. During weeks 5 and 6, the energy content of the diet was reduced to 60% of habitual intake. At the end of weeks 1, 4 and 6, measurements of body mass, body composition, and muscle performance were obtained under controlled laboratory conditions using dual energy x-ray absorptiometry (DXA), leg extension and leg press fixed resistance machines (Cybex International, Illinois, USA) and isokinetic dynamometry technology.

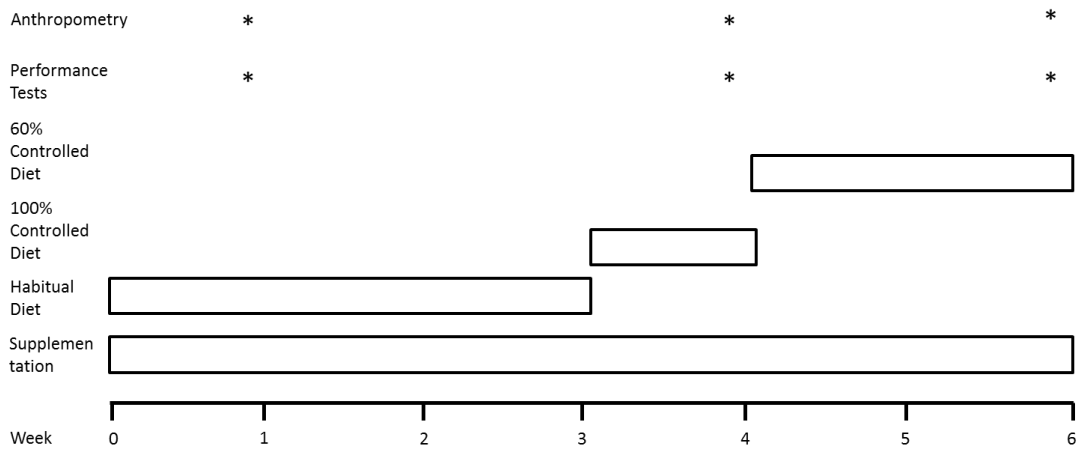


Figure 3.1 — Schematic overview of study design. Weeks 0-3, participants consumed their habitual diet under free-living conditions. Week 4, all food items were supplied by researchers to ensure participants consumed a diet constituting 100% of habitual energy intake and a macronutrient composition of 50% carbohydrate, 35% fat and 15% protein. Weeks 5 and 6, all food items provided to ensure energy-restricted diet was equivalent to 60% of habitual energy intake

3.3.2 Participant recruitment and ethical approval

Twenty healthy (no known metabolic disorders as determined by health questionnaire) young resistance-trained males were recruited from local sports clubs. All participants had undertaken resistance training for at least the previous six months, were currently training ≥ 2 times per wk, and were not consuming supplements containing n-3PUFA at the time of study enrolment. Participants were asked to continue their habitual training throughout the 6-week study period and remain from eating fish any more than 1 time per week. The West of Scotland Research Ethics Service approved the study procedures.

3.3.3 Dietary control and supplementation

In a single blinded fashion, participants were divided equally between supplement conditions, consuming 2 × 200 mL volume juice-based drinks (1 × morning and 1 × evening) daily over the 6 week supplementation period (Smartfish Sports Nutrition, Ltd). Drinks were provided to match energy and macronutrient composition of the two diets. However, the FO beverage contained an additional 2g of n-3PUFA per drink. Supplements were taste-matched and equal in protein and carbohydrate content. The experimental supplement condition contained 2g of fish oil (~1g of EPA and ~1g of DHA) whereas the placebo condition did not contain fish oil. The additional energy provided by the fish oil supplement in the experimental condition was accounted for by modifying the energy content of the background diet.

3.3.4 Trial days and measurements

Testing sessions commenced at ~07:00 on weeks 1 (day 7), 4 (day 27) and 6 (day 41) following an overnight fast and having consumed 500 ml of water 1-2 h prior to arriving at the laboratory. Participants were instructed to empty their bladder before body weight was measured using standard laboratory scales (Seca Quadra 808, Birmingham, UK) with participants wearing underwear only. Body composition was measured using a narrowed fan-beamed dual-energy x-ray absorptiometry (iDXA GE Healthcare) with analysis performed

using GE Encore 13.40.038 Software (GE Healthcare). All DXA scans followed procedures previously described by Rodriguez-Sanchez & Galloway (2015) and were performed by the same trained technician.

3.3.5 Muscle strength and endurance

The first test of muscle strength was a single leg isokinetic/eccentric maximum voluntary contraction (MVC) of the knee flexors using an isokinetic dynamometer (Biodex Corporation, New York). Participants were seated on the dynamometer with their upper body, hips and thigh securely strapped into the seat and the hip at a 90º angle to the legs. The lower leg was attached to the arm of the dynamometer 1 cm above the lateral malleolus ankle joint with the axis of rotation of the dynamometer arm aligned with the lateral femoral condyle. The dynamometer arm was set to start and stop at angles 90° and 0° respectively at the knee joint. Participants were asked to use maximum effort resist the dynamometer arm from moving the knee joint from a 90° to a 0° angle. Each participant performed 3×3 sets/reps of this MVC protocol with a 60 second rest between sets. Each participant's greatest peak torque from the 3 sets were recorded.

Following 5 min rest, unilateral 1RM for leg extension was assessed using a previously validated protocol (Baechle and Earle, 2008) on a fixed resistance machine (Cybex International Inc, Cybex International, MA). Seat and knee position was recorded during testing session and was replicated during weeks 4 and 6. On the same day, following a 10 min rest period, unilateral muscular endurance was measured. Participants completing as many repetitions as possible on leg extension and leg press, with resistance set at 60% of individual baseline 1RM. Participants completed repetitions at their own pace but were instructed to cease exercising as soon as a rest between repetitions was required. In total, testing sessions were completed within 180 min.

3.3.6 Diet

During weeks 1-3, all participants consumed their habitual diet but were asked to refrain from eating oily fish to ensure that supplementation accounted for changes in blood lipid profiles. During week 1, energy intake and energy expenditure were measured. Energy intake was measured using a 3-d food report. On the same days as the food report, energy expenditure was measured using the physical activity questionnaire (Bouchard et al., 1983) and from Actiheart data (CamNtech Ltd, Papworth Everard, England). All measures of energy intake and expenditure were averaged to give a 100% energy value. During week 4, participants were instructed to consume only the food provided by researchers that contained 100% of their habitual diet with a macronutrient composition of 50% carbohydrate, 35% fat and 15% protein. The energy content of the supplement was taken into account when calculating the habitual energy intake of each participant. The only exceptions were water and diet soft drinks that could be consumed ad libitum. Participants were asked to provide feedback on the volume of food consumed. If a participant reported feeling hungry, the energy content of the diet was increased. Conversely, if the volunteer was unable to eat all food provided, the energy content of the diet was reduced. Participants also were asked to monitor their body weight throughout week 4 to ensure body weight stability. During wks 5 and 6, dietary energy intake was reduced to 60% of habitual intake, however macronutrient composition remained constant. Throughout wks 4-6, foods rich in omega-3 fatty acids such as oily fish (tuna, salmon, mackerel), walnuts and margarine were omitted from the diet. The diets were individually tailored to compensate for individual eating patterns and preferences and therefore maximise diet compliance. During wks 4-6, participants were asked to return any food that was not consumed to researchers for weighing. Returned food was then weighed and an energy content was calculated. The energy content not consumed by the

participant was added on to the following day's diet. Every attempt was made to provide participants with the confidence to honestly report any noncompliance without any consequences.

3.3.7 Blood analysis

Approximately 1 mL of venous blood was dispensed onto specialized Whatman 903 blood collection cards (GE Healthcare Ltd, Forest Farm Industrial Estate, Cardiff, CF 14 7YT, UK). Cards were dried for 3h after which the dried whole blood sample was detached from the collection device using forceps and placed into a screw-cap vial containing 1mL of methylating solution (1.25M methanol/HCl). Vials were then placed in a hot block at 70°C for one hour. The vials were allowed to cool to room temperature before adding 2 mL of distilled water and 2 mL of saturated KCl solution. Fatty acid methyl esters (FAME) were then extracted using 1 × 2 mL of isohexane + BHT followed by a second extraction using 2mL of isohexane alone. This extraction method has been previously validated as a reliable measure of whole blood fatty acid composition in our own laboratories (Bell et al., 2011). FAME were then separated and quantified by gas liquid chromatography (ThermoFisher Trace, Hemel Hempstead, England) using a 60 m × 0.32 mm × 0.25 µm film thickness capillary column (ZB Wax, Phenomenex, Macclesfield, UK). Hydrogen was used as carrier gas at a flow rate of 4.0 mL·min−1 and the temperature program was from 50 to 150°C at 40°C·min−1 then to 195°C at 2°C·min−1 and finally to 215°C at 0.5°C·min−1. Individual FAME were identified compared to well characterized in house standards as well as commercial FAME mixtures (Supelco™ 37 FAME mix, Sigma- Aldrich Ltd., Gillingham, England).

3.3.8 Data presentation and statistical analysis

Data were analyzed using Statistical Package for Social Sciences 21 (IBM SPSS, Chicago, IL). All data were found to be normally distributed based on the Shapiro-Wilk test. Differences across time for body composition, muscle strength and muscular endurance were analyzed by a mixed-design, two-way (time and supplement group) ANOVA. Two between-subject variables (FO and PLA) and either 3 within-subject (weeks 1 (habitual diet), 4 (100% diet) and 6 (60% diet) time-points) or 2 within-subjects (week 4 and week 6) variables were modelled within the two-way ANOVA. Where a significant time × supplement group interaction was detected, a Tukey posthoc test was performed to locate the timepoint(s) whereby differences existed between supplement groups. Statistical significance was set at the level of ≤0.05. Cohen's effect sizes (d) were calculated to compare differences between conditions. Effect sizes of 0.2 were considered small, 0.5 considered medium and >0.8 were considered large (Cohen 1988). All data were expressed as means ± SD, unless otherwise stated.

3.4 Results

3.4.1 Supplement Control

All participants consumed all of the supplements provided to them. No adverse events occurred due to the fish oil or placebo supplementation.

3.4.2 Dietary intake during energy restriction

No differences in energy or macronutrient intakes were observed between FO and CON during the habitual diet, 100% diet and 60% diet periods ($p > 0.05$, **Figure 3.2**). Energy intake was lower during the 60% diet period compared to the 100% diet period in both conditions ($p < 0.001$).

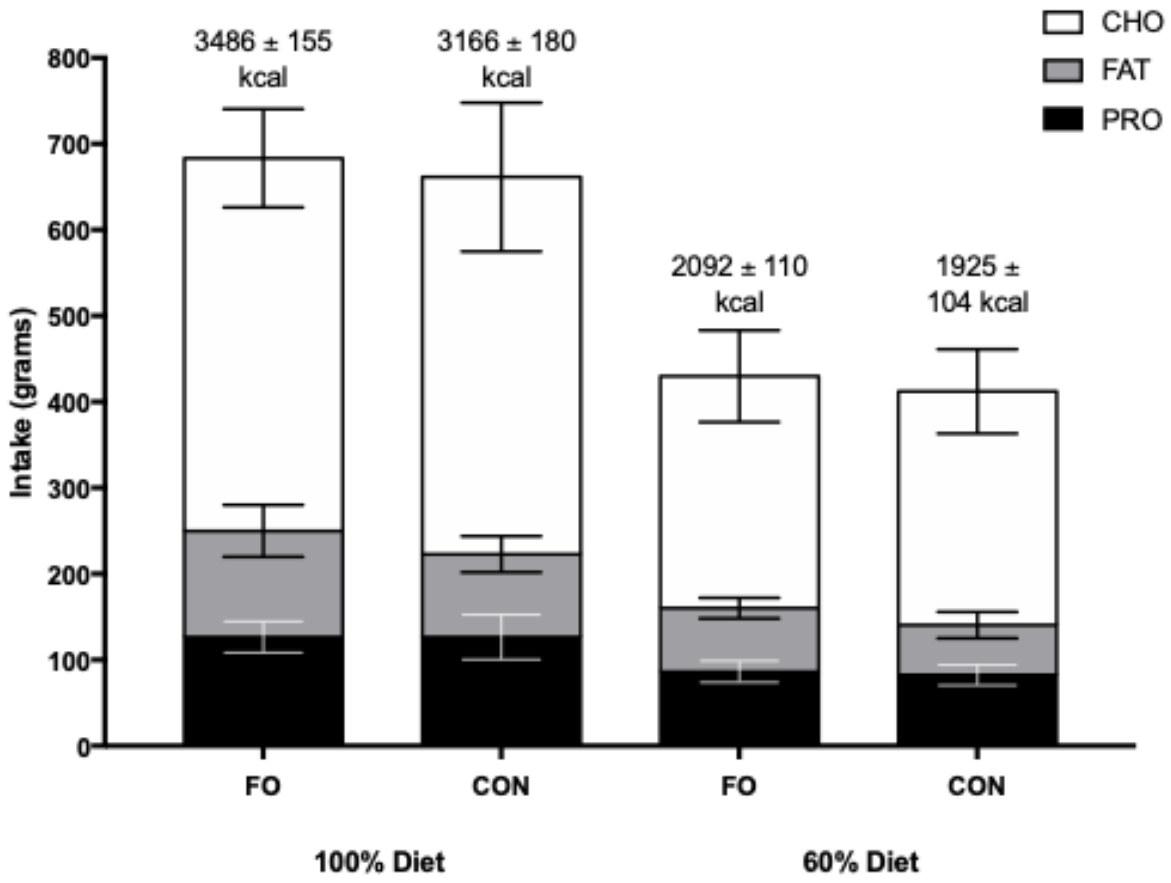


Figure 3.2 – Energy intake (kcal) and macronutrient composition (grams) during the 100% diet period and the 60% diet period in fish oil (FO) and control (CON) supplement groups. Values are means ± SD. CHO, carbohydrate; PRO, protein.

3.4.3 Blood n-3PUFA composition

Baseline (pre) blood % n-3PUFA/totalPUFA composition was similar between groups ($p < 0.01$, **Figure 3.3**). Blood n-3PUFA composition increased by ~60% following 6 wks of supplementation in FO, whereas no change was observed in CON. At the individual level, blood % n-3PUFA/totalPUFA composition increased in all 10 participants after supplementation in FO.

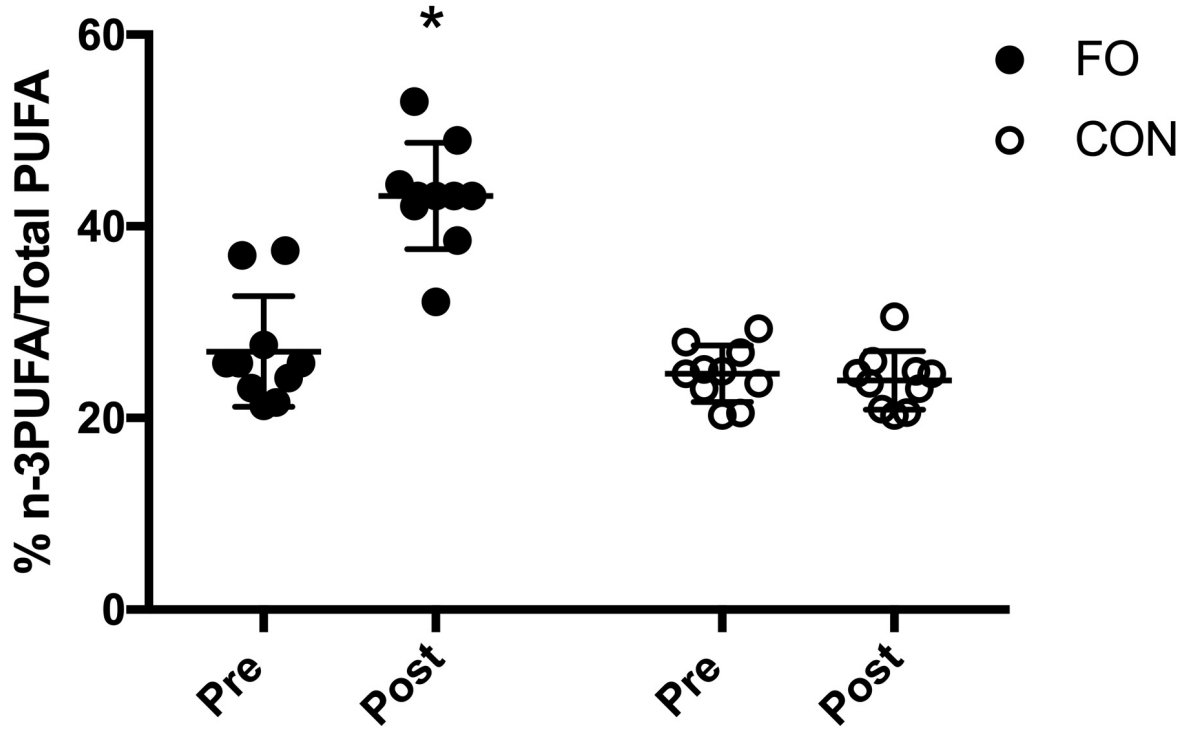
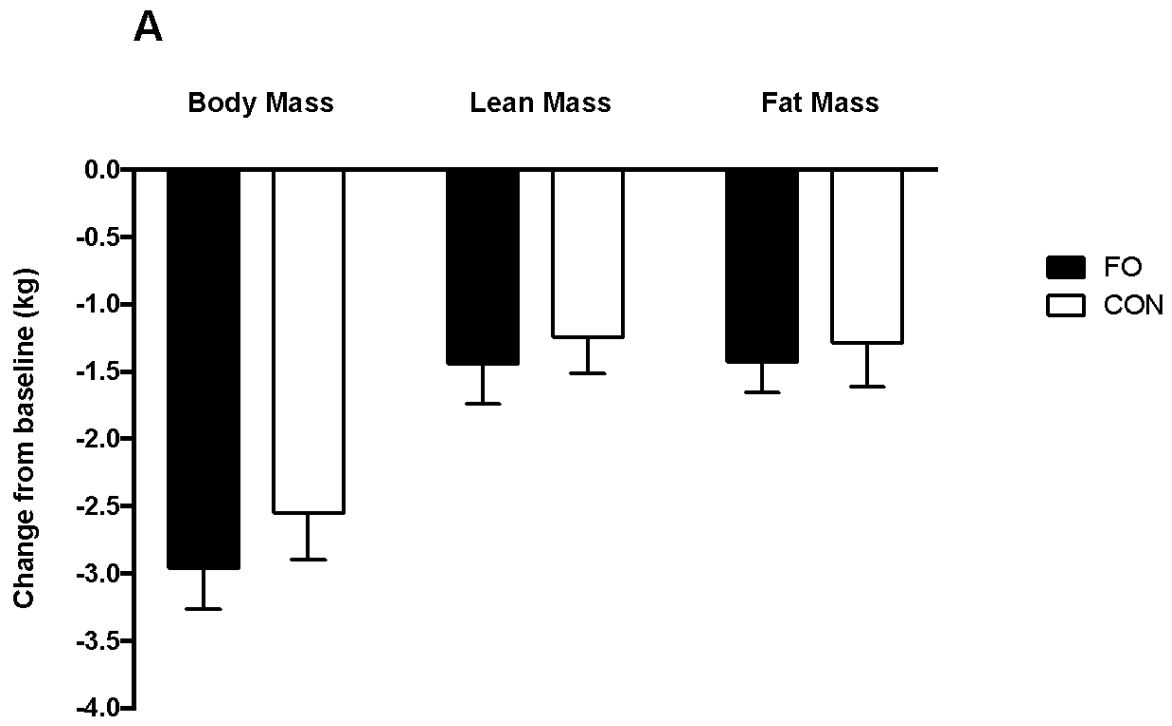
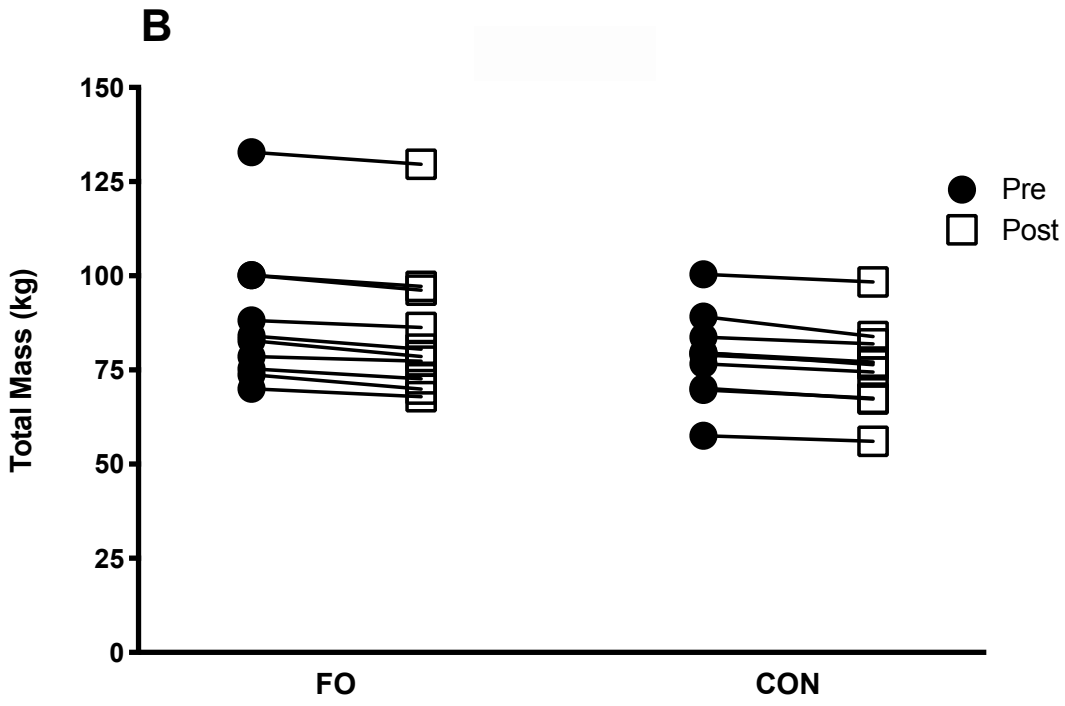


Figure 3.3 - Blood % n-3PUFA/totalPUFA composition before (Pre) and after (Post) 6 wk of supplementation. Data are expressed as means ± SD and also as individual values. *Significant difference vs. baseline (Pre) in corresponding supplement group.

3.4.4 Body composition

Total body mass (pre: 83.6 ± 3.6 kg; post: 80.8 ± 3.5 kg, $p < 0.001$), lean body mass (pre: 64.4 ± 2.3 kg; post: 63.0 ± 2.3 kg, $p < 0.001$) and fat mass (pre: 15.8 ± 1.6 kg; post: 14.4 ± 1.6 kg, $p < 0.001$) for all participants, all decreased from baseline (pre) following 2 weeks of energy restriction (**Figure 3.4A**), with no differences between conditions. Individual changes in body mass, lean body mass and fat mass are presented in **Figure 3.4B-D**.

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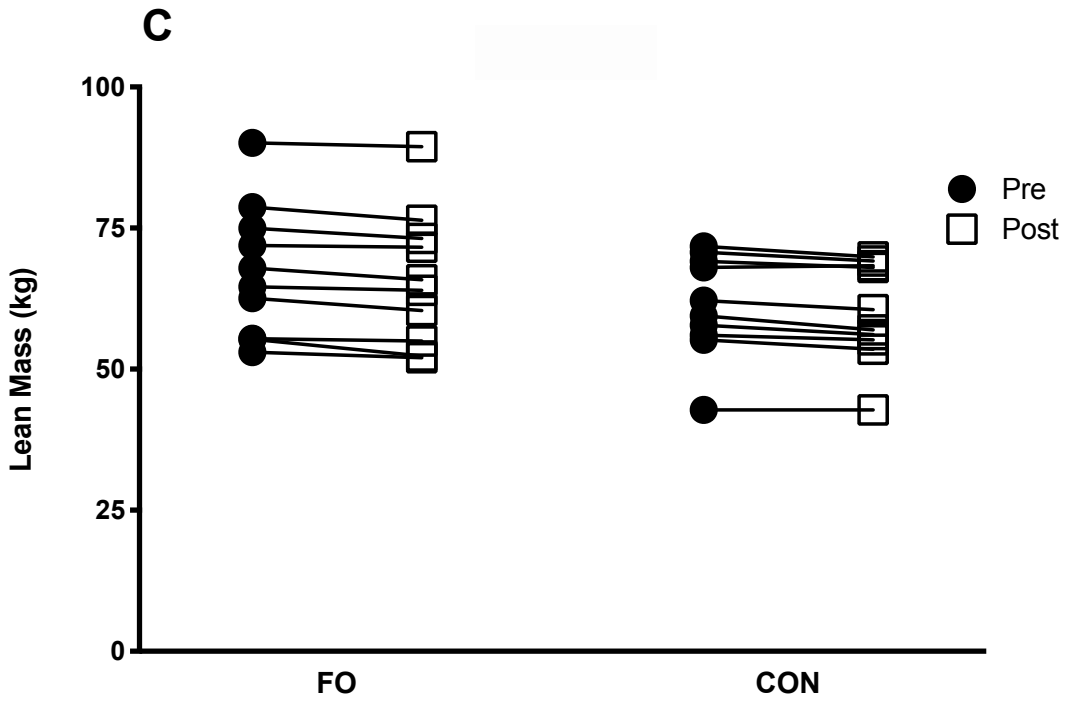
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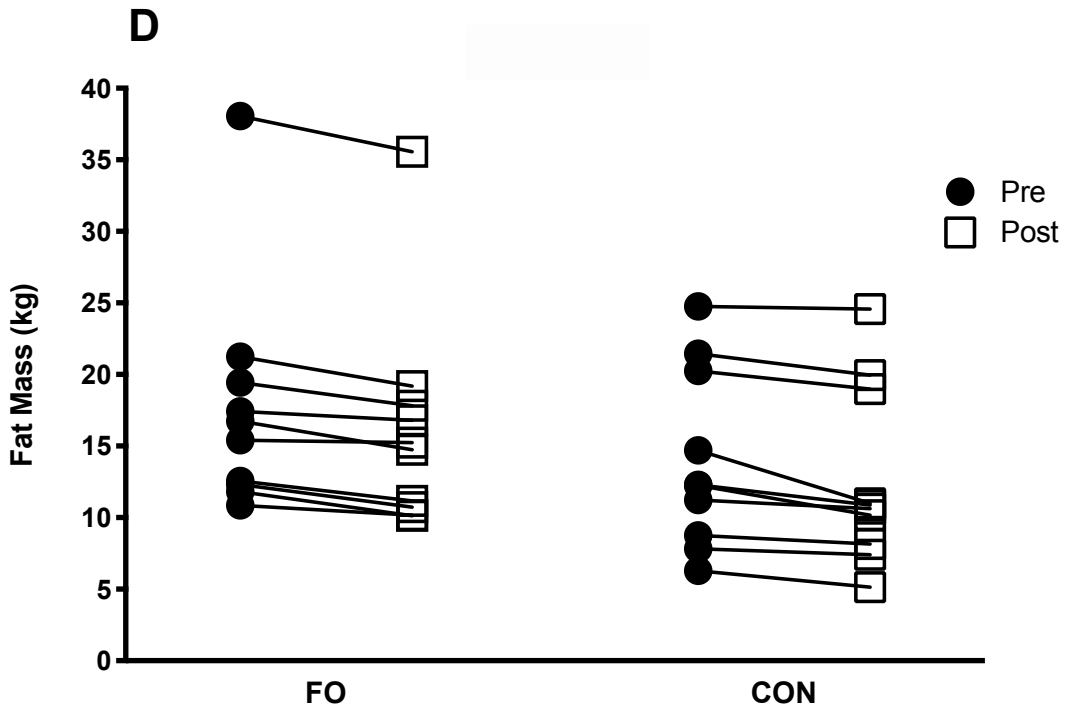

Figure 3.4 – Group (A) and individualised changes in total body mass (B), lean body mass (C), and fat mass (D) from baseline (average of the two measurements collected during wk 1 (Habitual diet) and 4 (100% diet) prior to weight loss) following 2 wk of 40% energy restriction in fish oil (FO) and control (CON) supplement groups. Values are means ± SEM.

3.4.5 Muscle strength

Leg press and leg extension 1RM remained constant between week 1 and week 4 for both dominant and non-dominant legs (all $p > 0.30$, **Figure 3.5**). Leg extension 1RM for the non-dominant leg increased by 6.1 ± 3.4 % following energy restriction (weeks 4-6) compared with energy balance (weeks 0-4) in FO ($p < 0.05$, $d = 0.29$), with no changes in CON across the 6 week period. Leg extension 1RM for the dominant leg tended to increase following energy restriction in FO ($p = 0.092$, $d = 0.29$), whereas no changes were observed in CON. No differences in leg press 1RM for either leg were observed between weeks 4 (pre 100% diet period) and 6 (post 60% diet period) in either supplement group.

There were no differences in MVC for the dominant leg across diet periods or between supplement groups (**Figure 3.6**). MVC for the non-dominant leg decreased by 5.7 ± 7.9 % from week 1 to 4 ($p = 0.03$, $d = 0.31$) and by 7.4 ± 11.8 % from week 1 to 6 ($p = 0.016$, $d = 0.42$), with no differences between supplement groups.

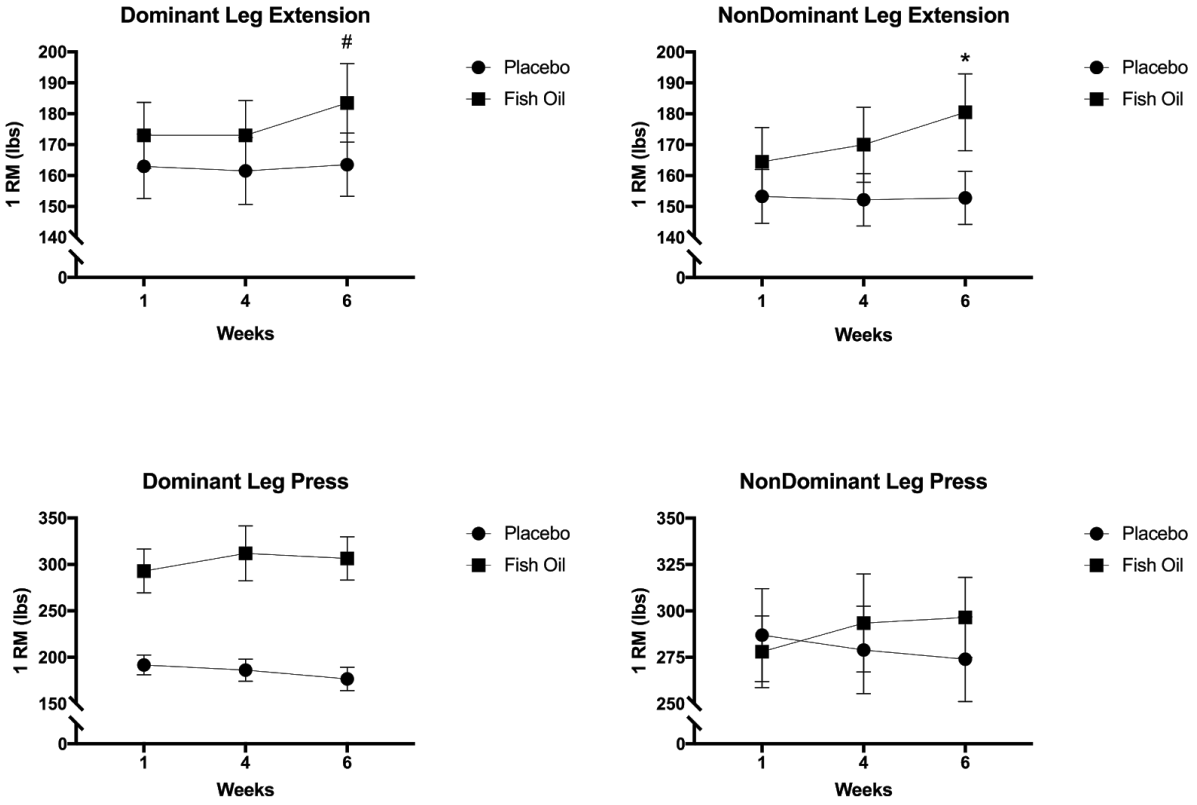

Figure 3.5 – One repetition maximum for (A) dominant leg extension, (B) non-dominant leg extension, (C) dominant leg press and (D) non-dominant leg press during wks 1 (habitual diet), 4 (100% diet) and 6 (60% diet) of study. Shaded area represents the 2 wk period of energy restriction. Values are means ± SEM. *Significant difference compared to wks 1 and 4 in corresponding supplement condition ($p < 0.05$).

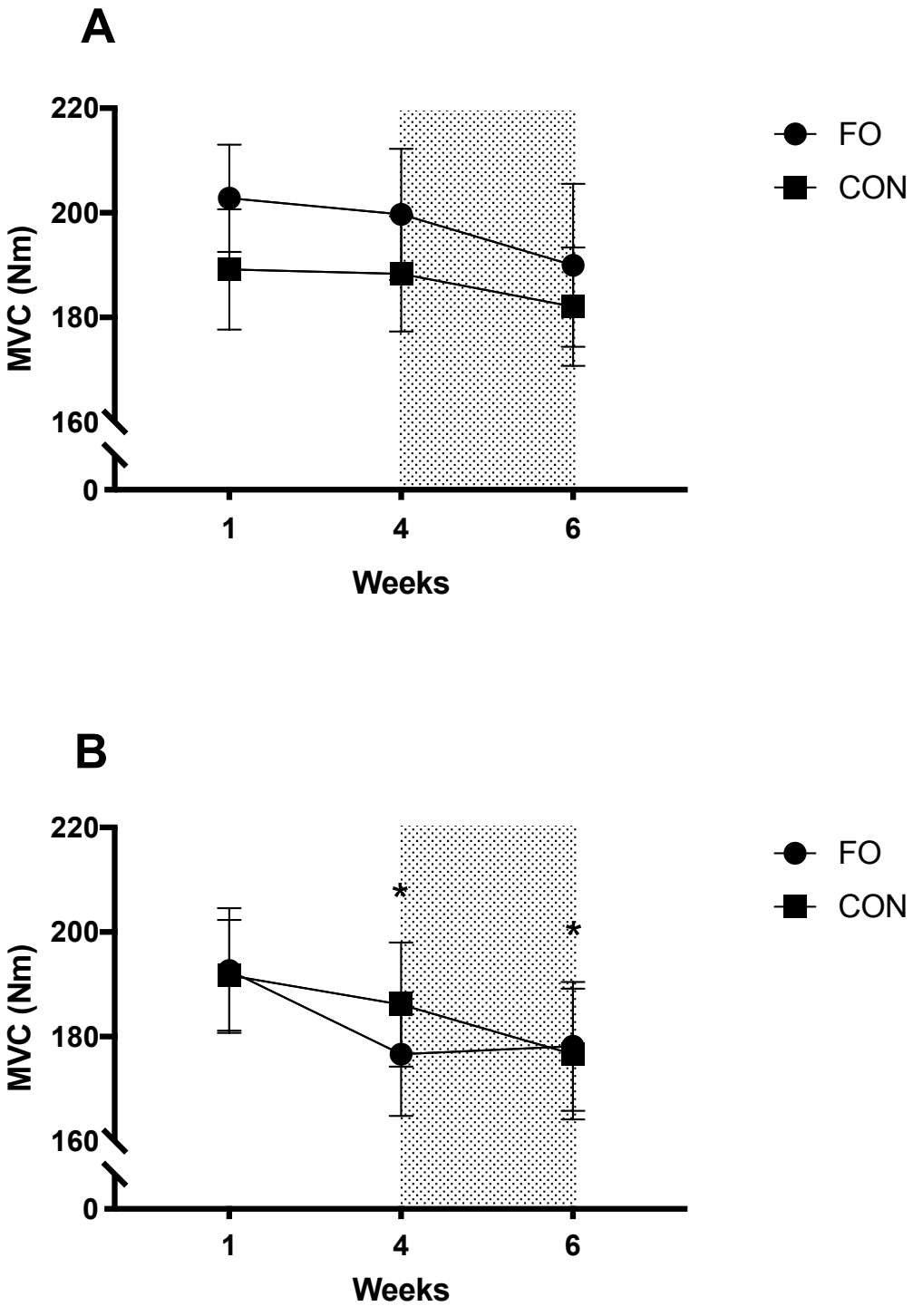

Figure 3.6 – Maximum Voluntary Contraction of dominant and non-dominant legs during wk 1 (habitual diet), wk 4 (100% diet) and wk 6 (60% diet). Shaded area represents 2 wk period of energy restriction. Values are mean ± SEM. A, dominant leg. B, non-dominant leg. *Significant difference compared to week 1.

3.4.6 Muscular Endurance

There were no differences in muscular endurance across across diet periods or between supplement groups in either the dominant or non-dominant leg for leg extension or leg press exercises (all $p > 0.05$, **Figure 3.7**).

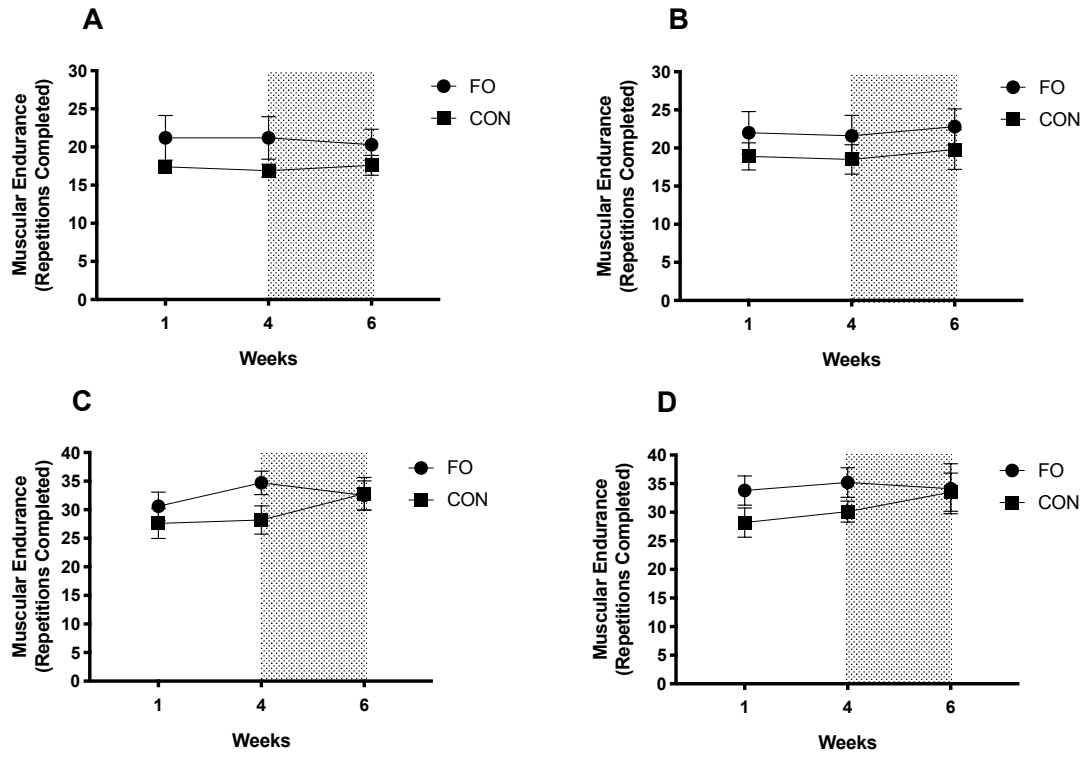

Figure 3.7 – Muscular endurance for (A) leg extension on dominant leg, (B) leg extension on non-dominant leg, (C) leg press on dominant leg, and (D) leg press on non-dominant leg during wks 1 (habitual diet), 4 (100% diet) and 6 (60% diet) of study protocol. Shaded area represents a 2 wk period of energy restriction. Values are means ± SEM.

3.5 Discussion

The primary aim of this study was to investigate the influence of dietary fish oil-derived n-3PUFA supplementation on changes in body composition and muscle strength during a short-term period of weight loss in resistance-trained young men. Our findings indicate that n-3PUFA supplementation resulted in a partial improvement in muscle strength following 2 wk of 40% energy restriction, i.e. a small improvement in 1RM leg extension in FO, but changes in MVC we similar between FO and CON groups. However, refuting our original hypothesis, n-3PUFA supplementation failed to modulate changes in body composition or attenuate the decline in muscle endurance induced by short-term weight loss. The practical implications of these preliminary data remain unclear, but suggest that dietary n-3PUFA supplementation may maintain, if not improve, some components of muscle strength during short-term weight loss in athletes competing in weight category sports and/or sports that depend on a high power-to-body mass ratio. However, the causal mechanism(s) that underpin this muscle adaptive response does not appear to be related to the modulation of lean body mass.

Changes in muscle strength induced by diet and exercise training are often associated with changes in muscle mass. Despite the improvement in 1RM leg extension with n-3PUFA supplementation following the 2 wk period of diet-induced weight loss, the decline in fat-free mass was comparable between supplement groups. Our laboratory previously demonstrated that 4 wks of fish oil supplementation markedly increased n-3PUFA concentrations in the muscle cell (McGlory et al., 2014). The uptake of n-3PUFA into the muscle cell membrane has been suggested to prime the muscle translational machinery inside the cell to respond to anabolic stimuli in both young (Smith et al., 2011b) and older (Smith et al., 2011a) adults. Moreover, a recent study demonstrated that fish oil supplementation attenuated the decline

in muscle mass following 2 wk of limb immobilisation in trained young women, as mediated by a greater integrated response of MPS (McGlory et al., 2019). Given that the primary locus of control for regulation muscle mass in resistance-trained young is MPS (Atherton & Smith, 2012), we hypothesised that any improvement in muscle strength during weight loss with n-3PUFA supplementation would be mediated by the preservation of lean body mass. However, in the present study, non-dominant leg extension 1RM increased by >6% with n-3PUFA supplementation following 2 wk of diet-induced weight loss, despite a 1.4 kg decrease in FFM. This apparent disconnect between muscle strength and FFM is not uncommon (Dankel et al., 2017) and cannot be explained from this proof-of-principle study. Nonetheless, even if the incorporation of n-3PUFA into phospholipid layer of the muscle cell membrane led to an upregulation of the muscle protein synthetic machinery, it did not appear to mediate the improvement in muscle strength observed following diet-induced weight loss in the n-3PUFA group.

Suring extreme periods of catabolism, as experienced in the present study, it may be possible that dietary fatty acids may be used for energy production rather than anabolic processes such as MPS. Previous research has found that calorie restriction results in an increase in fatty acid oxidation (Bruss et al., 2010). An increase in fatty acid oxidation leads to a further increase in ATP production, a molecule that provides energy for metabolism throughout the body. Due to the extreme situation of a 40% reduction in calories, the fate of fatty acid maybe be shifted towards fatty acid oxidation to produce energy not available due to the calorie restriction. The shift towards energy production may explain the lack of differences in lean mass changes between the high fat group and the low fat group. Further studies, should examine fatty acid oxidation during calorie restriction with an increase in dietary fat.

A feasible alternative explanation for the improvement in 1RM leg extension following energy restriction with n-3PUFA supplementation, in the absence of any changes in lean body mass, may relate to neuromuscular function. Consistent with this notion, a previous study demonstrated a reduction in electro-mechanical delay, defined as the time taken for a specific muscle to respond to a stimulus, with n-3PUFA supplementation, albeit in older adult women (Rodacki et al., 2012). From a mechanistic standpoint, DHA is an essential component of the phospholipid membrane of neurons localised in brain tissue (Lauritzen et al., 2001). Moreover, previous animal and human studies have reported strong associations between increased DHA concentrations in brain tissue (Brenna et al., 2009), improvements in brain function and increased muscle strength (Pentikainen et al., 2017). In the present study, we report a marked increase in blood n-3PUFA concentrations following 4 wk of n-3PUFA supplementation containing 2g of DHA per day. On the basis of comparable findings using a rodent model (Valentini et al., 2017), it is reasonable to assume that DHA concentrations also increased in the neural tissue of our resistance-trained men. Although speculative, these data provide preliminary support for the notion that neuromuscular mechanisms may underpin the current observation of a maintenance, if not increase, in muscle strength following an energy-restricted diet in the n-3PUFA group.

The divergent response of leg press strength and leg extension strength to weight loss between groups further supports the notion that n-3PUFA supplementation enhanced neural adaptations. Although we observed an improvement in leg extension 1RM with n-3PUFA supplementation, no difference in strength was observed for leg press 1RM between groups. A possible explanation for this differential finding relates to the recruitment and activation of different muscle groups between exercises. Based on electromyography data, only the quadricep muscle group is activated during leg extension (Jakobsen et al., 2012), whereas

multiple muscle groups, including gastrocnemius, quadriceps and gluteus maximus are activated during the leg press (Da Silva et al., 2008). We report a regional decline in FFM in both left and right legs following weight loss in both groups (supplementary table). Expressed relative to total muscle mass activated during the exercise test, the decline in FFM in muscles activated during the leg extension was less than leg press. Hence, we speculate that the incorporation of DHA into neural tissue altered the interaction between the central nervous system and muscle tissue, potentially improving firing rate and recruitment of motor neurons during the 1RM leg extension. Further research is warranted to substantiate this notion and to examine the influence of n-3PUFA supplementation on neuromuscular activity during leg press and leg extension exercises both under conditions of weight maintenance and weight loss.

The effective modification of body composition during weight loss with nutrition, and more specifically with manipulation of dietary protein, has been demonstrated previously in both clinical (Mojtahedi et al., 2011; Kempen et al., 1995) and athletic (Walberg et al., 1988; Areta et al., 2014) populations. In this regard, the protein content of an energy-restricted diet has been shown to modulate the magnitude of muscle loss during weight loss (Mettler et al., 2010; Longland et al., 2016). Recent evidence suggests an interactive effect of protein and n-3PUFA in the regulation of muscle protein metabolism. For example, Smith et al. (2011b) demonstrated a potentiated response of MPS to the intravenous infusion of amino acids following 8 weeks of n-3PUFA supplementation in young adults. Based on this observation, it is possible that a higher dietary protein intake was required in the present study for n-3PUFA supplementation to elicit a protective effect on lean body mass during weight loss. By design, in the present study dietary protein intake was reduced during the 2 wk weight loss period in proportion to the energy deficit imposed. The basis for this methodological decision was to

examine a proof-of-concept, i.e. testing the impact of n-3PUFA supplementation on body composition and muscle strength during weight loss rather than saturate any beneficial response with a high protein intake. Accordingly, during the energy restriction period, protein intake was 5% lower in FO and 7% lower in PLA compared to the habitual diet, equating to a reduction of 310 and 340 kcals, respectively. We also cannot discount the possibility that the combination of an energy deficit and reduction in dietary protein intake may have potentiated the decline in lean body mass during weight loss in both groups, thus countering any potential enhancement of n-3PUFA on MPS. Consistent with this notion, a negative nitrogen balance was reported for at least 10 days during a period of reduced protein intake (Quevedo et al., 1994). This negative nitrogen balance is indicative of a net loss of protein at the whole-body level. Conversely, increasing the protein content of the diet is known to increase nitrogen balance during energy restriction (Walberg et al., 1988). The measurement of nitrogen balance was beyond the scope of the present study. However, based on previous work (Oi et al., 1987), we speculate that participants in both supplement conditions were in negative nitrogen balance. Future research in athletes is warranted to examine the influence of n-3PUFA supplementation on changes in body composition and muscle performance during energy restriction within a more practical situation when combined with a protein intake that meets recently published guidelines (Witard, Garthe & Philips, 2018).

Although there are many strengths to the present study, including dietary control and blood omega-3 concentrations, there are also limitations. Firstly, DEXA was the only measure of body composition and we acknowledge that DEXA measurements alone are not sufficient for the accurate assessment of muscle mass (Wilson 2013). Therefore, although all attempts were made to standardize the DEXA protocol (i.e. body positioning, hydration status), we cannot discount the possibility that our study incurred a type II statistical error with regards

to examining the influence of FO supplementation on changes in body composition during short-term weight loss. Combining DEXA with measurements of bioelectrical impedance and air displacement plethysmography for calculating the 4-compartmental model of body composition may provide more accurate results. Secondly, the participants that took part in this study were not a homogenous group. Although participants were resistance trained for at least 6 months, there were individuals with different resistance abilities with different experience as well as being different types of athletes, i.e. power athletes or team-sports athletes. Thirdly, although we were able to fully control diet, we could only reliably control diet during the energy restriction phase for two weeks. A longer period of energy restriction may have allowed for further differences between the groups to have been observed.

To conclude, dietary supplementation with 4g/d of n-3PUFA may maintain, or even improve, leg extension 1RM strength following 2 wk of energy restriction. However, this strength adaptation was not mediated by the increased preservation of lean body mass during diet-induced weight loss. The practical application of these preliminary data remains unclear but implies a potential role for n-3PUFA supplementation in improving muscle performance during weight loss in athletic populations. However, follow-up mechanistic work is warranted to establish the influence of n-3PUFA supplementation on changes in body composition, muscle performance and neuromuscular function during more prolonged periods of energy restriction in athletic populations.

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Chapter 4 - Coingesting curcumin with fish oil fails to facilitate the incorporation of omega-3 polyunsaturated fatty acids into blood or skeletal muscle tissues in healthy young men

4.1 Abstract

A 2-4 week period of fish oil supplementation is required to detect physiological changes in the incorporation of omega-3 polyunsaturated fatty acids (n-3PUFA) into red blood cells and the phospholipid membrane of skeletal muscle cells, respectively. This latency period in n-3PUFA uptake may be partially attributed to elevated oxidation levels. Therefore,

the primary aim of this study was to investigate the influence of adding curcumin as an antioxidant compound to fish oil on the time-course response of n-3PUFA incorporation into whole red blood cells and the phospholipid membrane of skeletal muscle cells. Sixteen healthy, recreationally active, males were assigned to one of two supplement groups. Group A consumed supplements containing 3.6 g/day of n-3PUFA plus 1 g/day of curcumin (FO+AO) and group B consumed supplements containing 3.6 g/day of n-3PUFA only in fish oil form (FO). Both groups consumed 2 × 200 mL beverages daily. Venous blood samples were collected weekly over the entire supplementation period and muscle biopsies were collected at baseline, and at weeks 2 and 6 for assessment of changes in lipid composition. Muscle function, electromyography and electromechanical delay were measured in the quadriceps muscles of both legs at baseline and at weeks 3 and 6 after beginning supplementation. The % EPA and DHA/ total fatty acids in whole red blood cells increased from baseline by 115% at week 3 in both groups ($p < 0.001$), with no further changes during weeks 3-6 ($p = 0.186$). The % EPA and DHA/ total fatty acids in the muscle phospholipid membrane increased from baseline by 61% at week 2 in both groups ($p < 0.001$), with a further 51% increase observed from weeks 2-6 in both groups ($p < 0.001$). Maximal voluntary contraction and electromyography remained unchanged over the 6 week supplementation period in both groups. Electromechanical delay did not change throughout the 6 week period apart from in the *vastus lateralis* of the non-dominant leg which decreased by 21% at week 3 in both groups, but returned to baseline at week 6. To conclude, adding curcumin to a fish oil-based supplement failed to modulate the time-course response of n-3PUFA incorporation into whole red blood cells or the phospholipid membrane of skeletal muscle cells, translating into no changes in muscle function.

4.2 Introduction

Omega-3 polyunsaturated fatty acid (n-3PUFA) supplementation has been associated with a myriad of health benefits, including improvements in heart (Bhathena et al., 1991) and brain function (Dyall and Michael-Titus, 2008). The anti-inflammatory properties of n-3PUFA are often proposed to mediate these health benefits (Li et al., 2005). More recently, dietary n-3PUFA supplementation has been linked with improvements in muscle function

(Ochi, Tsuchiya and Yanagimoto, 2017) and neuromuscular function (Lewis et al., 2015), as well as modulating muscle protein metabolism in healthy individuals (Smith et al., 2011; Lalia et al., 2017; McGlory et al., 2019). In this regard, fish oil derived n-3PUFA supplementation was shown to increase the stimulation of muscle protein synthesis in response to a single bout of resistance exercise (Lalia et al., 2017). This upregulated response of muscle protein synthesis was observed in parallel with an increase in n-3PUFA composition in the muscle phospholipid membrane (Smith et al., 2011). Given the potential role of n-3PUFA supplementation in regulating muscle protein metabolism, there is increasing interest in strategies to increase the magnitude and rate of n-3PUFA incorporation into skeletal muscle with fish oil supplementation.

The profile of tissue n-3PUFA incorporation has been extensively characterized across several tissues, including erythrocytes, mononuclear cells and adipose tissue (Browning et al., 2012; Fielding 2011). Conversely, information regarding the uptake and incorporation of n-3PUFA into skeletal muscle is limited to few studies (McGlory et al., 2014, Metherel et al., 2009). McGlory et al (2014) demonstrated that 1 wk of high dose (5g/day) fish oil supplementation was required to increase n-3PUFA composition in whole blood cells, with further increases in blood n-3PUFA composition after 2 wks of fish oil supplementation. However, no further increase in whole blood cell n-3PUFA composition was observed from wks 2 to 4. By comparison, 2 wks of n-3PUFA supplementation was required to increase n-3PUFA composition in skeletal muscle cells with further increases in skeletal muscle n-3PUFA composition after 4 wks of fish oil supplementation (McGlory et al., 2014). The supplementation period in this study was truncated at 4 wks. Thus, the duration of fish oil supplementation required to maximize the incorporation of n-3PUFA into skeletal muscle cells remains unknown. Moreover, in this previous study (McGlory et al., 2014), n-

3PUFA composition were measured in whole skeletal muscle cells, rather than the phospholipid membrane of the skeletal muscle cell that is implicated in regulating lipid raft size and resolvin synthesis involved in the reduction of inflammation (Rockett et al., 2012; Mas et al., 2012). Taken together, these data suggest that during the initial stages of a fish oil supplementation regimen, the resulting change in muscle n-3PUFA composition is slower compared with the blood, reflecting the divergent turnover rates between blood and muscle tissue n-3PUFA composition.

The metabolic fate of exogenous n-3PUFA also includes degradation via ß-oxidation (Fielding, 2011) whereby, in the presence of oxygen, two carbon fragments are cleaved from fatty acids to generate acetyl-CoA (Houten & Wanders, 2010). This acetyl-CoA compound then enters the tricarboxylic acid cycle for complete oxidation. The majority of body tissues, including skeletal muscle, degrade fatty acids via the process of ß-oxidation. Therefore, by reducing n-3PUFA oxidation, it is theoretically possible to increase the magnitude and rate of n-3PUFA incorporation into whole blood cells and the phospholipid membrane of skeletal muscle tissue during a period of fish oil supplementation.

Curcumin is a yellow pigment commonly found in turmeric and is a member of the ginger family. Traditionally used in South Asian cooking, curcumin exhibits profound antioxidant properties (Wright, 2002). Cellular damage caused by oxidative stress can be caused by reactive oxygen species. While low levels of reactive oxygen species act as signaling molecules for cells, elevated levels of reactive oxygen species can initiate an inflammatory response. Curcumin displays antioxidant properties, primarily due to their free-radical scavenging activity, reducing the abundance of circulating free radicals (Deogade and Ghate, 2015). This upregulated free-radical scavenging activity occurs mainly due to the structure of curcumin, having two phenolic groups and methoxyl groups.

Therefore, the capacity for curcumin to reduce oxidation may increase the uptake and incorporation rate of n-3PUFA into whole blood and the phospholipid membrane of skeletal muscle.

The primary aim of this study was to investigate the influence of adding curcumin as an antioxidant nutrient to a fish oil based supplement on the time-course of incorporation of n-3PUFA into the phospholipid membrane of whole blood cells and skeletal muscle cells. The secondary aim was to investigate the influence of adding curcumin to a fish oil supplement on muscle function and neuromuscular function over a 6 wk study period. We hypothesised that 6 wks of combined fish oil and curcumin supplementation would facilitate the tissue incorporation (magnitude and rate) of n-3PUFA into whole blood cells and the phospholipid membrane of skeletal muscle cells, as well as promote improvements in muscle function and neuromuscular function compared with a fish oil only supplement condition.

4.3 Methods

4.3.1 Participant Details

Sixteen healthy, recreationally active males (aged 23.3 ± 3.2 yrs; body mass 78.7 ± 4.2 kg; height 181.1 ± 7.5 cm) who participate in exercise at least twice per week, were recruited from the local area to participate in the present study. Eligibility criteria included participants who were not consuming n-3PUFA containing supplements at the time of study enrolment.

Participants were asked to continue their habitual training throughout the 6-week study period. The University of Stirling Research Ethics Committee approved the study procedures. Written, informed consent was obtained prior to commencing the experiment.

4.3.2 Study Design

Using a double-blinded parallel study design, participants were assigned to one of two groups: a combined fish oil and antioxidant containing supplement group (FO+AO) or a fish oil only supplement group (FO). Participants consumed their assigned supplement twice daily and maintained their habitual diet throughout the 6 week period. The trial involved participants visiting the laboratory once per week for 6 weeks, totalling seven laboratory visits. On arriving at the laboratory on the baseline visit (week 0), participants completed assessments of maximum voluntary contraction and electromechanical delay. Initial baseline assessment of skeletal muscle and blood lipid profiles also were conducted at week 0 to determine the n-3PUFA composition of the skeletal muscle phospholipid membrane and red blood cell composition. Participants underwent blood tests to measure whole blood n-3PUFA concentrations on each visit to the laboratory. Phospholipid membrane n-3PUFA skeletal muscle concentrations were measured at baseline and at weeks 2 and 6. Maximum voluntary contraction (MVC) and EMD were measured at weeks 0, 3 and 6.

4.3.3 Dietary supplementation

In a single blinded fashion, participants were assigned to one of two supplement groups. Group A consumed supplements containing 3.6 g/day of n-3PUFA and 1 g/day of curcumin (FO+AO) and group B consumed supplements containing 3.6 g/day of fish oil only (FO). Participants consumed 2 × 200 mL volume juice-based drinks (1 × morning and 1 × evening) daily over the 6 week supplementation period (Smartfish Sports Nutrition, Ltd).

Participants were asked to return all empty cartons to ensure compliance of supplementation.

4.3.4 Blood sampling and n-3PUFA analysis

Blood samples were obtained from an antecubital vein using the venepuncture method. All blood samples were drawn into evacuated 2 mL vacutainers containing serum (Vacutainer Systems, Becton, Dickson and Company, UK). Whole blood samples were then dispensed as two circular collection spots on Whatman 903 blood collection cards (GE Healthcare Ltd., Forest Farm Industrial Estate, Cardiff, CF 14 7YT, UK). The cards were left open and allowed to dry for 3 h, after which the dried whole blood sample was detached from the collection device using forceps and placed into a screw-cap vial containing 2 mL of methylating solution (1.25 M methanol/HCl). The vials were placed in a hot block at 70 1C for 1 h before being allowed to cool to room temperature. Next, 3 mL of iso-hexane + 0.01% BHT and 4 mL of 50 % saturated KCl solution were added to the vials and shaken. Thereafter, 2.5ml of the top organic phase was washed with 5ml of iso-hexane and the fatty acid methyl esters (FAME) were washed with 5 mL of iso-hexane/diethyl ether (95:5). Finally, the solvent was evaporated under nitrogen before the FAME was redissolved in 200µl of iso-hexane and transferred to the gas chromatograph (GC) for analysis.

4.3.5 Muscle n-3PUFA composition analysis

Total lipid content was extracted from the muscle by modifying a method described previously (Folch, Lees and Sloane Stanley, 1957). In brief, frozen muscle biopsy samples (20–60 mg) were placed in a pre-weighed reacti-vial and an accurate reading tissue mass recorded to 5 decimal places. The reacti-vials were capped and placed on ice and then 1 mL of

chloroform/methanol (C:M, 2:1) was added to each vial. Each muscle sample was homogenised using a hand-held IKA-Werke Ultra-turrax T8 homogeniser (Fisher, Loughborough, UK). The probe was rinsed with 3 mL of C:M, 2:1, and the solution was added to the reacti-vial before being placed on ice for 1 h. Next, 1 mL 0.88% KCl was added to the solution, shaken and allowed to stand for 10 min to remove non-lipid impurities. The vials were then centrifuged at 400 *g* for 5 min before removal of the aqueous layer. The lower solvent layer was removed using a Pasteur pipette and filtered through a 5.5 mm Whatman No. 1 filter, pre-washed with C:M (2:1) into a 7 mL bottle, and dried under nitrogen. The lipid was then re-dissolved in 1 mL of C:M, 2:1, and transferred to a preweighed 1.7 mL bottle. The 1.7 mL bottle was then rinsed with 0.5 mL C:M, 2:1, and this was added to the 1.7 mL bottle. The lipid was dried under nitrogen and desiccated overnight in a vacuum desiccator after which the lipid was reweighed and dissolved in 0.5ml of C:M, 2:1+0.01% butylated hydroxyl toluene (BHT) at a concentration of 2 mg mL^{1}. Next, 50 mg of each lipid was placed 1.5 cm from the bottom of a 20 × 20 cm thin-layer chromatography plate 2 cm apart f, before the plate was developed in a glass tank of iso-hexane/diethyl ether/acetic acid (80:20:1). The plate was then sprayed with 0.1% 2,7-dichlorofluorescein in 97% aqueous methanol following development and the phospholipid bands were marked under UV light. Each phospholipid band was scrapped into a 15 mL test tube. Thereafter, 2 mL of 1% sulphuric acid in methanol was added to each tube before being flushed with nitrogen and incubated on a hot block at 50̊ C overnight. The following day the tubes are cooled to room temperature before 2.5 ml of potassium bicarbonate and 5ml of iso-hexane/diethyl ether + 0.01 BHT are added to each tube. The tubes were shaken and then centrifuged for 3 minutes at 1400 rpm. The upper organic phase was transferred to a clean test tube. A total of 5mL of iso-hexane/diethyl ether was re-extracted to the lower aqueous phase before being shaken and centrifuged as detailed

above. The second upper phase was combined with the first extraction before being dried under nitrogen. Finally, 0.2 mL of iso-hexane + 0.01 BHT was re-dissolved in the methyl esters before being transferred to 0.3 mL GLC autosampler vials. These vials were transferred onto the GC for analysis.

4.3.6 MVC, EMG and EMD

A single leg isometric/eccentric maximum voluntary contraction (MVC) of the knee flexors was used to assess muscle function, electromyography (EMG) and brain function (EMD). Participants were seated on the dynamometer with their upper body, hips and exercising thigh securely strapped into the seat. The lower leg was attached to the arm of the dynamometer 1 cm above the lateral malleolus ankle joint with the axis of rotation of the dynamometer arm aligned with the lateral femoral condyle. Upon hearing a buzzer, participants maximally contracted, producing an MVC force measurement. EMG activity was assessed using WhiteSensor WS ECG surface electrodes (Ambu Ltd., St Ives, UK) with an intra-electrode distance of 2 cm positioned over vastus lateralis and vastus medialis muscles. Prior to electrode placement, the quadricep area of interest was shaved and abraded as per Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines. The dynamometer arm was set at a 60° knee flexion (0° = full knee extension). 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 1.0 Hz high band filters. Signals were analyzed offline (Acqknowledge, v3.9.1.6, Biopac Systems, Inc. Goleta, CA, USA). Muscle activation was evaluated by calculating EMG root mean square (RMS) from the maximum voluntary contraction. RMS was analysed for all three MVC and the peak value was selected

for analysis. EMD was calculated from the time difference between the activation of the muscle and the onset of force from 2 standard deviations.

4.3.7 Data presentation and statistical analysis

Data were analyzed using Statistical Package for Social Sciences 21 (IBM SPSS, Chicago, IL). Shaprio-Wilk test was performed to analyse for normality. Differences across time for blood n-3PUFA concentrations, skeletal muscle phospholipid membrane n-3PUFA concentrations, MVC, EMG and EMD were analyzed by a mixed-design, two-way repeated measures (time × group) ANOVA with two between-subject factors (FO+AO and FO) and either 7 for blood n-3PUFA concentrations or 3 for skeletal muscle phospholipid membrane n-3PUFA concentrations, MVC, EMG and EMD within-subjects factors (time). Where a significant main effect of time or time × group interaction between-groups was detected, Tukey posthoc tests were performed to detect the time-points at which differences between supplement groups existed. Statistical difference was assumed at the level of ≤0.05. All data are expressed as means ± SEM.

4.4 Results

4.4.1 Time-course of lipid changes in blood

A full breakdown of blood fatty acid profiles is presented in Table 4.1 for the FO+AO group and Table 4.2 for the FO group. The % EPA/ total fatty acid content and % DHA/ total fatty acid content followed a similar trend over the 6 week timeframe, increasing by 183% in

EPA and 45 % DHA from week 0 to week 1 ($p < 0.001$). From weeks 1 to 2 there were further increases by 13% in EPA and 12 % DHA ($p < 0.001$). However, no further increase in % EPA/ total fatty acid content or % DHA/ total fatty acid content was observed from weeks 3-6 (Figure 4.1 A, Figure 4.1 B). Moreover, the differences in the timecourse or amplitude of % EPA/ total fatty acid or % DHA/ total fatty acid content was not different between groups.

Expressed as % EPA + DHA/ total fatty acids, values increased by 77% from week 0-1 ($p<0.001$), by 12%from week 1-2 ($p < 0.001$), and by 8% from week 2-3 ($p < 0.001$), with no further changes from week 3-6 ($p = 0.135$, figure 4.1 C). Moreover, there were no differences in the time-course of % EPA + DHA/ total fatty acids between groups.

Expressed as % total n-3PUFA/ total fatty acids, values increased each week from baseline up until week 3. No change in % total n-3PUFA/total fatty acids was observed from weeks 3-6 (Figure 4.1 D). There were no differences between the supplement groups. The % n-3 HUFA/ total HUFA increased from week 0-1 by 39% and continued to increase until week 4 before plateauing to week 6 (Figure 4.1 E).

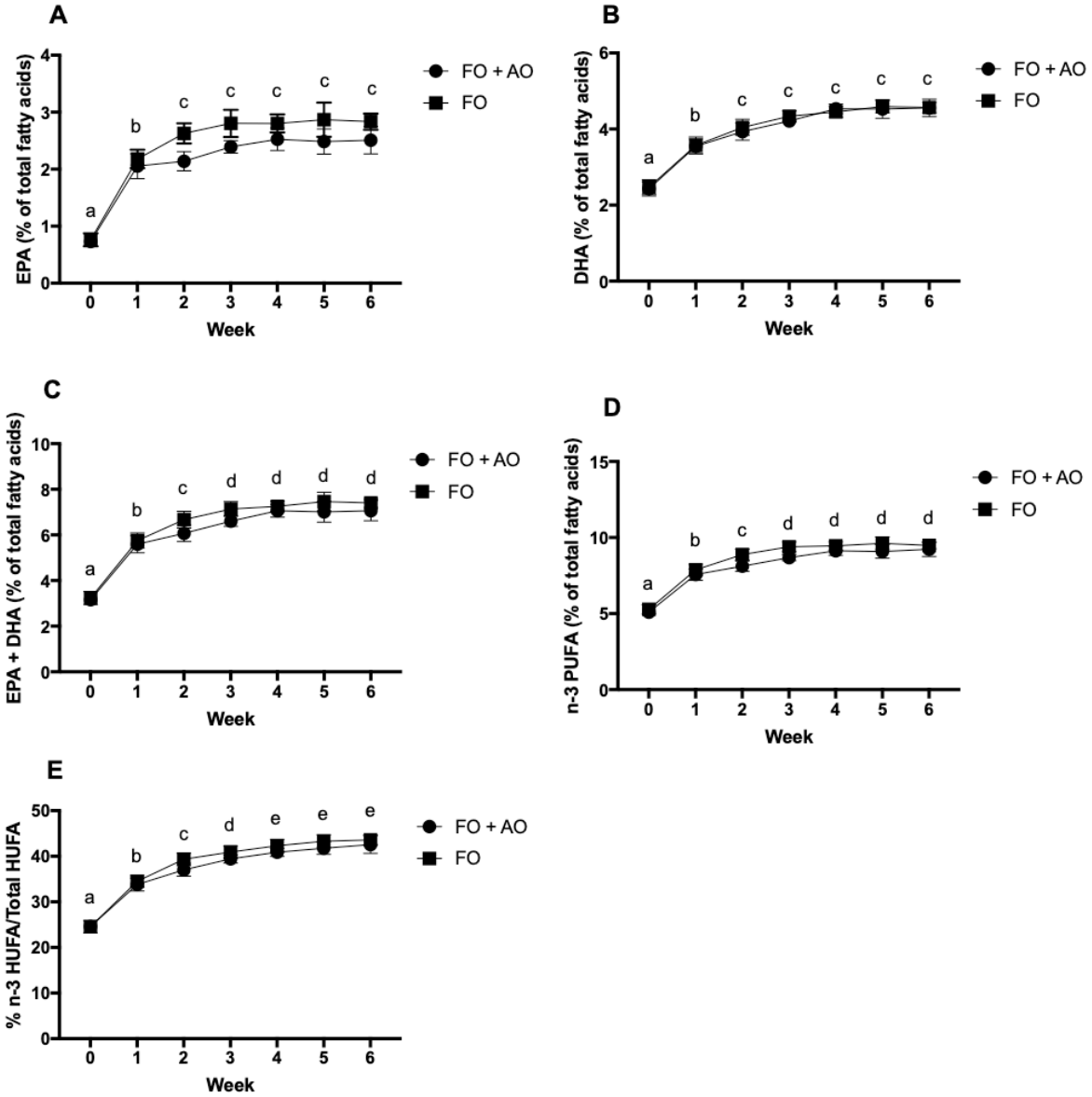

Figure 4.1 - Blood lipid changes over the 6 week study protocol. Data are expressed as means ± SEM. *A*, Eicosapentanoic acid (EPA). *B*, Docosahexaenoic acid (DHA). *C*, EPA + DHA. *D*, n-3 polyunsaturated fatty acid (n-3 PUFA). *E*, % n-3 highly unsaturated fatty acids to total highly unsaturated fatty acids (n-3 HUFA/ Total HUFA). FO+AO, Fish oil and antioxidant containing supplement beverage, FO, Fish oil only supplement beverage. Means that do not share the same letter are significantly different.

Table 4.1 - Blood lipid composition at week 0 to week 6 of participants in the FO+AO group throughout the 6 week study period. Values are percentage of total lipid composition presented as means ± SEM. *PUFA*, polyunsaturated fatty acids. *DMA*, dimethyl aldehyde.

Fatty acid	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
14:0	0.5 ± 0.07	0.47 ± 0.05	0.51 ± 0.08	0.41 ± 0.04	0.46 ± 0.05	0.53 ± 0.07	0.55 ± 0.08
15:0	0.19 ± 0.01	0.19 ± 0.01	0.2 ± 0.02	0.19 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.21 ± 0.01
16:0	19.89 ± 0.4	20.01 ± 0.31	19.69 ± 0.37	19.74 ± 0.41	19.93 ± 0.47	20.25 ± 0.3	20.31 ± 0.58
18:0	12.28 ± 0.13	11.66 ± 0.24	11.86 ± 0.23	11.6 ± 0.20	11.77 ± 0.11	11.63 ± 0.32	11.92 ± 0.17
20:0	0.26 ± 0.01	0.25 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
22:0	0.74 ± 0.03	0.76 ± 0.02	0.77 ± 0.03	0.79 ± 0.03	0.79 ± 0.03	0.77 ± 0.02	0.78 ± 0.03
24:0	1.25 ± 0.05	1.28 ± 0.06	1.29 ± 0.05	1.32 ± 0.05	1.31 ± 0.06	1.24 ± 0.05	1.29 ± 0.07
Total saturated	35.12 ± 0.49	34.63 ± 0.27	34.58 ± 0.43	34.32 ± 0.51	34.74 ± 0.53	34.89 ± 0.48	35.35 ± 0.71
16:1n-9	0.29 ± 0.02	0.27 ± 0.02	0.28 ± 0.02	0.29 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.02
16:1n-7	0.96 ± 0.1	1.01 ± 0.13	0.93 ± 0.12	0.97 ± 0.11	0.92 ± 0.11	0.94 ± 0.1	1.01 ± 0.17
18:1n-9	17.95 ± 0.5	16.73 ± 0.36	17.37 ± 0.36	16.81 ± 0.39	16.4 ± 0.44	16.91 ± 0.87	17.06 ± 0.41
18:1n-7	0.26 ± 0.03	0.29 ± 0.04	0.31 ± 0.06	0.31 ± 0.07	0.31 ± 0.02	0.34 ± 0.06	0.34 ± 0.05
20:1n-9	8.61 ± 0.02	8.32 ± 0.01	8.42 ± 0.01	8.3 ± 0.02	8.31 ± 0.01	8.37 ± 0.01	8.5 ± 0.01
24:1n-9	1.33 ± 0.03	1.47 ± 0.06	1.51 ± 0.04	1.58 ± 0.07	1.61 ± 0.08	1.58 ± 0.07	1.61 ± 0.08
Total monounsaturated	22.29 ± 0.52	21.35 ± 0.47	21.96 ± 0.5	21.5 ± 0.5	21.04 ± 0.52	21.64 ± 0.94	21.83 ± 0.49
18:2n-6	19.69 ± 0.59	18.92 ± 0.72	18.65 ± 0.56	19.06 ± 0.43	18.69 ± 0.61	18.69 ± 0.63	18.11 ± 0.92
18:3n-6	0.28 ± 0.04	0.19 ± 0.02	0.23 ± 0.04	0.23 ± 0.06	0.18 ± 0.04	0.16 ± 0.02	0.21 ± 0.04
20:2n-6	0.23 ± 0.01	0.21 ± 0.01	0.2 ± 0.02	0.19 ± 0.02	0.2 ± 0.01	0.19 ± 0.02	0.17 ± 0.02
20:3n-6	1.71 ± 0.09	1.48 ± 0.09	1.39 ± 0.08	1.34 ± 0.09	1.29 ± 0.1	1.27 ± 0.09	1.3 ± 0.09
20:4n-6	10.46 ± 0.26	10.61 ± 0.22	9.82 ± 0.23	9.62 ± 0.2	9.63 ± 0.19	9.21 ± 0.41	8.98 ± 0.26
22:4n-6	1.55 ± 0.07	1.45 ± 0.09	1.42 ± 0.08	1.35 ± 0.08	1.27 ± 0.06	1.2 ± 0.1	1.17 ± 0.05
22:5n-6	0.28 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.21 ± 0.01	0.2 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
Total n-6 PUFA	34.19 ± 0.63	33.12 ± 0.73	31.94 ± 0.65	32.01 ± 0.39	31.47 ± 0.52	30.9 ± 0.51	30.12 ± 0.94
18:3n-3	0.45 ± 0.07	0.41 ± 0.02	0.43 ± 0.03	0.43 ± 0.03	0.44 ± 0.04	0.45 ± 0.09	0.48 ± 0.07
20:4n-3	0.06 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.1 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
20:5n-3	0.73 ± 0.1	2.05 ± 0.2	2.14 ± 0.16	2.39 ± 0.1	2.53 ± 0.18	2.49 ± 0.21	2.51 ± 0.22
22:5n-3	1.42 ± 0.05	1.47 ± 0.05	1.52 ± 0.03	1.54 ± 0.05	1.54 ± 0.05	1.54 ± 0.1	1.59 ± 0.06
22:6n-3	2.43 ± 0.17	3.54 ± 0.18	3.93 ± 0.21	4.21 ± 0.14	4.53 ± 0.12	4.53 ± 0.23	4.56 ± 0.21
Total n-3 PUFA	5.09 ± 0.24	7.56 ± 0.35	8.12 ± 0.32	8.68 ± 0.19	9.13 ± 0.28	9.09 ± 0.42	9.22 ± 0.44
16:0DMA	1.11 ± 0.04	1.15 ± 0.04	1.17 ± 0.03	1.2 ± 0.03	1.28 ± 0.04	1.23 ± 0.08	1.22 ± 0.03
18:0DMA	1.67 ± 0.05	1.66 ± 0.07	1.69 ± 0.06	1.72 ± 0.04	1.77 ± 0.06	1.69 ± 0.11	1.7 ± 0.04
18:1DMA	0.52 ± 0.03	0.52 ± 0.03	0.54 ± 0.03	0.56 ± 0.03	0.57 ± 0.03	0.57 ± 0.04	0.56 ± 0.02
Total DMA	3.3 ± 0.09	3.33 ± 0.13	3.4 ± 0.09	3.49 ± 0.07	3.62 ± 0.11	3.49 ± 0.22	3.48 ± 0.06

Table 4.2 - Blood lipid composition at week 0 to week 6 of participants in the FO group throughout the 6 week study period. Values are percentage of total lipid composition presented as means ± SEM. *PUFA*, polyunsaturated fatty acids. *DMA*, dimethyl aldehyde.

Fatty acid	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
14:0	0.48 ± 0.06	0.56 ± 0.11	0.51 ± 0.07	0.49 ± 0.05	0.59 ± 0.06	0.61 ± 0.05	0.56 ± 0.09
15:0	0.19 ± 0.01	0.21 ± 0.03	0.20 ± 0.02	0.19 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.02
16:0	19.53 ± 0.33	19.73 ± 0.44	20.02 ± 0.34	19.29 ± 0.30	20.47 ± 0.40	20.3 ± 0.37	20.4 ± 0.29
18:0	12.04 ± 0.14	12.12 ± 0.32	11.92 ± 0.33	11.88 ± 0.35	11.49 ± 0.30	11.72 ± 0.24	11.46 ± 0.16
20:0	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
22:0	0.79 ± 0.03	0.79 ± 0.02	0.78 ± 0.03	0.78 ± 0.02	0.77 ± 0.04	0.78 ± 0.03	0.80 ± 0.03
24:0	1.39 ± 0.05	1.4 ± 0.05	1.36 ± 0.05	1.37 ± 0.04	1.32 ± 0.07	1.33 ± 0.07	1.36 ± 0.06
Total saturated	34.68 ± 0.33	35.07 ± 0.51	35.06 ± 0.50	34.26 ± 0.55	35.11 ± 0.41	35.21 ± 0.48	35.06 ± 0.42
16:1n-9	0.33 ± 0.05	0.40 ± 0.11	0.35 ± 0.05	0.33 ± 0.04	0.34 ± 0.05	0.33 ± 0.03	0.33 ± 0.05
16:1n-7	0.98 ± 0.09	0.93 ± 0.10	1.08 ± 0.10	0.88 ± 0.07	1.09 ± 0.10	1.07 ± 0.07	1.02 ± 0.06
18:1n-9	18.31 ± 0.43	16.98 ± 0.50	17.34 ± 0.56	16.92 ± 0.60	16.95 ± 0.36	17.19 ± 0.37	16.81 ± 0.45
18:1n-7	1.45 ± 0.05	1.41 ± 0.06	1.45 ± 0.06	1.45 ± 0.04	1.47 ± 0.05	1.47 ± 0.06	1.49 ± 0.05
20:1n-9	0.26 ± 0.01	0.32 ± 0.02	0.33 ± 0.02	0.35 ± 0.02	0.33 ± 0.01	0.32 ± 0.02	0.32 ± 0.02
24:1n-9	1.46 ± 0.05	1.51 ± 0.06	1.52 ± 0.05	1.57 ± 0.03	1.59 ± 0.04	1.62 ± 0.06	1.67 ± 0.06
Total monounsaturated	22.79 ± 0.51	21.55 ± 0.54	22.06 ± 0.66	21.49 ± 0.68	21.75 ± 0.46	22.00 ± 0.40	21.65 ± 0.55
18:2n-6	19.01 ± 0.76	17.69 ± 0.53	17.48 ± 0.29	18.40 ± 0.67	17.80 ± 0.65	17.57 ± 0.50	18.3 ± 0.74
18:3n-6	0.23 ± 0.02	0.22 ± 0.03	0.20 ± 0.03	0.17 ± 0.02	0.19 ± 0.03	0.20 ± 0.01	0.18 ± 0.02
20:2n-6	0.23 ± 0.01	0.22 ± 0.01	0.20 ± 0.02	0.19 ± 0.02	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
20:3n-6	1.86 ± 0.11	1.66 ± 0.11	1.49 ± 0.07	1.47 ± 0.08	1.42 ± 0.09	1.46 ± 0.06	1.34 ± 0.06
20:4n-6	10.75 ± 0.20	10.55 ± 0.28	9.73 ± 0.34	9.71 ± 0.31	9.35 ± 0.31	9.12 ± 0.27	9.13 ± 0.38
22:4n-6	1.49 ± 0.08	1.42 ± 0.08	1.29 ± 0.08	1.24 ± 0.08	1.14 ± 0.07	1.08 ± 0.06	1.04 ± 0.06
22:5n-6	0.30 ± 0.02	0.27 ± 0.02	0.26 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Total n-6 PUFA	33.88 ± 0.67	32.02 ± 0.55	30.66 ± 0.45	31.43 ± 0.43	30.31 ± 0.69	29.82 ± 0.58	30.38 ± 0.80
18:3n-3	0.48 ± 0.03	0.42 ± 0.04	0.45 ± 0.04	0.50 ± 0.05	0.47 ± 0.05	0.41 ± 0.03	0.41 ± 0.04
20:4n-3	0.08 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.10 ± 0.01
20:5n-3	0.76 ± 0.11	2.18 ± 0.15	2.63 ± 0.16	2.80 ± 0.22	2.80 ± 0.15	2.87 ± 0.28	2.83 ± 0.13
22:5n-3	1.48 ± 0.05	1.60 ± 0.03	1.62 ± 0.03	1.63 ± 0.04	1.60 ± 0.02	1.63 ± 0.04	1.58 ± 0.05
22:6n-3	2.47 ± 0.18	3.57 ± 0.21	4.04 ± 0.20	4.34 ± 0.13	4.45 ± 0.13	4.59 ± 0.15	4.57 ± 0.13
Total n-3 PUFA	5.28 ± 0.29	7.88 ± 0.34	8.88 ± 0.35	9.39 ± 0.30	9.45 ± 0.25	9.62 ± 0.39	9.49 ± 0.20
16:0DMA	1.12 ± 0.05	1.18 ± 0.06	1.13 ± 0.06	1.15 ± 0.06	1.16 ± 0.05	1.14 ± 0.03	1.17 ± 0.04
18:0DMA	1.75 ± 0.07	1.79 ± 0.10	1.72 ± 0.10	1.76 ± 0.09	1.70 ± 0.08	1.70 ± 0.08	1.73 ± 0.07
18:1DMA	0.51 ± 0.03	0.51 ± 0.04	0.51 ± 0.03	0.52 ± 0.03	0.51 ± 0.02	0.51 ± 0.01	0.53 ± 0.02
Total DMA	3.38 ± 0.14	3.48 ± 0.19	3.35 ± 0.18	3.43 ± 0.17	3.37 ± 0.14	3.35 ± 0.12	3.43 ± 0.13

4.4.2 Time-course of lipid changes in the phospholipid membrane of skeletal muscle

A full breakdown of the phospholipid membrane fatty acid profiles is displayed in Tables 4.3 FO+AO group) and 4.4 (FO). The % EPA/ total fatty acid content (Figure 4.2 A), %DHA/ total fatty acid content (Figure 4.2 B), % EPA + DHA/ total fatty acids (Figure 4.2 C), % total n-3PUFA/ total fatty acids (Figure 4.2 D) and % n-3 HUFA/ total HUFA (Figure 4.2 E) all followed a similar timecourse over the 6 week supplementation period, on average increasing by 38% from weeks 0 - 2 ($p < 0.001$) and by 35% from weeks 2 - 6 ($p < 0.001$). No differences between groups were observed for any changes of muscle lipid profile.

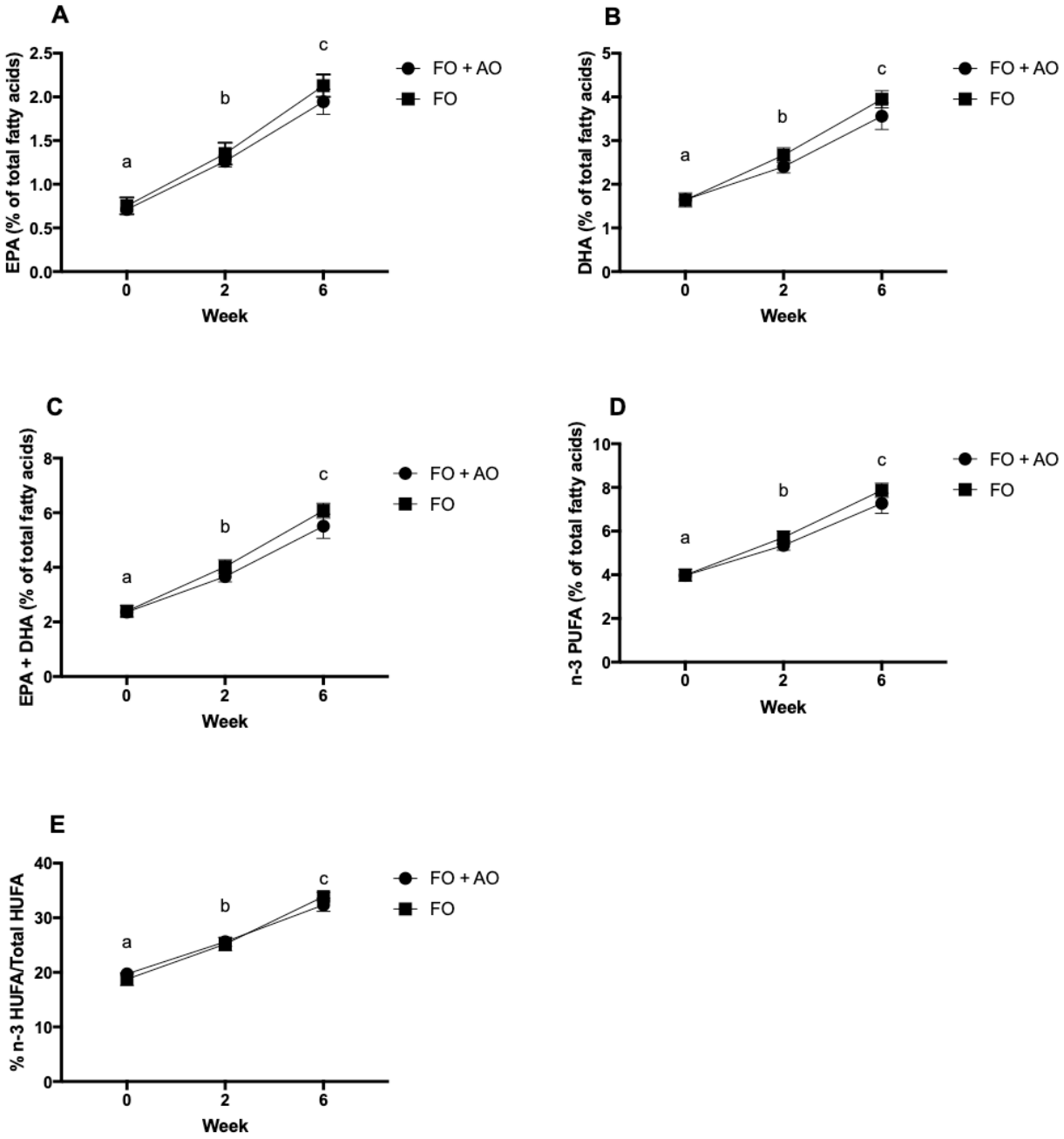

Figure 4.2 – Phospholipid membrane of skeletal muscle lipid changes over the 6 week study protocol. Data are expressed as means ± SEM. *A*, Eicosapentanoic acid (EPA). *B*, Docosahexaenoic acid (DHA). *C*, EPA + DHA. D, n-3 polyunsaturated fatty acid (n-3PUFA). *E*, % n-3 highly unsaturated fatty acids to total highly unsaturated fatty acids (n-3 HUFA/ Total HUFA). FO+AO, Fish oil and antioxidant supplement beverage, FO, Fish oil only supplement beverage. Means that do not share a letter are significantly different.

Table 4.3 – Phospholipid membrane of skeletal muscle lipid composition at week 0, 2 and 6 of participants in the FO+AO group throughout the 6 week study period. Values are percentage of total lipid composition presented as means ± SEM. *PUFA*, polyunsaturated fatty acids. *DMA*, dimethyl aldehyde.

Fatty acid	Week 0	Week 2	Week 6
14:0	0.51 ± 0.05	0.48 ± 0.03	0.48 ± 0.02
15:0	0.24 ± 0.01	0.22 ± 0.02	0.23 ± 0.02
16:0	17.83 ± 0.18	17.85 ± 0.26	18.06 ± 0.22
18:0	13.74 ± 0.31	13.75 ± 0.29	13.43 ± 0.39
20:0	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
22:0	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
24:0	0.18 ± 0.01	0.20 ± 0.01	0.18 ± 0.01
Total saturated	32.75 ± 0.14	32.74 ± 0.19	32.61 ± 0.33
16:1n-9	0.23 ± 0.02	0.19 ± 0.02	0.19 ± 0.01
16:1n-7	0.48 ± 0.05	0.47 ± 0.04	0.45 ± 0.04
18:1n-9	6.76 ± 0.32	6.22 ± 0.28	5.97 ± 0.38
18:1n-7	1.87 ± 0.07	1.93 ± 0.10	1.96 ± 0.08
20:1n-9	0.09 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
24:1n-9	0.34 ± 0.05	0.36 ± 0.05	0.39 ± 0.04
Total monounsaturated	9.76 ± 0.41	9.31 ± 0.36	9.11 ± 0.41
18:2n-6	28.56 ± 0.84	28.02 ± 0.57	26.32 ± 0.89
18:3n-6	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.01
20:2n-6	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
20:3n-6	1.40 ± 0.04	1.33 ± 0.06	1.26 ± 0.06
20:4n-6	12.92 ± 0.59	12.53 ± 0.36	12.59 ± 0.60
22:4n-6	0.59 ± 0.03	0.53 ± 0.03	0.40 ± 0.03
22:5n-6	0.34 ± 0.01	0.31 ± 0.01	0.26 ± 0.01
Total n-6 PUFA	44.02 ± 0.69	42.91 ± 0.59	41.00 ± 0.58
18:3n-3	0.24 ± 0.02	0.26 ± 0.02	0.24 ± 0.02
20:4n-3	0.00 ± 0.00	0.02 ± 0.01	0.04 ± 0.01
20:5n-3	0.71 ± 0.07	1.26 ± 0.06	1.95 ± 0.13
22:5n-3	1.37 ± 0.05	1.40 ± 0.06	1.49 ± 0.06
22:6n-3	1.65 ± 0.13	2.40 ± 0.13	3.56 ± 0.28
Total n-3 PUFA	3.97 ± 0.19	5.35 ± 0.20	7.27 ± 0.43
16:0DMA	5.65 ± 0.25	5.77 ± 0.21	5.92 ± 0.25
18:0DMA	2.15 ± 0.11	2.18 ± 0.11	2.26 ± 0.13
18:1DMA	1.70 ± 0.07	1.75 ± 0.06	1.84 ± 0.08
Total DMA	9.50 ± 0.38	9.69 ± 0.34	10.02 ± 0.41

Table 4.4 - Phospholipid membrane of skeletal muscle lipid composition at week 0, 2 and 6 of participants in the FO group throughout the 6 week study period. Values are percentage of total lipid composition presented as means ± SEM. *PUFA*, polyunsaturated fatty acids. *DMA*, dimethyl aldehyde.

Fatty acid	Week 0	Week 2	Week 6
14:0	0.48 ± 0.02	0.41 ± 0.03	0.55 ± 0.08
15:0	0.21 ± 0.01	0.20 ± 0.01	0.27 ± 0.04
16:0	17.27 ± 0.09	17.42 ± 0.15	17.89 ± 0.26
18:0	14.25 ±0.34	13.68 ± 0.19	13.66 ± 0.13
20:0	0.10 ± 0.01	0.08 ± 0.00	0.08 ± 0.01
22:0	0.21 ± 0.02	0.17 ± 0.01	0.21 ± 0.02
24:0	0.24 ± 0.03	0.18 ± 0.01	0.21 ± 0.01
Total saturated	32.76 ± 0.37	32.14 ± 0.13	32.88 ± 0.34
16:1n-9	0.20 ± 0.01	0.18 ± 0.01	0.35 ± 0.12
16:1n-7	0.47 ± 0.04	0.39 ± 0.03	0.50 ± 0.06
18:1n-9	6.87 ± 0.31	5.75 ± 0.25	6.05 ± 0.33
18:1n-7	2.03 ± 0.04	1.96 ± 0.04	2.07 ± 0.04
20:1n-9	0.14 ± 0.03	0.13 ± 0.00	0.14 ± 0.01
24:1n-9	0.43 ± 0.07	0.29 ± 0.02	0.35 ± 0.02
Total monounsaturated	10.14 ± 0.39	8.71 ± 0.33	9.47 ± 0.39
18:2n-6	27.45 ± 0.78	26.82 ± 0.76	25.35 ± 0.61
18:3n-6	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
20:2n-6	0.13 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
20:3n-6	1.37 ± 0.05	1.39 ± 0.06	1.26 ± 0.05
20:4n-6	13.76 ± 0.45	14.05 ± 0.46	12.92 ± 0.36
22:4n-6	0.60 ± 0.04	0.53 ± 0.04	0.39 ± 0.02
22:5n-6	0.38 ± 0.02	0.35 ± 0.02	0.28 ± 0.01
Total n-6 PUFA	43.76 ± 0.66	43.31 ± 0.55	40.38 ± 0.49
18:3n-3	0.25 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
20:4n-3	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01
20:5n-3	0.75 ± 0.09	1.35 ± 0.12	2.13 ± 0.12
22:5n-3	1.34 ± 0.07	1.47 ± 0.06	1.57 ± 0.06
22:6n-3	1.64 ± 0.15	2.67 ± 0.16	3.95 ± 0.18
Total n-3 PUFA	3.99 ± 0.26	5.71 ± 0.28	7.87 ± 0.30
16:0DMA	5.62 ± 0.20	6.14 ± 0.21	5.67 ± 0.39
18:0DMA	2.05 ± 0.09	2.22 ± 0.09	2.10 ± 0.08
18:1DMA	1.67 ± 0.06	1.77 ± 0.06	1.64 ± 0.08
Total DMA	9.35 ± 0.31	10.13 ± 0.29	9.41 ± 0.47

4.4.3 Maximum voluntary contraction

Dominant leg MVC did not change throughout the 6 week study period (Figure 4.3 A). However, the FO+AO group was significantly higher in force production than the FO group throughout the study period ($p = 0.029$). Similarly, non-dominant leg MVC did not change throughout the 6 week protocol. However, MVC for the FO+AO was significantly higher than the FO group at all timepoints ($p < 0.05$) (Figure 4.3 B).

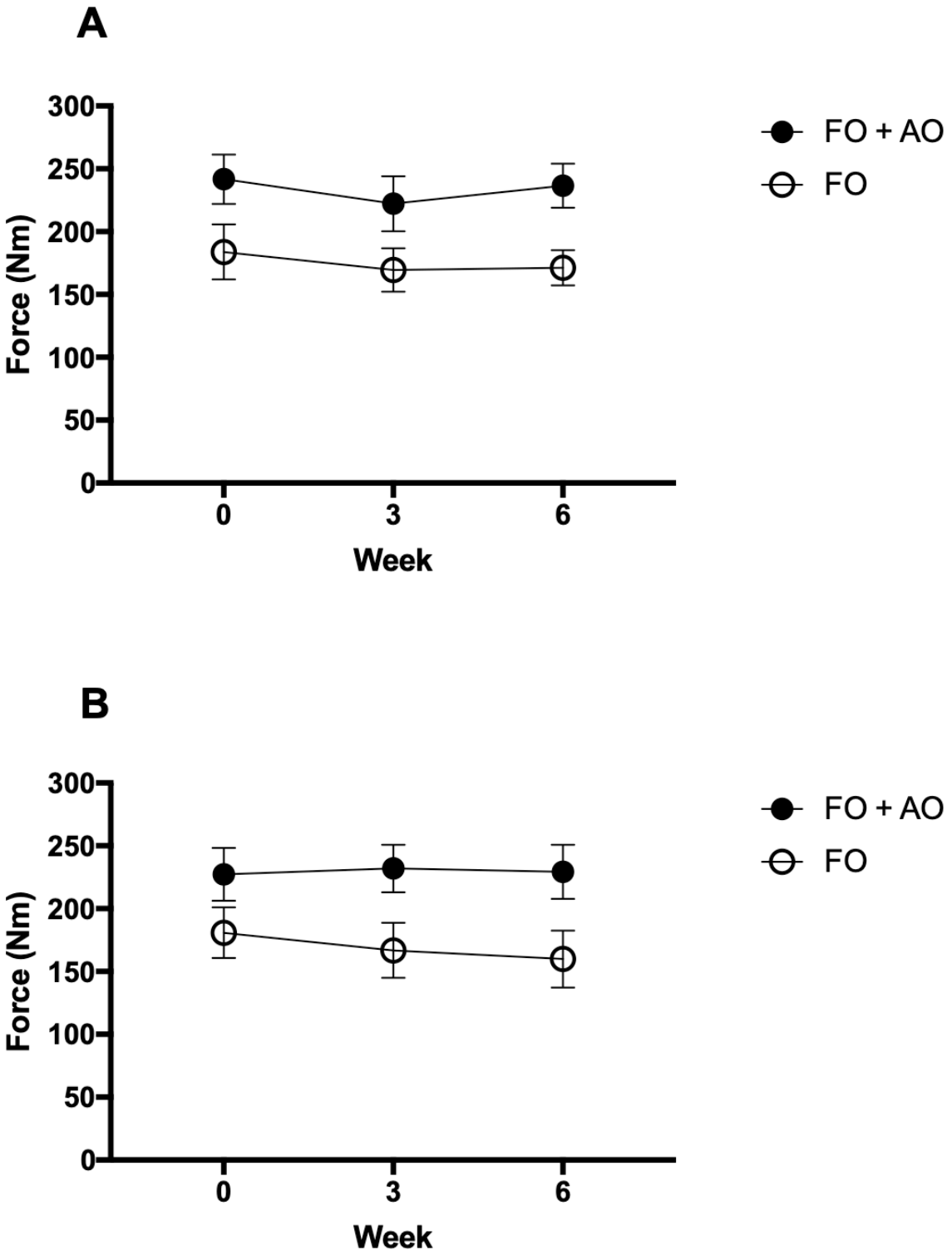

Figure 4.3 - Maximum voluntary contraction over the 6 week study protocol. Data are expressed as means ± SEM. *A*, MVC in the dominant leg. *B*, MVC in the non-dominant leg. FO+AO, Fish oil and antioxidant supplement beverage, FO, Fish oil only supplement beverage.

4.4.4 Electromyography

There was no impact of n-3PUFA supplementation, with or without the addition of curcumin, on EMG activity of both dominant and non-dominant leg for the VM or the VL throughout the 6 week supplement period (Figure 4.4).

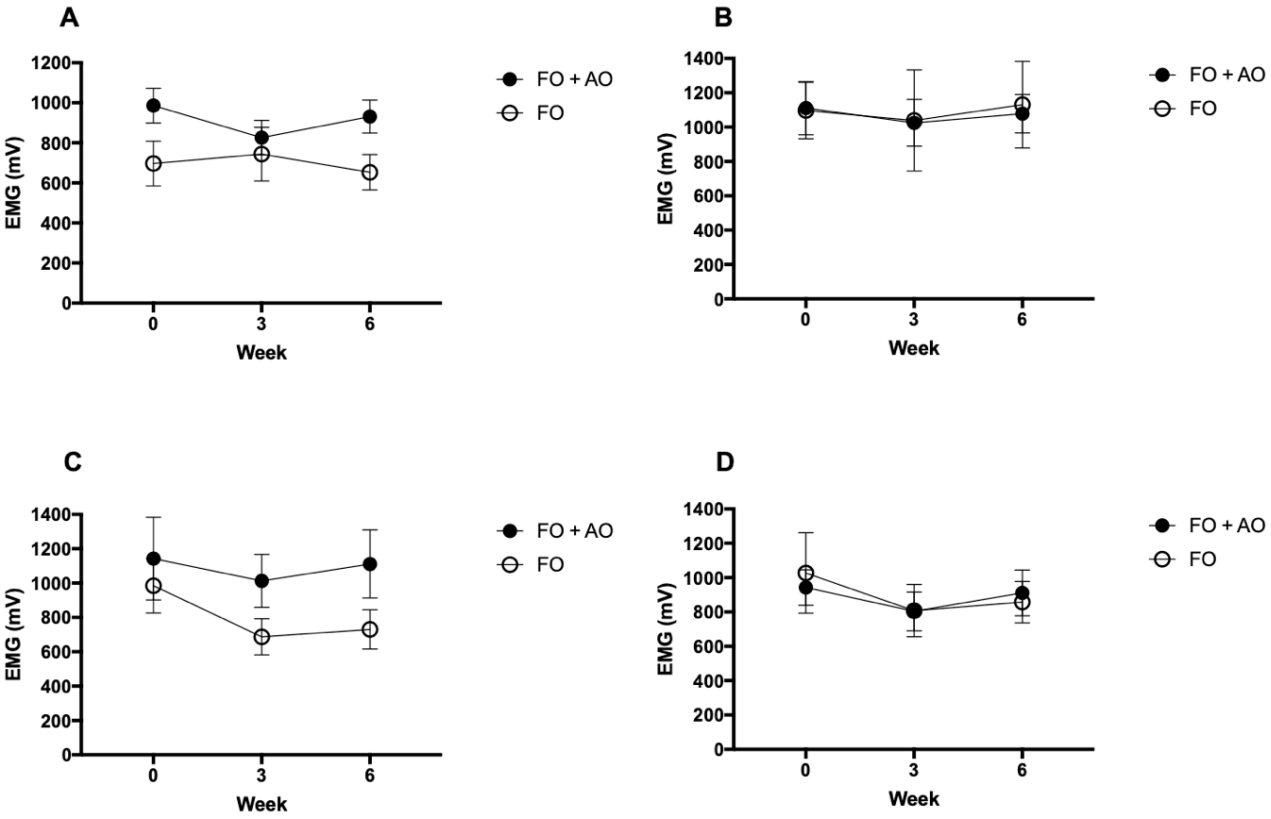

Figure 4.4 - Electromyography over the 6 week study protocol. Data are expressed as means ± SEM. *A*, EMG in the dominant Vastus Lateralis. *B*, EMG in the non-dominant Vastus Lateralis. *C*, EMG in the dominant Vastus Medialis. *D*, EMG in the non-dominant Vastus Medialis. FO+AO, Fish oil and antioxidant supplement beverage, FO, Fish oil only supplement beverage.

4.4.5 Electromechanical delay

Dominant leg VL EMD did not alter in group or in time throughout the 6 week study period (Figure 4.5 A). However in the non-dominant VL, EMD significantly decreased in time between weeks 0 and 3 by 21% (p = 0.045) but significantly increased between weeks 3 and 6 (p = 0.004) (Figure 4.5 B). There were differences between the groups. No differences in group or time were observed in both the dominant and non-dominant vastus medialis throughout the 6 week study period (Figure 4.5 C and D).

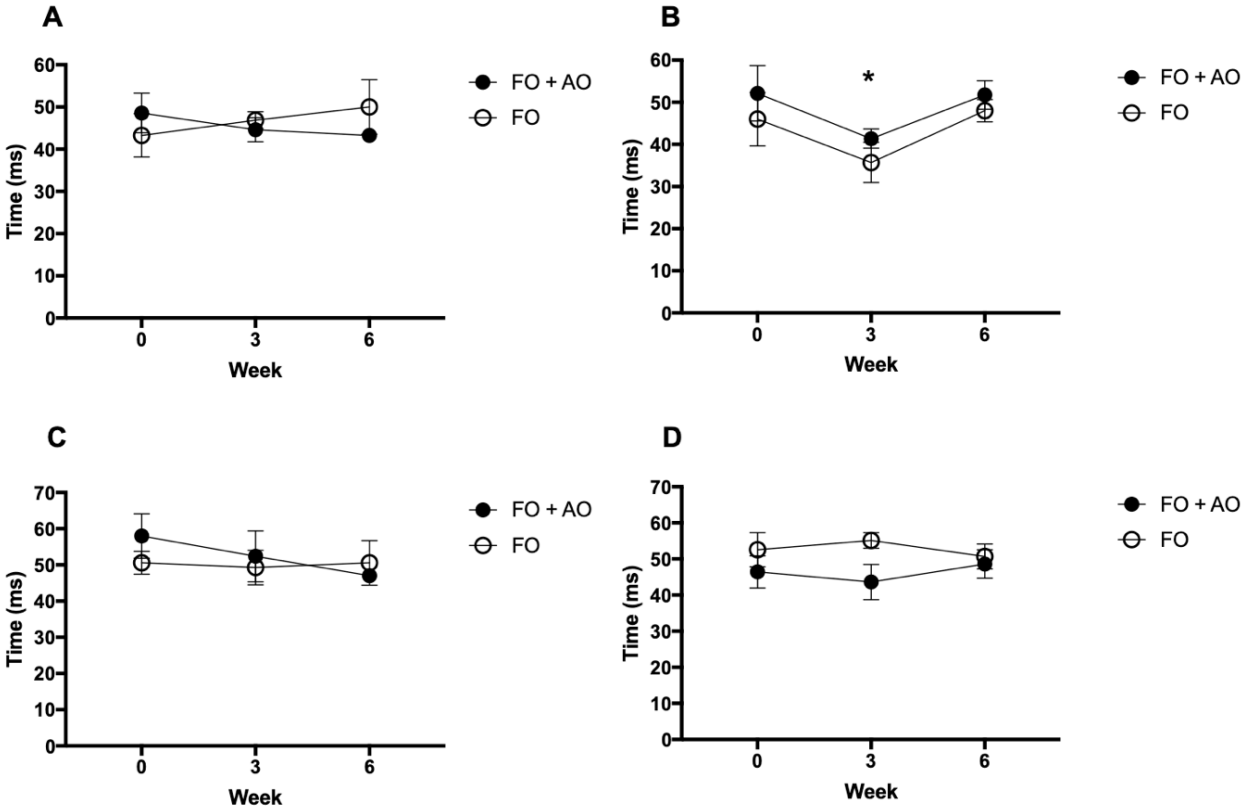

Figure 4.5 - Electromechanical delay over the 6 week study protocol. Data are expressed as means ± SEM. *A*, EMD in the dominant Vastus Lateralis. *B*, EMD in the non-dominant Vastus Lateralis. *C*, EMD in the dominant Vastus Medialis. *D*, EMD in the non-dominant Vastus Medialis. * significantly different to week 0 and week 6. FO+AO, Fish oil and antioxidant supplement beverage, FO, Fish oil only supplement beverage.

4.5 Discussion

This study was designed to investigate the influence of coingesting curcumin as an antioxidant nutrient with a fish oil-based supplement on the time-course of changes in n-3PUFA incorporation into whole blood cells and the phospholipid membrane of skeletal muscle cells. Refuting our original hypothesis, we demonstrate that n-3PUFA incorporation into blood and skeletal muscle tissue was not modulated over the 6 wk period by adding 1 g/day of curcumin to a fish oil-based supplement containing 3.6g/day of n-3PUFA. This study also demonstrated that the incorporation of n-3PUFA into the phospholipid membrane of skeletal muscle did not influence muscle function or neuromuscular function over the 6 wk study period. Hence, from a practical perspective, adding 1 g of curcumin to a daily fish oil supplement is not an effective strategy to augment the magnitude or rate of n-3PUFA incorporation into the metabolically active tissues of blood and skeletal muscle. Second, in the absence of exercise training, 6 wk of n-3PUFA supplementation, with or without the addition of curcumin, does not improve muscle function and neuromuscular function in healthy young men.

Following further research, it is probable that the lack of differences in results is not as much as a surprise as first anticipated. Initially, the original hypothesis was based upon curcumin, as an antioxidant, using its ability to free radical scavenge and therefore reduce n-3PUFA oxidation. However, it is clear that free radical scavenging does not lead to a reduction in ß-oxidation and the addition of curcumin to n-3PUFA would not, to our knowledge, influence ß-oxidation rates. Measuring the ß-oxidation rates of n-3PUFA within the blood would have been important in gaining a full understanding of any influence the addition of curcumin had made on n-3PUFA incorporation. Analysing a biomarker within the blood such as DNA/RNA damage or lipid peroxidation would be a sensible approach for future research when examining n-3PUFA oxidation. Another explanation or the lack of findings may relate

to the relatively modest 1 g dose of curcumin ingested daily throughout the 6 wk supplementation period. The threshold dose of curcumin to elicit an antioxidant response is currently unknown in humans. Accordingly, previous studies have implemented various curcumin dosing regimens, ranging from 10mg/day - 2 g/day administered as a single dose or as repeat boluses over a 12 wk period (Chilelli et al., 2016; Santos-Parker et al., 2017). Given such variations in dosing regimen, it is unclear whether the ingestion of 1g/day of curcumin for 6 wks was sufficient to negate the oxidation of n-3PUFA with fish oil supplementation. Future research is warranted to determine the dose-response relationship between curcumin intake and antioxidant status.

An alternative explanation for the lack of modulation in tissue n-3PUFA incorporation with curcumin coingestion may relate to the high dose of fish oil administered in the present study. Although speculative, we acknowledge that ingesting 3.6 g/day of fish oil over the 6 wk period may have served to saturate the tissue incorporation of n-3PUFA, particularly in whole blood cells. In the present study, a plateau in whole blood cell n-3PUFA composition was reached from 3-6 weeks, suggesting that n-3PUFA oxidation rates were increased after 3 weeks. Further research is warranted to examine the influence of adding antioxidants to a lower dose of fish oil on the magnitude and rate of n-3PUFA incorporation into whole blood cells and the phospholipid membrane of skeletal muscle tissue. This insight is of practical relevance given that commonly consumed n-3PUFA-rich food sources such as canned tuna contain markedly lower levels of n-3PUFA (i.e., a 100g serving of canned tuna contains ~0.3g of n-3PUFA), of which a proportion of the n-3PUFA will have oxidised..

A strength of the present study relates to characterising the time-course of n-3PUFA incorporation at regular intervals during 6 wks of fish oil supplementation in blood and

skeletal muscle tissue. A previous study demonstrated that 5 g/day of fish oil supplementation increased the n-3PUFA composition of whole blood cells within 1 wk, with blood n-3PUFA composition further increasing up to 2 wks, before reaching a plateau between 2 and 4 wks (McGlory et al., 2014). Consistent with this observation, we observed an elevation in blood n-3PUFA composition after 1 wk of fish oil (3.6g/day) supplementation, which increases further at 3 wks before a plateau was reached between wks 3 and 6. Taken together, these data suggest that between 2 and 3 weeks of high dose fish oil supplementation is sufficient to saturate the incorporation of n-3PUFA into whole blood cells. McGlory et al., (2014) found an increase in n-3PUFA incorporation into skeletal muscle after 2 wks of supplementation with a further increase after 4 wks of fish oil supplementation. We also observed an increase in n-3PUFA incorporation into the phospholipid membrane of skeletal muscle after 2 wks with a further increase after 6 wks of n-3PUFA supplementation. In the present study, we did not directly assess n-3PUFA oxidation rates. This measurement would have afforded greater insight into the metabolic fate of n-3PUFA. Therefore, we can only speculate that the fate of additional exogenous n-3PUFA was likely primarily oxidation. Rather than oxidation, it also is feasible that a proportion of n-3PUFA ingested from wk 3 onwards was redirected to other tissues such as adipose tissue (Fielding, 2011) and the bowel (Stenson et al., 1989). Future research should profile oxidation rates in response to n-3PUFA supplementation over short-term (up to 3 wks) and longer-term (>3 wk) periods of fish oil supplementation.

Marked differences in tissue turnover rates between whole blood cells and skeletal muscle cells (Shemin & Rittenberg, 1946; Spalding et al., 2005) have been proposed to explain the divergent timecourse of n-3PUFA incorporation between blood and muscle tissue. Consistent with previous work (McGlory et al., 2014), we observed a slower rate of n-

3PUFA incorporation into skeletal muscle tissue compared with whole blood. Whereas McGlory et al., (2014) reported a ~50% increase in skeletal muscle EPA composition after 2 wks of fish oil supplementation, we observed a 62% increase in muscle phospholipid EPA composition at the same time interval, despite our participants consuming 1.7 g less EPA per day. This apparent discrepancy in muscle tissue EPA incorporation between past (McGlory et al., 2014; Metherel et al., 2009) and present studies may be attributed to methodological differences in cellular components measured in skeletal muscle. Whereas McGlory et al., (2014) measured the n-3PUFA composition of the whole muscle cell, by design we measured changes in n-3PUFA composition of the phospholipid membrane of muscle cells. Measurements of muscle phospholipid membrane composition negated the possibility of n-3PUFA incorporation into other muscle cell organelles such as mitochondria (Gerling et al., 2019). Moreover, modulating the n-3PUFA composition of the skeletal muscle phospholipid membrane is proposed to mediate the upregulation of muscle function and reduce local inflammation (Calder, 2012). Although the present study was not designed to characterise the dose-response of n-3PUFA incorporation with ingested fish oil, based on the comparison with McGlory et al (2014), we speculate that 3.6 g/day of n-3PUFA is sufficient to observe an increase in n-3PUFA incorporation into both whole blood cells and the phospholipid membrane of skeletal muscle tissue. Future studies are warranted to examine the dose-response relationship between ingested n-3PUFA and tissue-specific incorporation rates of n-3PUFA.

In addition to measuring changes in whole blood and skeletal muscle lipid composition with n-3PUFA supplementation, we also obtained measurements of neuromuscular function and muscle functional capacity over 6 wks of fish oil supplementation. In this regard, previous studies have demonstrated improvements in

neuromuscular function with n-3PUFA supplementation (Rodacki et al., 2012; Ochi, Tsuchiya & Yanagimoto, 2017; Lewis et al., 2015). The causal mechanism proposed to underpin the action of n-3PUFA supplementation to improve neuromuscular function involves the modulation of acetylcholine via modification of membrane fluidity. In this regard, acetylcholine plays a key role in neurotransmission at the neuromuscular junction leading to an increased firing rate of muscle contraction. However, this mechanism has only been demonstrated in rat ileum and not in a human model (Patten et al., 2002). In theory, the increased speed of muscle contraction results in a reduced EMD (Patten et al., 2002). However, in the present study, we did not observe any improvements in either EMG or EMD over the 6 wk period of n-3PUFA supplementation, with or without the addition of curcumin. Two factors may explain this null finding. First, our supplementation period only lasted 6 wks. Previous studies reported improvements in neuromuscular function after 90 days of n-3PUFA supplementation (Rodacki et al., 2012). Thus, it is possible that the duration of n-3PUFA supplementation was insufficient to elicit an improvement in neuromuscular function. Second, previous studies that observed an improvement in EMD with fish oil supplementation recruited untrained participants and implemented a resistance training programme throughout the n-3PUFA supplementation period (Rodacki et al., 2012). Participants in the present study were physically-active and were asked to maintain their training throughout the study. Thus, it remains unclear whether n-3PUFA supplementation serves to improve peripheral neuromuscular function in young, physically active populations.

Although the present study has several strengths such as providing novel insight into the dosing strategy of n-3PUFA by directly analysing the phospholipid membrane of skeletal muscle n-3PUFA composition, there are limitations to the study. First, although we obtained

skeletal muscle samples that give us an insight into the incorporation of n-3PUFA into the phospholipid membrane of skeletal muscle, the moderately invasive nature of muscle biopsies restricted how regularly we measured lipid composition in skeletal muscle. In past (McGlory et al., 2014) and present studies, 2 weeks of fish oil supplementation was required to increase skeletal muscle phospholipid n-3PUFA composition above baseline levels, with skeletal muscle n-3PUFA composition continuing to rise after 6 weeks of supplementation. Hence, the supplementation period required to saturate the n-3PUFA composition of skeletal muscle remains unknown. Second, in the present study, we recruited healthy recreationally-active males to allow for a direct comparison with similar previous n-3PUFA incorporation studies (McGlory et al 2014). However, the findings in this study may not translate to all population groups. Given that blood lipid profiles differ between males and females (Metherel et al., 2009), it is feasible that sex-differences in rates of n-3PUFA incorporation also may differ. Further research is warranted to examine the incorporation rates of n-3PUFA into blood and skeletal muscle in physically-active females, as well as other more compromised populations such as older adults.

To conclude, the addition of 1 g/day of curcumin to 3.6g/day of n-3PUFA supplementation failed to facilitate the magnitude and rate of n-3PUFA incorporation into whole blood cells and the phospholipid membrane of skeletal muscle cells in physically-active young men. We also observed no influence of n-3PUFA, regardless of the addition of curcumin, on neuromuscular function or muscle strength as determined by isokinetic dynamometry. These data suggest that adding 1 g/day of curcumin to a fish oil-based supplement containing 3.6 g/day of n-3PUFA is not an effective strategy to facilitate the incorporation of n-3PUFA into metabolically-active blood and muscle tissues.

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Chapter 5 – General Discussion

The general aim of this thesis was to examine the nutritional role of dietary n-3PUFA supplementation in the context of sports performance. In terms of exercise recovery, **Chapter 2** investigated the influence of n-3PUFA supplementation on indirect markers of muscle damage and muscle repair in response to a single, intense bout of eccentric exercise. Previous scientific evidence supports a beneficial, or at least not harmful, role for protein ingestion in the context of reducing muscle damage and/or accelerating muscle repair during acute recovery from eccentric exercise. Previous literature has demonstrated a reduction in inflammation with n-3PUFA supplementation. Thus, it is intuitive that combining n-3PUFA with protein and carbohydrate with a synergistic focus may improve recovery from muscle damaging exercise. The idea to study a combination of nutrients, rather than n-3PUFA independently, provided real world application, as this practice is often undertaken by athletes following exercise. For example, carbohydrate ingestion to facilitate the replenishment of muscle glycogen and protein ingestion to stimulate muscle remodelling following exercise. To this end, although several studies have investigated the influence of n-3PUFA supplementation on recovery from muscle damage, few studies have examined the influence of combining n-3PUFA with other nutrients on muscle damage recovery. By collecting direct and indirect measurements of muscle damage, muscle soreness and muscle function during acute recovery from an intense bout of eccentric exercise, we examined the efficacy of n-3PUFA supplementation to protect the muscle from damaging exercise and/or accelerate muscle repair.

This thesis also investigated the application of n-3PUFA in the context of promoting training adaptation. Using a weight loss model in trained young men, and given previous evidence regarding the anabolic action of n-3PUFA, study 2 investigated the influence of n-

3PUFA supplementation on changes in body composition and performance during a short-term period of diet-induced weight loss. This thesis hypothesised that n-3PUFA supplementation would enhance the "quality" of weight loss, i.e., promote the loss of fat mass while maintaining lean body mass, during energy restriction in resistance-trained young men. By examining body composition, muscle function and muscular performance during a short-term period of weight loss whilst supplementing with n-3PUFA, we determined the capacity for n-3PUFA supplementation to preserve muscle mass during the catabolic situation of diet-induced weight loss.

Study 3 focussed on the mechanisms of action of n-3PUFA incorporating into both whole blood cells and phospholipid membrane of skeletal muscle cells, with a view to optimising the dosing regimen of n-3PUFA ingestion. The mechanism most commonly proposed to underpin the physiological role of n-3PUFA in skeletal muscle relates to the incorporation of the n-3 species, EPA and DHA, into the muscle phospholipid membrane. In theory, reducing the oxidation of n-3PUFA during supplementation will facilitate the magnitude and rate of n-3PUFA incorporation into skeletal muscle tissue. Accordingly, the design of study 3 included the addition of antioxidants to n-3PUFA with a view to facilitating the rate of n-3PUFA uptake and incorporation into both blood and skeletal muscle tissues.

Given the recent emergence of n-3PUFA as a popular nutritional strategy among athletes to promote recovery, training adaptation and performance, the overarching aim of this thesis was to investigate the application of n-3PUFA for sports performance. The specific objectives of this thesis were:

1. **To determine the influence of adding n-3PUFA to a protein- and carbohydrate-containing beverage on recovery from intense eccentric exercise in soccer players.**

2. **To determine the influence of n-3PUFA supplementation during a 2 wk period of diet-induced energy restriction on changes in body composition, muscle function and muscular performance.**

3. **To determine the influence of adding antioxidants to an n-3PUFA based supplement on the time-course of changes in blood and skeletal muscle lipid profile over a 6 wk period**

4. **To determine the influence of 6 wks of n-3PUFA supplementation, with or without the addition of antioxidants, on changes in neuromuscular function in recreationally active young men.**

This general discussion presents a critical appraisal of all the studies included in this thesis and attempts to translate findings into practical recommendations. The general discussion will discuss how n-3PUFA supplementation influences sporting performance including muscle recovery and adaptation, with improvements influenced by the incorporation of n-3PUFA into the phospholipid membrane of skeletal muscle.

5.1 Omega-3 fatty acid supplementation and exercise recovery

The principal finding of **Chapter 2** was the benefit to recovery when trained soccer players undertook a 6 wk period of n-3PUFA supplementation. The improvements in recovery demonstrated with n-3PUFA supplementation included a decrease in muscle soreness and a decrease in creatine kinase as a putative blood marker of muscle damage. Two theoretical mechanisms may underpin the improvement in exercise recovery with n-3PUFA supplementation. First, n-3PUFA ingestion may have facilitated the speed of muscle repair. Second, n-3PUFA ingestion may have attenuated the degree of muscle damage experienced by soccer players. Based on the attenuated increase in plasma CK concentrations measured during acute (0-72 h) recovery from eccentric exercise, the most likely causal mechanism underlying the improved muscle recovery in soccer players was due to the 6 wk 'priming' period of n-3PUFA supplementation. The priming period allowed for the incorporation of EPA and DHA into the phospholipid membrane of the muscle cell, thus stabilising the integrity of the cell membrane (McGlory et al., 2014) and reducing the damage experienced by the soccer players rather than promoting faster repair of damaged muscle tissue. As such, by stabilising the phospholipid membrane of the skeletal muscle cell prior to the muscle damage stimulus, the muscle cell may have been protected during the muscle damage protocol, resulting in reduced muscle damage and subsequent reduction in perceived feelings of muscle soreness. In essence, our data suggest that participants ingesting n-3PUFA experienced a reduced level of damage to the muscle architecture.

Previous literature examining the influence of n-3PUFA supplementation in the context of athlete performance have prescribed various doses of n-3PUFA. The protocol we

adopted in **Chapter 2** required participants to supplement with 4 g/day of n-3PUFA for 6 wks prior to muscle damaging exercise. Previous work has established that n-3PUFA supplementation results in the incorporation of EPA and DHA into skeletal muscle tissue (McGlory et al., 2014; Smith et al., 2011a; Smith et al., 2011b). In this study, we chose to supplement with 4 g/day of n-3PUFA over a 6 wk period. This supplementation period was based on findings of McGlory et al (2014) who observed an increase in skeletal muscle n-3PUFA concentrations following 4 wks of 5g/day of n-3PUFA supplementation. Given that we supplemented with a lower dose of n-3PUFA, the supplementation period was extended to 6 wks to ensure a sufficient incorporation of n-3PUFA into the skeletal muscle phospholipid membrane. We were unable to directly measure skeletal muscle n-3PUFA concentration because the collection of muscle biopsies would likely have contributed to the muscle damage experienced with eccentric exercise. However, based on the 58% increase in blood n-3PUFA concentrations following supplementation and previous literature showing an increase in n-3PUFA blood and skeletal muscle concentration using a similar dosing strategy (McGlory et al., 2014), we are confident that an increase in muscle n-3PUFA concentration occurred.

Measurements of blood CK concentrations is a common approach to examining muscle recovery (Clifford et al., 2017; Damas et al., 2016; Bell et al., 2016). However, measurements of plasma CK concentrations provide indirect markers of muscle damage that rely on several assumptions. When interpreting CK as a blood marker of muscle damage, the assumption is made that the increased CK concentration is from the site of muscle damage (Koch, Pereira & Machado, 2014). Future research should adopt direct measurements of skeletal muscle fibre disruption to measure the damage and recovery process following the exercise bout. Z-band streaming is a direct measure of muscle damage that may provide more

insight into the muscle damage repair process. Moreover, the measurement of z-band streaming using a toluidine blue-stained light microscope methodology would allow for the direct measurement of muscle damage. In this regard, z-band streaming has previously been used as a tool to measure muscle damage (Damas et al., 2016). However, it would be difficult to measure z-band streaming whilst adopting a similar protocol as the present study due to the nature of muscle biopsies and the potential impact of the muscle biopsy on subsequent performance over the following days. However, future research should adopt a single-legged muscle damage protocol in order to eliminate the issue of muscle biopsies disrupting the recovery process.

The application of n-3PUFA supplementation in sports nutrition extends beyond acute exercise recovery, and includes training adaptation. As discussed, the primary finding in **Chapter 2** was that 6 wks of n-3PUFA supplementation elicited a protective effect on exercise induced muscle damage. However, it may be possible that accelerating recovery by protecting the muscle from physiological stress is not optimal for long term training adaptation. For instance, whereas reducing inflammation may be beneficial for improving recovery (Howatson et al., 2010), local inflammation is an important initial step in the muscle adaptation process (Teixeira et al., 2009). Therefore, in **Chapter 3** we attempted to examine the role n-3PUFA supplementation in the context of muscle adaptation. As shown in **Chapter 2**, we observed a protective effect with a sustained 6 wk supplementation period of n-3PUFA in a recovery setting. Previous human studies demonstrate a protective effect of n-3PUFA supplementation in catabolic situations by ameliorating the loss of muscle mass (McGlory et al., 2019; Murphy et al., 2011), and in animal models by ameliorating the loss of myosin heavy chain content (You et al., 2011a; You et al., 2011b). Therefore, in **Chapter 3** we adopted a

protocol to establish whether n-3PUFA supplementation exhibited a protective effect on muscle mass during a catabolic situation such as short term diet-induced weight loss in athletes. Our study design included a 40% decline in energy intake over a 2 wk period with two different groups. One group ingested an n-3PUFA containing beverage daily, whereas the other group ingested a carbohydrate control beverage. The overall macronutrient breakdown remained the same between the groups, equivalent to 50% carbohydrate, 35% fat and 15% protein. Although the 40% decrease in energy intake was substantial, it is not an uncommon protocol adopted by athletes involved in weight category sports such as weightlifting or boxing, in order to fit a certain category (Kasper et al., 2018). Moreover, such an energy deficit has been adopted in comparable human studies over time periods of similar or longer duration (Mettler et al., 2010; Pasiakos et al., 2014). However, we demonstrated that in the context of short-term weight loss, n-3PUFA does not protect the muscle cell from breakdown compared to a carbohydrate placebo.

Due to the pronounced energy deficit, we only adopted a 2 wk period of weight loss in athletes to enhance participant retention and compliance to the study. However, it is possible that the weight loss period was not of sufficient duration to observe the optimal effects of n-3PUFA supplementation on body composition. Recent research that adopted a similar protocol demonstrated that increasing dietary protein intake while undergoing a 21 day weight loss period spared lean body mass and promoted the loss of fat mass (Pasiakos et al., 2014). This protocol demonstrated a significant decline in body mass after 21 days, suggesting that 21 days of weight loss was necessary to observe changes in body composition. Further research should adopt a similar protocol to the present study but increase the

duration of weight loss in order to maximize the response n-3PUFA supplementation influences body composition.

During the weight loss period, even though there were no differences in body composition between n-3PUFA and the placebo supplementation groups and all participants experienced a decline in lean body mass, participants who supplemented with n-3PUFA recorded an increase in dominant leg press following the weight loss period. The mechanism that underpins this increase in muscle strength and decrease in lean mass remains to be fully elucidated. However, it is feasible that a neuromuscular adaptation with 6 wks of n-3PUFA supplementation may have influenced muscle strength. In support of this notion, previous studies report an improvement in brain function and an increase in neuromuscular adaptation with sustained n-3PUFA supplementation in humans (Rodacki et al., 2012; Johnson et al., 2008). Therefore, in **Chapter 4**, neuromuscular and muscle function were assessed with 3.6 g/day of n-3PUFA supplementation in combination with antioxidants. However, neuromuscular and muscle function did not improve with n-3PUFA supplementation regardless of whether n-3PUFA supplementation was coingested with antioxidants. The lack of improvement in neuromuscular function and isometric force production is consistent with some (Rodacki et al., 2012) but not all (Lewis et al., 2015) studies. Although improvements in neuromuscular function have been reported previously in well trained athletes (Lewis et al., 2015), these findings were analysed using magnitude based inferential statistics which has been well scrutinised (Welsh & Knight, 2015). Lewis et al., (2015) do report a likely beneficial increase in muscle activation of the vastus lateralis with n-3PUFA supplementation after 21 days of n-3PUFA supplementation. However, upon retesting on the same visit, a very likely harmful effect of n-3PUFA supplementation on muscle activation of the vastus lateralis is

reported when compared to an olive oil placebo. These findings suggest a large variation in electromyography findings. Therefore, taken together with findings from the present study, it is still unclear whether n-3PUFA supplementation improves both neuromuscular function and isometric force production in a healthy male population.

The incorporation of EPA and DHA into the phospholipid membranes of skeletal muscle cells have been shown to modify cell function by leading to a reduction in inflammation (Healy et al., 2000). In **Chapter 4**, we demonstrated a similar rate of incorporation of EPA and DHA into whole blood cells over 6 wks of n-3PUFA supplementation. Therefore, throughout this thesis we adopted a 1:1 ratio of EPA and DHA with regards to the n-3PUFA composition of the fish supplement. However, recent research has examined the biological fate of EPA and DHA independently, demonstrating that EPA blunts muscle protein breakdown (MPB) in C2C12 myotubes (Kamolrat & Gray, 2013). This blunting of MPB with EPA supplementation is consistent with our notion that n-3PUFA exerts a protective effect on the muscle cell, as seen by our observations of a decrease in creatine kinase concentrations (as a marker of muscle damage) and subsequent muscle soreness in **Chapter 2**. However, it should be acknowledged that in a practical sense, blunting MPB also may be inhibitory for muscle adaptation (Phillips et al., 2002). Therefore, the balance between improving recovery and improving muscle adaptation should be considered when supplementing with n-3PUFA.

Within this body of work, we demonstrated a benefit of n-3PUFA supplementation on exercise recovery but also for adaptation. However, from an athlete perspective, there are practical issues when supplementing with n-3PUFA. As detailed above, the most plausible mechanism(s) that underpins the improvement in muscle recovery is the incorporation of n-3PUFA into the phospholipid membrane of the muscle cell. However, one issue with n-3PUFA

supplementation is the time delay with entering the muscle phospholipid membrane, mainly due a high concentration of ingested n-3PUFA being oxidised within the body. Therefore, in **Chapter 4** we attempted to reduce oxidation in order to establish an optimal dosing regimen. We hypothesised that the addition of 1 g/day of curcumin, that exhibits antioxidant properties, to 3.6g/day n-3PUFA supplementation would reduce the n-3PUFA oxidation and therefore increase the rate and magnitude of n-3PUFA incorporation into the muscle phospholipid membrane. Interestingly, the addition of antioxidants to n-3PUFA in a beverage supplement did not influence the rate of n-3PUFA incorporation into both whole blood cells and the phospholipid membrane of skeletal muscle. These data suggest that adding antioxidants, in this case curcumin, confers no advantage in the context of a dosage strategy for optimising n-3PUFA incorporation into blood and skeletal muscle. It should be noted that we did not measure the oxidation of each supplement. Therefore, we are unable to conclude why the curcumin supplement had no influence on oxidation of n-3PUFA. However, we can assume the influence of curcumin on oxidation rates is negligible due to the incorporation rates of n-3PUFA into whole blood and the phospholipid membrane of skeletal muscle. Further research should examine the influence of adding other antioxidant sources to n-3PUFA on oxidation rates in human plasma via a liquid chromatography methodology.

Although past (McGlory et al., 2014; Browning et al., 2012) and present findings provide insight into the duration of n-3PUFA supplementation required before n-3PUFA concentrations increase within the muscle cell and whole blood cell, research is yet to examine the washout period following n-3PUFA supplementation. In the absence of information regarding the washout period following n-3PUFA supplementation, research studies are unable to conduct blinded crossover study design protocols. Being unable to

perform crossover designed studies does not allow participants to be their own control, therefore not allowing for maximal control of participant variables, such as baseline blood and skeletal muscle n-3PUFA concentration. Further research is warranted to establish a washout period from n-3PUFA supplementation and therefore informing future n-3PUFA research.

Our data (**Chapter 4**) adds to the present literature in the context of increasing n-3PUFA incorporation into blood and skeletal muscle cells. All experimental studies presented within this thesis supplemented participants with a 1:1 ratio of EPA to DHA. Generally, research involving n-3PUFA supplementation within the context of sporting performance adopted a supplementation regimen ranging from 2-5 g/day. Therefore, an optimal dosage of n-3PUFA is yet to be established. Not only does this gap in knowledge make it difficult to distinguish findings between studies, it also makes it difficult for practitioners to prescribe a dosage of n-3PUFA not only for athletes but for the general public. For example, although the protocols of muscle damage were different, Gray et al., (2014) experienced no improvement in muscle soreness with 3 g/day of n-3PUFA, however we observed a decrease in muscle soreness with 4 g/day of n-3PUFA supplementation. Therefore, a practitioner may come to the conclusion that 4g/day is more effective than 3 g/day but this is not the case for other aspects of sporting performance as well as brain and heart health (Tatsuno et al., 2013; Fakhrzadeh et al., 2010). Further research should attempt to identify an optimal dosage of n-3PUFA supplementation for specific situations, i.e. recovery and adaptation, in order for context specific recommendations to be communicated. Moreover, another common debate when discussing n-3PUFA supplementation is ingesting via tablet/beverage or ingesting via food. Taking a food first approach, but still achieving 2-5 g/day of EPA and DHA, is challenging.

In this regard, individuals would need to consume 10 cans of tuna a day in order just to reach 2g/day of n-3PUFA. Therefore, for athletes, it may be a more realistic approach to ingest n-3PUFA from a capsule or beverage form.

Athletes often use nutrition as a tool to perform better and minimise their chances of injury by maximising their ability to recover. During periods of fixture congestion in soccer (2 or more matches a wk), the injury rate during match play was 24.7% higher than in non-congested periods of the season (Dellal et al., 2013). Fixture congestion has also been shown to reduce soccer-specific performance, compared with non-congested fixture periods (Rollo et al., 2014). Therefore, soccer players attempt to optimise performance by speeding up the recovery process so they are less susceptible to injury and can still perform at a high level and one of the key tools for soccer players to achieve this is through nutrition. Protein ingestion is the most common nutritional strategy for improve the recovery process following bouts of intense exercise. However, the evidence for protein ingestion in promoting recovery is mixed (Hoffman et al., 2010; White et al., 2008). The anti-inflammatory properties of n-3PUFA may make it a viable nutritional component to ingest to improve recovery from muscle damage experienced during soccer play. In this regard, the aim of **Chapter 2** was to investigate whether adding n-3PUFA to a protein and carbohydrate beverage would improve markers of recovery in soccer players following a bout of intense muscle damaging exercise. We demonstrated that following 6 wks of n-3PUFA supplementation, adding n-3PUFA to a protein and carbohydrate beverage decreased muscle soreness and blood concentrations of CK but did not influence soccer specific sporting performance. However, the beneficial effect of n-3PUFA supplementation during periods of recovery may not be facilitative for muscle adaptation. Therefore, we aimed to understand the mechanism in which n-3PUFA

supplementation influenced muscle adaptation. The beneficial effect of supplementing n-3PUFA during catabolic situations, such as immobilisation, has previously been shown (McGlory et al., 2019). Therefore, in **Chapter 3** we investigated the influence of n-3PUFA supplementation during periods of weight loss in athletes. However, contrary to our original hypothesis, adding n-3PUFA to the diet during a short-term weight loss period did not influence body composition changes. However, an improvement in a strength measure even during a period of lean muscle loss may suggest that n-3PUFA supplementation alters neuromuscular function. We therefore theorise that the strength increase found in the participants ingesting n-3PUFA was from neuromuscular adaptation. This theory is backed up by previous research that has found improvement in EMD with sustained n-3PUFA supplementation (Rodacki et al., 2012). However, this improvement in EMD has only been observed in older female participants. Therefore, in **Chapter 4** we examined the influence of 6 wks of n-3PUFA supplementation on neuromuscular adaptation, and more specifically EMD and EMG. However, over the 6 wk period no improvement in EMG and EMD were observed, although a decrease in non-dominant VL EMD decreased after 3 wks, it returned back to baseline after 6 wks. Therefore, it can be concluded that n-3PUFA supplementation in healthy young men does not improve neuromuscular adaptation. At present, it is unclear why strength increased during a period of loss of muscle mass (**Chapter 3**) however no improvement in neuromuscular function was observed in similar participants (**Chapter 4**).

5.2 Limitations

This thesis provides novel insight into the influence of n-3PUFA supplementation on sporting performance. However, it is important to note that there are limitations to the data presented in this thesis. First, the inclusion criteria for the participants throughout this thesis,

i.e. active males, only encompasses part of the population, therefore the results of this thesis can only be interpreted to participants who fit the criteria of the population group examined in the studies.

All the participants in this thesis were males. It is well established that females have a different blood lipid profile to males (Metherel et al., 2009). Recent research examining the sex differences found that n-3PUFA supplementation improves muscle torque and muscle quality in females compared to males (Da Boit et al., 2017). Although researched in older adults, n-3PUFA has shown to improve muscle function and quality in female participants compared to males (Da Boit et al., 2017). A main theory, although speculative, as to why an improvement in muscle quality and function improved in females compared to males may be due to a greater capacity for improvement compared to males, therefore making the females more responsive to the n-3PUFA supplementation. Therefore, it is difficult to translate these results to a female population.

In older adults, the muscle response to anabolic stimuli has been shown to be impaired compared with younger individuals (Drummond et al., 2008). Therefore, the findings in this thesis cannot necessarily be directly extrapolated to both female and older adult populations. Further research is warranted to explore the role of n-3PUFA supplementation on sporting performance and health in female and older adult populations.

5.3 Future Research

This body of work demonstrates that n-3PUFA supplementation combined with protein and carbohydrate reduces muscle soreness following muscle damaging exercise in soccer

players. However, this thesis also reveals that supplementing with n-3PUFA during periods of energy restriction does not alter body composition but may partially improve muscle strength. This thesis also observed similar rates of incorporation of n-3PUFA into blood and skeletal muscle with or without the aid of antioxidants as well as no improvement in neuromuscular and muscle function. However, many questions still remain, as listed below.

1. What is the washout period of n-3PUFA supplementation in both blood and skeletal muscle?
2. Would longer periods of weight loss with added n-3PUFA improve body composition in athletes?
3. Would assessing direct measures of muscle damage (i.e. z-band streaming, sarcolemma disruption) provide better understanding into the influence of n-3PUFA on muscle recovery?
4. How would ingesting n-3PUFA effect recovery, adaptation and neuromuscular function in female participants?

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Appendices