An Examination of Neuromuscular Fatigue and Adaptations to Exercise and the Effect of Seal Oil Omega-3 Fatty Acid Supplementation in Trained and Untrained Males

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Abstract

Exercise performance is controlled and limited by the neuromuscular system. In response to exercise training, the neuromuscular system is able to rapidly adapt to novel exercise. However, few studies have investigated the effect of nutritional interventions on neuromuscular adaptations to exercise in trained or untrained males. Omega-3 polyunsaturated fatty acids (N-3 PUFA) are integrally involved in the development and maintenance of nerve tissue. Therefore, I hypothesize that N-3 PUFA supplementation will enhance neuromuscular adaptations to training in trained and untrained males.

First, a comparison of neuromuscular fatigue from a 10 km cycling time trial between trained cyclists and untrained cyclists demonstrated that both groups showed neuromuscular fatigue proportional to their training experience. Then, two studies examined the effect of seal oil N-3 PUFA supplementation on neuromuscular adaptations to training. Untrained males performed two-weeks of sprint interval training (SIT), while trained individuals complete three-weeks of sport specific training. Seal oil N-3 PUFA supplementation did not enhance
neuromuscular adaptations to SIT, as the training caused significant neuromuscular fatigue. Seal oil N-3 PUFA supplementation increased muscle activation (+22% SD 20 (ΔN-3 PUFA vs. ΔPlacebo)) and attenuated sprint cycling fatigue in well-trained males (+4.8% SD 3.4 (ΔN-3 PUFA vs. ΔPlacebo)).

In conclusion, seal oil N-3 PUFA supplementation appears to enhance neuromuscular adaptations to training in trained but not untrained males.
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List of Abbreviations

AA – Amino acid
AKT – Protein kinase B
ARA – Arachidonic acid
Ca$^{2+}$ – Calcium
CNS – Central nervous system
DHA – Docosahexaenoic acid
DPA – Docosapentaenoic acid
EMD – Electromechanical delay
EMG - Electromyography
EPA – Eicosapentaenoic acid
H+ - Hydrogen ions
HR – Heart rate
LFF – Low-force frequency fatigue
mA – M-wave amplitude
mAUC – M-wave area under the curve
mD –M-wave duration
mTor – Mammalian target of rapamycin
MVC – Maximum voluntary contraction
N-3 PUFA – Omega-3 polyunsaturated fatty acid
NMJ – Neuromuscular junction
P$\text{i}$ – Inorganic phosphate
PLA – Placebo
PTEN – Phosphatase and tensin homolog
QTW – Potentiated twitch force
RFD – Rate of force development
RM – Repetition maximum
RPE – Rating of perceived exertion
SIT – Sprint interval training
tAUC – Twitch area under the curve
tHRT – Twitch to half relaxation
tPF – Twitch peak force
tRFD – Twitch rate of force development
TP – Twitch time to peak force
TT – Time trial
VA – Voluntary activation
VL – Vastus lateralis
VM – Vastus medialis
VO$_2$ – Volume of oxygen consumed
Wmax – Watt max
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1 Introduction

Human movement is regulated by the communication between the brain and skeletal muscles, referred to as the neuromuscular system. Nerve stimuli, or action potentials, that are required to stimulate a muscle to contract for movement originate in the motor cortex of the brain and are transmitted down the spinal cord to motor neurons that innervate the targeted muscle. During exercise, repeated muscle contractions can cause fatigue within the neuromuscular system that limits exercise performance. Neuromuscular fatigue can manifest in the brain, at the nerve-muscle junction or in the conduction of action potentials through the muscle. Exercise induced neuromuscular fatigue can be dependent or independent of metabolic perturbations within the muscle. After novel or challenging exercise the neuromuscular system can rapidly adapt to improve muscle or movement coordination, increase the innervation of the muscle and its force generating capacity regardless of structural or metabolic adaptations [1].

There has been limited investigation of nutritional interventions to enhance neuromuscular adaptations to training. Dietary supplementation with carbohydrates and protein, alone and in combination, have been well studied for enhancing endurance exercise performance, recovery from training, muscle protein synthesis and enhancing muscle strength [2,3]. In contrast, the study of fat supplementation is less prevalent compared to carbohydrates and protein and has been focused primarily on altering muscle metabolism during endurance exercise [4,5] or body composition [6].

In addition to being used as a fuel source, fats have many important roles throughout the body, including the development and maintenance of nerve tissue. Fats that the body requires for normal function can be synthesized endogenously with the exception of omega-3 (N-3) and omega-6 (N-6) polyunsaturated fatty acids (PUFA). These fats are classified as essential fatty
acids, as they must be sourced exclusively from the diet. Historically, N-3 and N-6 PUFAs were consumed at similar levels; however, advances in agricultural practices and food processing technology have contributed to changes in the modern Western diet [7,8]. Such changes include increased consumption of processed foods, oil seeds and grains that have contributed to a marked decrease in diet N-3 : N-6 PUFA [7,8]. Both N-3 and N-6 PUFAs are integral components of nerve membranes and for normal physiologic function [9]. Since modern intakes of N-3 PUFAs are much lower than N-6 PUFAs it is unclear if this dietary imbalance has impacted neuromuscular function during exercise or if N-3 PUFA supplementation could enhance neuromuscular function.

N-3 PUFAs occur in different fatty acid lengths and naturally occur in different food sources. The shortest N-3 PUFA is alpha-linoleic acid (ALA) (carbon length:double bonds) (18:3 N-3), which is derived from plant sources (e.g. flax, hemp, soy). Eicosapentaenoic acid (EPA) (20:5 N-3), docosapentaenoic acid (DPA) (22:5 N-3) and docosahexaenoic acid (DHA) (22:6 N-3) are all derived from marine sources. EPA and DHA are present in fish, while EPA, DPA and DHA are present in marine mammals (e.g. seal) [10].

Animal model research has identified that N-3 PUFAs are integrally involved in normal physiologic function and the development and maintenance of nerves [11-13]. The basis for research into the potentially ergogenic effect of N-3 PUFAs has emerged from clinical intervention studies that have demonstrated a neuro- and cardio-protective aspect of these fats [14-16]. As such, the effective use of N-3 PUFAs in different clinical settings provides a logical link to study their ergogenic potential for enhancing adaptations to training and exercise performance.

The overall purpose of this thesis is to examine if N-3 PUFA supplementation can enhance neuromuscular adaptations to training in males with different training backgrounds.
Chapter 2 of this thesis describes the neuromuscular system and how it functions during exercise and how exercise causes neuromuscular fatigue. Chapter 2.3 is adapted from a submitted systematic review of the literature that summarizes all placebo controlled randomized trials in humans that have investigated the effect of N-3 PUFA supplementation on adaptations to training and exercise performance.

Chapters 4-6 detail the laboratory experiments in this thesis. Chapter 4 compares baseline neuromuscular function and neuromuscular fatigue from a 10 km cycling time trial (TT) between a group of well-trained cyclists and recreationally-active but untrained cyclists. The 10 km cycling TT is a standard laboratory test to measure acute exercise performance and adaptations to training that is used in Chapters 5 and 6. Since the trained and untrained participants showed neuromuscular fatigue, it was important to investigate if N-3 PUFA supplementation could enhance neuromuscular adaptations to training in both groups.

Chapter 5 investigates how N-3 PUFA supplementation affects neuromuscular adaptations to sprint interval training (SIT). SIT is a low-volume, high-intensity training technique that promotes muscle metabolic adaptations in a more time-effective manner than traditional high volume low-intensity endurance training [17-20]. As such, it is an increasingly common training modality for new exercisers. While the muscle metabolic adaptations to SIT and detraining have been thoroughly examined [17-19,21], the neuromuscular adaptations to SIT are unknown. A two-part study was performed to describe the neuromuscular adaptations to SIT, followed by a randomized placebo-controlled double-blinded study to investigate the effect of N-3 PUFA supplementation on neuromuscular adaptations to SIT.

Chapter 6 investigates if N-3 PUFA supplementation could enhance neuromuscular adaptations to training in a group of well-trained athletes. Neuromuscular testing along with a
battery of laboratory based performance tests were used to examine changes in neuromuscular function and exercise performance.

Chapter 7 provides an overall summary of the research results along with the implication of these findings. Limitations of the studies are also discussed followed by an overall conclusion of the thesis.
2 Review of Literature

Section 2.3 ‘The effect of omega-3 fatty acids supplementation on training adaptations and exercise performance: a systematic review of the literature’ has been submitted for peer-reviewed publication

Contribution: As first author I designed the systematic review, performed the literature search and wrote the first draft of the manuscript.

N-3 PUFA supplementation has been investigated as a potential stimulator of adaptations and enhanced exercise performance in many different setting and through multiple areas of exercise physiology. This systematic review provides a summary of the trials performed in humans and identifies the specific areas where supplementation is effective.

2.1 Neuromuscular Function

The neuromuscular system is comprised of the brain, spine, and motor neurons that transmit action potentials, which stimulate muscle contractions. These action potentials are electrical signals that are transmitted through the dendrite of a neuron and then transmitted through the cell body and along the axon to the dendrite of a neighboring neuron. The neuronal membrane is a poor conductor of action potentials and to enhance the speed of action potential transmission, a myelin sheath surrounds nerve axons [22]. For a muscle contraction to occur, an action potential is generated in the central neuromuscular system where it is transmitted from the motor cortex in the brain, down the spinal column and along an alpha-motorneuron until it reaches a neuromuscular junction (NMJ). There are two types of alpha-motorneurons, a1 and a2, that innervate fast and slow twitch muscle fibres respectively [23]. This innervating neuron
determines both the muscle’s twitch and metabolic phenotype [23]. Each alpha-motorneuron and the group of muscle fibres it innervates, is referred to as a motor unit. When an action potential reaches the end of the alpha-motorneuron, the neurotransmitter acetylcholine is released into the synapse from the axon terminal to transmit the action potential stimulus across the neuromuscular junction (NMJ) to the muscle fibres. Upon successful transmission to the peripheral neuromuscular system, the action potential propagates from the motor end plate (muscle side of the NMJ synapse) and travels towards both ends of the muscle fibre along the sarcolemma (muscle cell membrane). At regular intervals along the sarcolemma are t-tubule openings, which are a network of tubes that allow the action potential to propagate into the middle of the muscle fibre [22]. As the action potential travels through t-tubules, the change in voltage activates dihydropyridine receptors, which stimulates the release of calcium (Ca\(^{2+}\)) from the sarcoplasmic reticulum via ryanodine receptors [24]. This increase in intramyocellular Ca\(^{2+}\) enables binding with the myofilament troponin, which causes a conformational change in the myofilaments such that troponin alters the position of tropomyosin to allow actin and myosin to bind together, enabling a muscle contraction [24]. During exercise, increased circulating H\(^+\), muscle lactate or depletion of muscle glycogen can cause group III-IV muscle afferents to provide feedback to the brain that lower voluntary muscle force production and impaired exercise performance [25]. While acute exercise-induced neuromuscular fatigue can impair exercise performance, the neuromuscular system is quite plastic and can rapidly adapt to novel exercise challenges to delay the onset of future fatigue and improve exercise performance [1].

2.1.1 Central Neuromuscular Adaptations to Exercise

In response to novel acute and chronic exercise, functional and morphological adaptations have been observed across the neuromuscular system that result in increased muscle strength and reduced neuromuscular fatigue [26-29]. Neuromuscular learning in the central
nervous system (CNS) plays an essential role in adaptations to training, as new motor programs are developed to increase motor unit recruitment in agonist muscles and reduce co-activation of antagonist muscles. These adaptations result in increased strength, endurance and movement efficiency [1,30,31]. The pervasiveness of neuromuscular adaptations to new exercise was demonstrated during the first four and eight-weeks of isometric resistance training in untrained men [32,33]. The authors showed that the quadriceps maximal voluntary contraction (MVC) force was 18% and 36% higher with no measurable change in limb girth [32,33]. These findings suggest that neuromuscular adaptations to training play a primary role in initial adaptations to training (Figure 1), however, a major limitations exists as changes in muscle metabolic properties were not investigated. While acute increases in muscle protein synthesis after exercise can not be ignored [34], it appears that hypertrophy associated strength gains require greater than eight weeks to be measured [35,36].

Increased CNS neuronal excitability has been observed within the motor cortex after four weeks of resistance training in untrained men and women [35]. This adaptation is thought to increase synaptic input and motor unit synchronization, which in turn lowers motor unit recruitment thresholds and ultimately increases motor unit firing rate causing greater force development and muscle force output. Furthermore, movement-related cortical potentials, a measurement of the post-synaptic potential of motor cortex dendrites during movement, were decreased following training [37]. This indicates central neural adaptations to training can reduce the neural drive required to generate a given force by a muscle. This adaptation might also increase total muscle stimulation during acute near maximal activity or prolong the ability to stimulate muscle during sub-maximal exercise [37].
Figure 1 – Proposed timeline of neuromuscular/myofibrillar adaptations to resistance exercise from Sale [1].
Animal studies provide a unique examination of exercise-associated adaptations at the alpha-motorneuron. After 10-weeks of treadmill running, the soleus and extensor digitorum longus of rats were dissected and compared to sedentary controls. The average diameter of nerves that innervated the soleus muscle increased by 14% [38]. The increased neuron size was accompanied by higher succinate dehydrogenase activity to facilitate increased neural metabolism necessary during activity.

Examination of motorneuron adaptations to one-week of treadmill running revealed significant reduction in mRNA associated with inhibitory nerve responses [39]. After 16-weeks of training, mRNA levels were consistent with the altered inhibitory response along with increased muscle innervation. This suggests that the neuromuscular system is able to rapidly adapt to acute and chronic exercise stress by modifying the expression of inhibitory genes and with complementary morphological adaptations to increase muscle innervation.

While it is not possible to directly measure morphological changes in the central neuromuscular system in humans, evidence of central neuromuscular adaptations, including those at the alpha-motorneuron, can be measured from changes in muscle voluntary activation. In the case of the quadriceps muscle, percent voluntary activation is measured using the interpolated twitch technique, where the femoral nerve is super-maximally stimulated during a MVC. The ratio of the increase in force from the superimposed twitch is compared to the force elicited from a stimulation 2-5 seconds after the contraction with the muscle in a potentiated state (Qtw), is considered an individual’s voluntary activation [40,41]. Percent voluntary activation is calculated as follows:

\[ VA(\%) = (1 – \text{superimposed twitch} / Q_{TW}) \times 100 \]
### 2.1.2 Peripheral Neuromuscular Adaptations to Exercise

The NMJ is a site where numerous neurophysiologic adaptations can translate into increased transmission of central neural stimulation to the peripheral neuromuscular system. Examination of NMJ morphology using fluorescent staining after 12-weeks of low and high-intensity endurance training revealed an increase in NMJ size in both exercise groups [42]. Surprisingly, no change in acetylcholine vesicle or receptor concentration was observed in either group, which may have been related to the age of the study animals (Sprague-Dawley rats <1 year) [42]. Visualization of the NMJ using immunofluorescent staining and laser scanning microscopy showed the high-intensity group had greater axonal sprouting with longer sprout length. Similar findings have been reported by other groups in response to 30 days of moderate intensity endurance training [43] and electrical stimulation of the sciatic nerve [44]. Exercise-induced axonal sprouting is also coupled with increased acetylcholine release capacity from the axon, increased acetylcholine receptor content on the muscle fibre motor endplate [45] and increased acetylcholinesterase concentration to more rapidly breakdown acetylcholine for re-uptake by the transmitting axon [46]. All of these adaptations increase the number of NMJs and the rate of action potential transmission from the alpha-motorneuron to the motor end plate [47], which can lead to increased voluntary activation of muscle fibres and also increased muscle action potential size during exercise [48]. Ultimately, greater stimulation from action potentials will lead to an increased rate of muscle contractions, rate of muscle force development and force output.

Action potential transmission through the t-tubule network and subsequent stimulation of sarcoplasmic calcium (Ca\(^{2+}\)) release/reuptake are important steps in peripheral neuromuscular system function. After 12-weeks of resistance training, there was no change in sarcoplasmic calcium release or Ca\(^{2+}\) ATPase activity in young women [49]. In contrast, elderly women who
completed the same training protocol showed a partial reversal of age-associated decrease in calcium release and Ca\(^{2+}\) ATPase activity. While there is limited research in this area, it appears that Ca\(^{2+}\) handling is not a limiting factor for peripheral neuromuscular adaptations to exercise.

Throughout the neuromuscular system there are numerous facets that are capable of rapid adaptations to exercise. Central adaptations within the motor cortex to increased alpha-motorneuron diameter can help increase muscle stimulation during exercise, while peripheral adaptations can continue to convert central stimulation into muscle contraction. Therefore, it appears that central and peripheral neuromuscular adaptations to exercise occur in a complementary manner.

2.1.3 Central Neuromuscular Fatigue

Central neuromuscular fatigue refers to fatigue originating between the motor cortex and the NMJ that results in decreased muscle activation. This translates into reduced stimulation from the central nervous system to the alpha motorneuron, which directly reduces the ability to voluntarily activate motor units in targeted muscle(s). Central neuromuscular fatigue occurs with higher rating of perceived exertion (RPE) resulting from increased blood lactate, H+ or muscle glycogen depletion, which can occur during prolonged sub-maximal or short intense exercise [50]. It has been previously suggested that the onset of central neuromuscular fatigue during exercise exists as a homeostatic safety mechanism to prevent biologic failure and internal damage in humans [51]. This model proposed by St. Clair and Noakes [51], is based on the brain’s ability to control the number of motor units recruited to perform a specific task. Since all motor units within a muscle are never activated at the same time [52], the body is never truly able to perform maximal exercise or exercise to exhaustion because of this central regulation. An individual’s perceived exertion has a direct effect on central neuromuscular fatigue [50]. Therefore, modulating perceived exertion might be necessary to approach maximal exertion. To
test this theory, Swart et al. [53] had well trained individuals cycle at a rating of 16 on the Borg RPE scale until their power output decreased below 70% of average power measured in the first three minutes of cycling. Prior to exercise, participants were given either an amphetamine supplement or a placebo, as amphetamines have been found to enhance athletic performance by attenuating perceived fatigue and increasing time to exhaustion [54,55]. During the amphetamine trial, participants’ cycling power output decreased at a slower rate compared to the placebo group, leading to a 32% longer exercise duration that was not due to any measured metabolic differences between the groups. This finding clearly indicates the placebo group experienced central neuromuscular fatigue with metabolic and cardio-respiratory reserves still accessible and unused, possibly to prevent self-injury. To quantify the level of central neuromuscular fatigue in well trained cyclists, Presland et al. [56] examined RPE and central neuromuscular fatigue during cycling to exhaustion at 70% VO\textsubscript{2}max. Exercise time and perceived exertion were positively related to each other, which also corresponded with significant central neuromuscular fatigue at the time of exhaustion that persisted for at least 30 minutes post-exercise. While peripheral factors, such as glycogen depletion could have influenced the onset of fatigue, participants showed a 30% decrease in MVC force and 13% reduction in voluntary activation, suggesting perceived exertion is related to the ability to centrally activate muscle.

The regulation of central fatigue is thought to occur through the Group III-IV muscle afferents [57]. These free nerve endings are stimulated by muscle contraction to provide feedback to the innervating motor neurons, motor cortex and brain regions associated with movement planning. Muscle feedback can alter the firing rate of motor units, resulting in the gradual decrease in muscle force production [58]. Exercise induced stimuli such as lactate, potassium and altered muscle perfusion can all cause negative feedback to attenuate exercise performance [59]. Amann et al. [60] investigated the effect of blocking the spinal transmission of
group III-IV afferents using the opioid fentanyl during a 5 km cycling time trial. During the first 2.5 km, cycling power was increased 6% compared to placebo and control, while in the final 2.5 km power was 11% lower. The fentanyl trial allowed for a higher accumulation of blood lactate and arterial hemoglobin desaturation, which ultimately lead to a greater peripheral fatigue as a result of deregulation of central neuromuscular control of exercise.

Central neuromuscular fatigue limits exercise performance as a result of metabolic perturbations and feedback from skeletal muscles. This appears to be a mechanism to preserve homeostasis and prevent irreversible damage to the body.

2.1.4 Peripheral Neuromuscular Fatigue

Peripheral neuromuscular fatigue describes exercise induced factors distal to the NMJ that cause a reduction in muscle force generating capacity [61]. There are two aspects that can independently or in concert cause peripheral neuromuscular fatigue during exercise: altered action potential transmission along the sarcolemma and impaired excitation-contraction coupling [48]. Muscle action potential transmission along the sarcolemma (M-wave) is an essential step for a muscle contraction, as only this stimulus can initiate the excitation-contraction coupling release of Ca$^{2+}$ required for actin/myosin interaction. A prolonged M-wave duration indicates a reduction a sarcolemma conduction velocity, whereas reductions in M-wave amplitude denotes NMJ fatigue either at the pre-synaptic cleft or motor end plate [62,63]. An obvious cause of altered M-wave form is damage to the muscle itself from the mechanical stress of exercise. In contrast, reduced sodium/potassium pump activity or impaired activation of sodium channels within the muscle fibre can reduce sarcolemma membrane excitability and conduction velocity [63]. In vitro experiments of muscle fibre conduction properties using mouse muscle preparations determined an increase in potassium concentration from 5 mM to 10 mM caused a decrease in action potential amplitude of 70% and increased duration by 100% [25]. Estimated
changes in electrolyte concentration suggest that after each muscle action potential, the extracellular sodium concentration decreases by 0.5 mM and extracellular potassium concentration increases by 0.28 mM [64]. Therefore, it is quite likely that changes in sarcolemma conduction velocity and excitability can occur early in exercise. After two hours of cycling at 65% of VO$_2$max, trained cyclists showed increased M-wave duration coupled with a decrease in amplitude, which resulted in lower muscle EMG measurements compared to pre-cycling [62]. This suggests peripheral neuromuscular fatigue was the result of both impaired nerve transmission across the NMJ and reduced nerve conduction velocity and excitability of the sarcolemma. In response to a single bout of treadmill running, rat muscle membrane content of sodium/potassium pumps was increased with a corresponding increase in mRNA [65]. This indicates membrane based adaptations can rapidly occur to improve sodium and potassium kinetics across the muscle membrane to reduce fatigue. This improved regulation of sodium and potassium in whole muscle has been shown to prevent the fatigue-associated changes in M-wave characteristics observed in single muscle fibre preparations [66].

Excitation-contraction coupling is the process of converting muscle action potential impulses to actin/myosin interaction and cross bridge cycling to generate muscle force [67]. During exercise, excitation-contraction coupling can become impaired from decreased sensitivity of t-tubule voltage sensing proteins and altered sarcoplasmic reticulum Ca$^{2+}$ handling [68]. Muscle action potential propagation down t-tubules is essential for the release of Ca$^{2+}$; however, during exercise the gradual increase in myoplasmic Ca$^{2+}$ concentration can slow and even block action potential transmission into the muscle fibre core [24]. As a result, Ca$^{2+}$ release can be impaired or inhibited, causing a failed contraction. In response to metabolic changes associated with muscle fatigue, such as the accumulation of hydrogen ions (H$^+$) from lactic acid production, ryanodine receptor channels open less frequently and for a shorter duration [69]. This lower Ca$^{2+}$
release can affect Ca\textsuperscript{2+} binding to troponin, which is necessary to facilitate actin/myosin interaction. In contrast, exhaustive exercise has been found to reduce the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase pump activity [70], thereby slowing the rate of muscle relaxation and contraction. Similar reductions in Ca\textsuperscript{2+} pump function were observed after 30 minutes of eccentric exercise at 60% of MVC [71]. While muscle force-generating capacity returned following exercise, Ca\textsuperscript{2+} pump function was impaired 60 minutes post-exercise. This impairment was likely a result of structural changes in the Ca\textsuperscript{2+}-ATPase pump protein or the phospholipid membrane of the sarcoplasmic reticulum [71]. Similar findings from single muscle fibre studies showed sarcoplasmic reticulum Ca\textsuperscript{2+} pump function can be depressed for at least 30 minutes after contractions; however, metabolic factors including ADP, H\textsuperscript{+} and P\textsubscript{i} could have influenced this finding [72].

It is clear muscle action potential transmission and excitation-contraction coupling are directly linked to peripheral neuromuscular fatigue. Interestingly, muscle damage and altered ion handling can cause impairments of both processes; however, the accumulation of metabolites appears to only impact excitation-contraction coupling. While maintaining optimal excitation-contraction coupling is necessary for actin/myosin interaction and force production, muscle action potential transmission is the rate-limiting step for muscle contractions to occur.
2.2 The Role of Omega-3 Fatty Acids in Nerve Function

The importance of dietary fatty acids was first identified by Burr et al. [12,13] during experiments with rats consuming diets devoid of fats. Upon re-feeding with N-3 PUFA ALA and N-6 PUFA linolenic acid, the animals began to regain weight and an overall healthy appearance. Since saturated fats fed to the animals did not promote regrowth, it was concluded that N-3 and N-6 PUFAs must be consumed from the diet and they cannot be synthesized endogenously [12].

A diet high N-6 PUFAs and low in N-3 PUFAs has been shown to impair nerve function. A clinical case study reports the presence of numbness, tingling, blurred vision and muscle weakness in a patient receiving a total parenteral nutrition diet low in N-3 PUFAs for five months [73]. These symptoms were reversed when ALA was added to the diet. Subsequently, an animal model study by Bourre et al. [11] revealed that a diet low in N-3 PUFA ALA caused abnormal nerve function measured from an electroretinogram compared to soybean oil fed controls. The authors also observed impaired task learning on a low N-3 PUFA diet. As such, N-3 PUFAs have been identified as integral components of nerve tissue, including brain and eye [74-76].

While ALA was used to resolve neurological deficits, our understanding of the relationship between N-3 PUFAs and nerve function has reveal that DHA is highly involved in the development and maintenance of nerve tissue, especially in the brain [77]. This occurs primarily by DHA incorporation into the phospholipid membrane combined with phosphatidylcholine [78]. Using an animal model of spinal cord injury, it has been shown that direct administration of DHA can support nerve repair and recovery [79], further demonstrating the importance of DHA and nerve function. From this investigation, DHA was shown to
modulate the protein kinase B (AKT) / mammalian target of rapamycin (mTor) pathway to cause nerve re-growth. Specifically, DHA promoted promoted nerve sprouting and neuroplasticity after spinal cord injury while down-regulating phosphatase and tensin homolog (PTEN) that suppresses neuroplasticity.

While there is clear evidence of the need for N-3 PUFAs in the human diet, there are currently no population based daily recommended intakes (DRIs) [80]. The American Dietetic Association and Dietitians of Canada joint position recommends ≥500 mg d⁻¹ of N-3 PUFA and 1.3-2.7 g d⁻¹ of ALA N-3 PUFA [81] and the Institute of Medicine and FDA have set an acceptable macronutrient distribution range of 0.6-1.2% of total energy for adults and an adequate intake (AI) of <1.6 g d⁻¹ for males >18 years and <1.1 g d⁻¹ for females >18 years [82]. There is evidence that Canadian [83] and Western [7,8] populations have a low N-3 PUFA intake and as a result many individuals, choose to supplement their diet to support their health [84]. Given the role of N-3 PUFAs in the development and maintenance of nerve structure and function, N-3 PUFA supplementation may enhance adaptations to training and exercise performance, especially through the neuromuscular system.

N-3 PUFA supplements currently available can be sourced from fish, krill, algae and seal oil. While krill oil N-3 PUFAs occurs in phospholipid form, it appears less bioavailable than the triglyceride structure of fish, algae and seal oil N-3 PUFAs [10,85]. The supplementation studies within this thesis use seal oil omega-3s because seal triacylglycerol molecules have N-3 PUFA fats primarily in the sn-1 and sn-3 positions, as opposed to the sn-2 position of fish oil N-3 PUFA [10,86]. Fats in the sn-3 position are preferentially cleaved by sublingual lipases, and the sn-1 fat is cleaved in the small intestine, while the sn-2 fatty acid is left for later esterification [87,88]. These structural differences are thought to enable chylomicrons and chylomicron remnants containing seal oil N-3 PUFA to have a higher rate of clearance from the blood
compared to fish oil intake [89], potentially increasing bioavailability. While digestibility or bioavailability between N-3 PUFA sources is not being examined within, the duration of supplementation of each experiment is short, and therefore the most absorbable form of N-3 PUFA is necessary to test the effect of supplementation on neuromuscular adaptations to training.
2.3 The effect of omega-3 fatty acids supplementation on training adaptations and exercise performance: a systematic review of the literature.

2.3.1 Abstract

Omega-3 polyunsaturated fatty acids (N-3 PUFA) have gained increasing popularity as a dietary supplement. This review evaluates the methodological quality and findings of trials investigating the effect of N-3 supplementation on training adaptations and exercise performance. The PubMed, Medline and Cochrane databases were searched from inception to July 30, 2015 for randomized, controlled human studies that investigated the effect of N-3 PUFA supplementation on exercise or athletic performance. Included studies were evaluated independently by two reviewers graded based on their methodological quality using the PEDro rating scale. Of the 36 trials that met the inclusion criteria, 25 showed a positive effect of N-3 PUFAs. N-3 PUFA supplementation had the following beneficial effects: 1) improved cardiovascular function with lower submaximal exercise heart rate, higher cardiac output and stroke volume and lower heart rate variability; 2) improved pulmonary function with increased forced expiratory volume both in normal individuals and those with exercise induced bronchoconstriction; 3) attenuation of exercise induced muscle damage and inflammation; and 4) enhanced training adaptations from increased muscle mass, strength and motor control. Adverse events were under reported in the trials reviewed, but those reported were consistent with the N-3 PUFA literature. All included trials were of moderate to high methodological quality. There is moderate to high quality evidence to support the use of N-3 PUFA supplementation to enhance training adaptations and exercise performance through improved cardiovascular and pulmonary function, attenuation of muscle damage and inflammation, increasing muscle mass and motor control.
2.3.2 Introduction

The omega-3 polyunsaturated fatty acids (N-3 PUFA) are essential nutrients that cannot be synthesized endogenously and must be sourced from the diet. Alpha-linoleic acid (ALA), the shortest N-3 PUFA with 18 carbons and three double bonds (18:3 N-3), is derived from plant sources (e.g. flax, canola). Eicosapentaenoic acid (EPA) is 20 carbons long with five double bonds (20:5 N-3) and is obtained from marine sources and marine oil supplements along with the 22 carbon, six double bond docosahexaenoic acid (DHA) (22:6 N-3). Interest in the clinical effects of N-3 PUFA was sparked by observational studies linking a high intake of N-3 PUFA to a low risk for ischemic heart disease [90]. Subsequent, larger cohort based cardiovascular trials have been null [16]. However, smaller intervention studies showing that N-3 PUFA’s have a wide range of physiological effects from altering nerve [14] and peripheral vascular functions [91] to increasing muscle mass [92], suggest that N-3 PUFA supplementation may have ergogenic potential for athletic performance and adaptations to training.

The objective of this review is to determine if N-3 PUFA supplementation can impair or enhance training adaptations and exercise performance, identify potential mechanisms of action on different aspects of physiology and discuss optimal dosing for potential beneficial effects.

2.3.3 Methods

2.3.3.1 Search Strategy

A search strategy was developed in accordance with the standards for the reporting of scientific literature (STARLITE) [93]. The STARLITE method was used for developing search methodology. Searches were carried out using PubMed, Medline and Cochrane Central Register of Controlled Trials from inception to July 30, 2015. The reference section of identified articles was also examined for potentially relevant inclusions. Searches were executed using the
following terms: ‘omega-3 fatty acid or omega-3 supplementation’, and ‘omega-3 fatty acid or omega-3 supplementation’ and ‘exercise’, ‘training’, ‘endurance’, ‘strength’, neuromuscular, ‘performance’ and ‘athlete’.

2.3.3.2 Study Selection

Articles were selected based on the following criteria adapted from Quesnele et al. [94]: 1) English language; 2) human studies; 3) determined the effect of long chain N-3 PUFA supplementation on training adaptations, exercise or athletic performance (measures of power, strength, endurance, fatigue, metabolism, sport specific measures, neuromuscular function, muscle damage, recovery from exercise and immune function); 4) randomized controlled trials (RCT); 5) published in a peer-reviewed journal. If a study investigated N-3 PUFAs with other potentially performance-enhancing compounds, the study was included only if a stratified analysis for N-3 PUFA was performed.

2.3.3.3 Data Collection and Analysis

One author (EJHL) performed the literature search and screened articles for consideration. EJHL and GDW independently reviewed the selected articles for eligibility if they related to N-3 PUFA supplementation for training adaptations and exercise performance. Eligible articles were then critically appraised based the Physiotherapy Evidence Database (PEDro) rating scale for RCTs [95] as previously described [94]. Trials with a score ≥7 were considered high quality. Studies with a score of 5-6 were considered as moderate quality and studies with a score ≤5 were considered poor quality.

The preferred reporting items for systematic reviews and meta-analysis (PRISMA), was used for reporting in this systematic review. Characteristics of each RCT including inclusion criteria, participants’ age and gender, N-3 PUFA source, dose and duration, primary outcome measure and any other interventions are summarized in Table 1.
2.3.4 Results

2.3.4.1 Search Results

The search process and selection of articles is shown in Figure 1. Using the specified search terms 573 titles were found, of which 59 articles were excluded as being duplicates and 447 were excluded based on the title not being relevant to the search strategy. The abstracts of the remaining 67 articles were screened; 18 were excluded as not meeting the inclusion criteria and the remaining 49 articles were reviewed in full. Of these articles, 13 were excluded from the review: one systematic review was strictly focused on clinical cardiovascular outcomes [96]; one trial examined N-3 PUFAs in combination with other supplements [97]; and 11 did not have adequate randomization [98-108]. Thus, a total of 36 peer-reviewed articles were included in this systematic review. Selected articles were then categorized by hypothesized N-3 PUFA mechanism of action: antioxidants status (n=6), cardiovascular function (n=6), post-exercise muscle damage, inflammation and/or immune function (n=11), pulmonary function (n=4) and training adaptations and performance (n=9).
Figure 1 - Flow diagram of literature search process.
<table>
<thead>
<tr>
<th>First author, year</th>
<th>N, sex, sport/population</th>
<th>Mean age</th>
<th>Groups</th>
<th>Supplementation</th>
<th>Duration</th>
<th>Outcome measure</th>
<th>Intervention</th>
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</thead>
<tbody>
<tr>
<td><strong>Anti-oxidants</strong></td>
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<tr>
<td>Filaire et al. 2010</td>
<td>22, male, trained judo athletes</td>
<td>22.3±1.4</td>
<td>N-3 Placebo</td>
<td>N-3 FO 2 g/d (0.6 g EPA, 0.4 g DHA); Pla gelatin, water glycerine</td>
<td>6-weeks</td>
<td>Oxidative stress and glutathione peroxidase activity</td>
<td>Judo training</td>
</tr>
<tr>
<td>Filaire et al. 2011</td>
<td>36, male, trained judo athletes</td>
<td>22.3±1.4</td>
<td>N-3 N-3+Vit Placebo</td>
<td>N-3 FO 2 g/d (0.6 g EPA, 0.4 g DHA); Vit (60 mg vit C, 30 mg vit E, 6 mg B-carotene); Pla gelatin, water, glycerin</td>
<td>6-weeks</td>
<td>Oxidant stress and antioxidant markers</td>
<td>Judo training</td>
</tr>
<tr>
<td>Martorell et al. 2013</td>
<td>15, male, professional soccer players</td>
<td>19.7±0.4</td>
<td>N-3 Placebo</td>
<td>N-3 DHA (1.14 g/d) in almond drink; Pla almond drink</td>
<td>8-weeks</td>
<td>Oxidative stress in neutrophils</td>
<td>Soccer training; 2-h sport specific drills</td>
</tr>
<tr>
<td>Martorell et al. 2014</td>
<td>15, male, professional soccer players</td>
<td>19.7±0.4</td>
<td>N-3 Placebo</td>
<td>N-3 DHA (1.14 g/d) in almond drink; Pla almond drink</td>
<td>8-weeks</td>
<td>Oxidative stress and PGE2</td>
<td>Soccer training; sport specific drills</td>
</tr>
<tr>
<td>McAnulty et al. 2010</td>
<td>48, male and female, trained cyclists</td>
<td>N-3 24.1±2.4 N-3+Vit 25.2±2.1 Vit 22.2±0.9 Pla 26.9±2.8</td>
<td>N-3 N-3+Vit Vit Placebo</td>
<td>N-3 FO 2 g EPA, 0.4 g DHA; Vit (3000 IU Vit A, 2 g vit C, 800 IU Vit E, 200 μg selenium); Pla soybean and</td>
<td>6-weeks</td>
<td>F2-isoprostanes</td>
<td>Cycling training; 3 days of 3-h cycling at 57% of</td>
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<tr>
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<td>Participants</td>
<td>Intervention</td>
<td>Eicosapentaenoic Acid (EPA)</td>
<td>Docosahexaenoic Acid (DHA)</td>
<td>Duration</td>
<td>Outcome Measures</td>
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<td>Poprzecki et al. 2009</td>
<td>24, male, recreationally active</td>
<td>N-3 21.0±0.9; Pla 20.7 ± 1.1</td>
<td>N-3 FO 1.3 g/d (0.39 g EPA, 0.26 g DHA); Pla gelatin capsules</td>
<td>6-weeks</td>
<td>Blood antioxidant status 1h cycling at 60% VO2 max</td>
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<tr>
<td>Buckley et al. 2009</td>
<td>25, male, Australian football athletes</td>
<td>N-3 21.7±1.0 Pla 23.2±1.1</td>
<td>N-3 FO 6.0 g/d (0.36 g EPA, 1.56 g DHA); Pla 6.0 g/d sunflower oil</td>
<td>5-weeks</td>
<td>Running time to exhaustion, HR during submaximal exercise 5-week of standardized training; submaximal treadmill run and run to exhaustion</td>
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<tr>
<td>Macartney et al. 2014</td>
<td>39, male, recreationally active</td>
<td>N-3 28±5 Pla 24±7</td>
<td>N-3 FO 2.0 g/d (EPA 0.14 g, 0.56 g DHA); Placebo 2.0 g/d soya bean oil (0.15 g ALA, 1.0 g omega-6)</td>
<td>8-weeks</td>
<td>HR at rest and during exercise, HR recovery 10-min submaximal cycling, 6 Wingates</td>
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<td>Ninio et al. 2008</td>
<td>17 male, 29 female, sedentary hypertensive and overweight</td>
<td>N-3 52 (40-63) N-3+Ex 48 (37-52) N-3 51 (31-</td>
<td>N-3 FO 6.0 g/d (0.36 g EPA, 1.56 g DHA); Pla 6.0 g/d sunflower oil</td>
<td>12-weeks</td>
<td>Resting HR, HRV and HR during submaximal exercise Ex groups performed 45-min aerobic exercise at 75% of age predicted HR 3d/wk</td>
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<td>Study</td>
<td>Subjects</td>
<td>Treatment</td>
<td>Duration</td>
<td>Main Outcomes</td>
<td>Exercise Protocol</td>
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<td>Peoples et al. 2008</td>
<td>16, male, trained cyclists</td>
<td>N-3 FO 8.0 g/d (0.8 g EPA, 2.4 g DHA); Pla 8.0 g/d olive oil</td>
<td>8-weeks</td>
<td>Cycling energy efficiency and HR</td>
<td>Off-season cycling training; 60-min cycling at 55% VO2max.</td>
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<td>N-3</td>
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<td>10-min low intensity and 10-min moderate intensity cycling</td>
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<td>Placebo</td>
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<td>Cycling training</td>
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<td>N-3 FO 8.0 g/d (0.8 g EPA, 2.4 g DHA); Pla 8.0 g/d olive oil</td>
<td>8-weeks</td>
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<td>Wasler et al. 2008</td>
<td>14, male, 7 female, healthy</td>
<td>N-3 FO 6.0 g/d (3.0 g EPA, 2.0 g DHA); Pla 7.2 g/d safflower oil</td>
<td>6-weeks</td>
<td>Blood pressure, CO, SV, exercise HR</td>
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<td></td>
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<td>Placebo</td>
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<td>Zebrowska et al. 2015</td>
<td>13, male, trained cyclists</td>
<td>N-3 FO 1.3 g/d (0.66 g EPA, 0.44 g DHA); Pla 1.3 g/d lactose monohydrate</td>
<td>3-weeks supplement, 2-week washout</td>
<td>Flow mediated dilation, arterial stiffness, nitric oxide and VO2max</td>
<td>Cycling training</td>
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<td>Study</td>
<td>Participants</td>
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<td>Bloomer et al. 2009</td>
<td>14, male, trained</td>
<td>25.5±4.8</td>
<td>N-3</td>
<td>Placebo Crossover</td>
<td>N-3 EPA (2.2 g/d) and DHA (2.2 g/d); Pla soybean oil; 6-weeks supplementation, 8-weeks washout; Resting and exercise induced inflammation; Stair climbing with weighted backpack for 60-min 4-weeks of paddling increasing distance from 6 000 m to 12,000 m</td>
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<td>Delfan et al. 2015</td>
<td>22, male, dragon boat paddlers</td>
<td>23.6±1.9</td>
<td>N-3</td>
<td>Placebo</td>
<td>N-3 FO 6.0 g/day (2.4 g EPA, 1.2 g DHA); Pla mineral oil; Inflammatory cytokines and helper T cell phenotype</td>
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<td>Gray et al. 2014</td>
<td>20, male, recreationally active</td>
<td>22.0±1.3</td>
<td>N-3</td>
<td>Placebo</td>
<td>N-3 FO 3 g (1.3g EPA, 0.3g DHA, 45 IU d-a tocopherol); Pla 3 g/d olive oil; Oxidative stress and muscle damage</td>
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<td>Lembke et al. 2014</td>
<td>69 male and female, recreationally active</td>
<td>18.6±1.2</td>
<td>N3</td>
<td>Placebo</td>
<td>N3 EPA+DHA 2.7 g/d; Pla high oleic sunflower oil; Muscle soreness, inflammation; 60 eccentric isokinetic elbow flexion contractions with non-dominant arm</td>
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<td>Study</td>
<td>Subjects/Methods</td>
<td>Intervention</td>
<td>Measurement</td>
<td>Duration</td>
<td>Key Outcomes</td>
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<td>Lenn et al. 2002</td>
<td>12 male, 9 female, sedentary</td>
<td>N-3 Soy isolate, Placebo</td>
<td>30-days Pain, inflammation</td>
<td>30-days</td>
<td>50 maximal isokinetic eccentric elbow flexion contractions</td>
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<td>Mickleborough et al. 2015</td>
<td>32, male, untrained</td>
<td>N-3 Placebo</td>
<td>30-days Muscle damage, inflammation and quadriceps force</td>
<td>30-days</td>
<td>20-min downhill running (-16%) at 70% VO₂max</td>
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<td>Nieman et al. 2009</td>
<td>19 male, 4 female, trained cyclists</td>
<td>N-3 Placebo</td>
<td>6-weeks Cycling performance and inflammation</td>
<td>6-weeks</td>
<td>Cycling training; 3 days of 3-h cycling at 57% of peak watts</td>
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<td>Santos et al. 2013</td>
<td>8 male marathon runners; 13 male; well-trained athletes</td>
<td>N-3 Control</td>
<td>60-days Lymphocyte proliferation, cytokine concentration</td>
<td>60-days</td>
<td>Marathon training; marathon</td>
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<tr>
<td>Tartibian et al. 2009</td>
<td>27, male, sedentary</td>
<td>N-3 Placebo</td>
<td>30-days Perceived pain and symptoms of delayed onset muscle soreness</td>
<td>30-days</td>
<td>40-min eccentric bench stepping</td>
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<td>Study</td>
<td>Participants</td>
<td>Intervention Details</td>
<td>Duration</td>
<td>Outcomes</td>
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<td>Tartibian et al. 2011</td>
<td>45, male, sedentary</td>
<td>N-3 29.3±6.2 Pla 31.1±4.9 C 28.7±7.1</td>
<td></td>
<td>Markers of muscle damage and inflammation</td>
<td>30-days eccentric bench stepping</td>
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<tr>
<td>Toft et al. 2002</td>
<td>20, male, marathon runners</td>
<td>N-3 29 (23-48) Pla 28 (24-43)</td>
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<td>Cytokine production</td>
<td>Marathon training; marathon</td>
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<tr>
<td>Mickleborough et al. 2003</td>
<td>5 male, 5 female, well-trained with EIB; 5 male, 5 female, well-trained</td>
<td>EIB 23.2±1.9 Healthy 22.4 ±1.7</td>
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<td>Pulmonary function and inflammatory markers</td>
<td>Treadmill run to exhaustion</td>
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<td>Mickleborough et al. 2006</td>
<td>10, male 6 female, recreationally active with EIB</td>
<td>23±1.6</td>
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<td>Inflammatory markers in blood and sputum</td>
<td>Treadmill run to exhaustion</td>
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<tr>
<td>Mickleborough et al. 2013</td>
<td>12, male, 8 female, asthmatic</td>
<td>22.6±2.1</td>
<td></td>
<td>Airway inflammation and bronchoconstrictor response</td>
<td>Eucapnic voluntary hyperventilation challenge</td>
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</table>

**Pulmonary**

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Intervention Details</th>
<th>Duration</th>
<th>Outcomes</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mickleborough et al. 2003</td>
<td>5 male, 5 female, well-trained with EIB; 5 male, 5 female, well-trained</td>
<td>EIB 23.2±1.9 Healthy 22.4 ±1.7</td>
<td></td>
<td>Pulmonary function and inflammatory markers</td>
<td>Treadmill run to exhaustion</td>
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<td>Mickleborough et al. 2006</td>
<td>10, male 6 female, recreationally active with EIB</td>
<td>23±1.6</td>
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<td>Inflammatory markers in blood and sputum</td>
<td>Treadmill run to exhaustion</td>
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<tr>
<td>Mickleborough et al. 2013</td>
<td>12, male, 8 female, asthmatic</td>
<td>22.6±2.1</td>
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<td>Airway inflammation and bronchoconstrictor response</td>
<td>Eucapnic voluntary hyperventilation challenge</td>
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<td>Participants</td>
<td>Intervention</td>
<td>Outcome</td>
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<tr>
<td>Tartibian et al. 2010</td>
<td>40 male, trained wrestlers</td>
<td>N-3 18.6±2.3 Pla 19.0±2.7</td>
<td>N-3 Placebo Active control 18.5±2.2 Inactive control 18.6±2.2</td>
<td>N-3 FO 1 g/d (0.180 g EPA, 0.12 g DHA); Pla matched capsules</td>
<td>12-weeks FEV1 and other markers of pulmonary function</td>
</tr>
<tr>
<td>Boss et al. 2009</td>
<td>16, male, sedentary</td>
<td>N-3 25±4.3 Con 22.5±2.1</td>
<td>N-3 Control N-3 FO 1.1g (EPA, 0.7g DHA)</td>
<td>4-weeks Insulin sensitivity, fat oxidation and endurance capacity</td>
<td>8-days of graduated cycling training</td>
</tr>
<tr>
<td>Gerling et al. 2014</td>
<td>30, male, recreationally active</td>
<td>N-3 22.7±0.8 Pla 20.6±0.4</td>
<td>N-3 Placebo N-3 FO 5.0 g/d (3.0 g EPA, 1 g DHA); Pla 3 g/d olive oil</td>
<td>12-weeks Whole body resting energy expenditure and substrate oxidation</td>
<td>10-weeks Aerobic capacity</td>
</tr>
<tr>
<td>Raastad et al. 1997</td>
<td>28, male, professional soccer players</td>
<td>N-3 23.5±2.7 Pla 23.6±3.3</td>
<td>N-3 Placebo N-3 FO 5.2 g/d (1.6 g EPA, 1.0 g DHA); Pla 5.2 g/d corn oil</td>
<td>10-weeks Aerobic capacity</td>
<td>Treadmill running AT, VO2max and time to exhaustion</td>
</tr>
<tr>
<td>Cornish et al. 2009</td>
<td>28 male, 23 female, untrained</td>
<td>N-3 M 63±7 N-3 F 67±7 Pla M 67±7 Pla F 65±7</td>
<td>N-3 Placebo N-3 Flax oil 30 mL/d (14.0 g ALA); Pla 30 mL/d corn oil</td>
<td>12-weeks Inflammation, muscle mass and strength</td>
<td>Resistance training 3 d/wk for 12-weeks</td>
</tr>
<tr>
<td>Study</td>
<td>Gender</td>
<td>Age</td>
<td>Intervention</td>
<td>Duration</td>
<td>Outcome</td>
</tr>
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</tr>
<tr>
<td>Smith et al. 2011</td>
<td>10 male, 6 female, untrained</td>
<td>N-3 71±1 Pla 71±2</td>
<td>N-3 FO 4.0 g/d (1.9 g EPA, 1.5 g DHA); Pla 4.0 g/d corn oil</td>
<td>8-weeks</td>
<td>Rate of muscle protein synthesis and anabolic signaling</td>
</tr>
<tr>
<td>Smith et al. 2015</td>
<td>15 male, 29 female, untrained</td>
<td>N-3 68±5 Pla 69±7</td>
<td>N-3 FO 4.0 g/d (1.9 g EPA, 1.5 g DHA); Pla 4.0 g/d corn oil</td>
<td>6-months</td>
<td>Muscle volume, strength and isokinetic power</td>
</tr>
<tr>
<td>Fontani et al. 2005</td>
<td>29 male, 32 females, recreationally active</td>
<td>N-3 33±7 Pla 33±3</td>
<td>N-3 FO 4.0 g/d (1.6 g EPA, 0.8 g DHA); Pla 4.0 g/d olive oil</td>
<td>35-days</td>
<td>Neuro-psychological function</td>
</tr>
<tr>
<td>Guzman et al. 2011</td>
<td>24, female, 23.58±5.22 professional soccer players</td>
<td>N-3 Placebo</td>
<td>N-3 DHA 3.5 g/d; Pla 3.5 g/d olive oil</td>
<td>4-weeks</td>
<td>Complex reaction time, precision, and efficiency</td>
</tr>
<tr>
<td>Rodacki et al. 2013</td>
<td>45, female, sedentary</td>
<td>64±1.4</td>
<td>N-3 FO 2.0 g/d (0.6 g EPA, 0.5 g DHA)</td>
<td>N-3 150 (60 days with no training, 90 days with training), N-3 90 (90 days with training)</td>
<td>Neuromuscular adaptations to resistance training</td>
</tr>
</tbody>
</table>
ALA: alpha linolenic acid; Con: control; EIB: exercise induced bronchoconstriction; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; FO: fish oil; MVC: maximum voluntary contraction; N-3: Omega-3 polyunsaturated fatty acid; PGE2: prostaglandin E2; Pla: placebo; Vit: vitamin; VO$_2$max: maximum volume of oxygen consumed.
2.3.4.2 Characteristics of Studies

Details of the included trials can be found in Table 1. Of the 36 trials, two recruited women only [26,109] and 11 included both males and females [110-120]. Trial participants ranged from sedentary with no previous training history to elite athletes. Fourteen trials examined the effects of N-3 PUFA supplementation in athletes and well-trained individuals. Sports represented in these trials included athletes from mixed endurance sports [114], cyclists [121-124], marathon runners [125,126], Australian Rules football players [127], paddlers [128], wrestlers [129], judo athletes [130,131] and soccer players [109,132-134].

2.3.4.3 Supplementation Source of Omega-3s

The source of N-3 PUFA supplements is shown in Table 1. Fish oil was used in 28 trials. Two trials used marine oil blends with New Zealand green lipped mussel [115,135]. One trial used ALA from flax seed oil [116]. Four trials used refined EPA and DHA only [117,118,120,136] and three trials used algal DHA [109,132,133]. The predominant placebo supplements used in studies were olive oil, corn oil, safflower oil or blends thereof. Studies that used soybean oil might have seen a placebo effect due to the ALA content. Two studies [129,137] did not mention the composition of their placebo supplement but did report the dose was similar to the N-3 PUFA group, while one study omitted the dose of placebo supplement administered [114].

2.3.4.4 Omega-3 Treatment Dose

The treatment dose of N-3 PUFA was reported in all studies and all studies used a constant dosing strategy with no loading periods. There was a wide range in supplementation dose and duration. Mickleborough et al. [135] provided the lowest dose (N-3 PUFA 0.4 g/d with 0.058 g EPA and 0.044 g DHA). Mickleborough et al. [113,114] provided the highest dose of N-
3 PUFA oil (20 g/d) that included the highest dose of essential fatty acids (3.2 g/d EPA, 2.2 g/d DHA). Supplementation duration lasted 3-weeks to 6-months.

The majority of studies used capsules as the route of supplement administration. Martorell et al. [132,133] used DHA added to an almond beverage. Cornish and Chilibeck [116] provide participants with ALA as flaxseed oil but did not specify if it was to be taken in capsules or added to food.

2.3.4.5 Outcome Measures

Given the wide focus of studies found, there were many different outcome measures reported. Only two studies identified a single variable as the primary outcome of the trial [132,138]. The trials examining N-3 PUFA as an antioxidant measured change in blood antioxidant status, lipid peroxidation and F2-isoprostanes. Trials examining the effect of N-3 PUFA on cardiovascular function measured resting heart rate, submaximal exercise heart rate, time to exhaustion, heart rate variability and vascular resistance. Studies investing the effect of N-3 PUFA on muscle damage examined creatine kinase, blood lactate, muscle soreness, cytokines, markers of inflammation, and quadriceps maximal voluntary force (MVC). Trials examined pulmonary function measured forced expiry volume in one-second (FEV1), forced inspiratory volume in one-second and markers of airway inflammation in blood and in sputum. Trials examining N-3 PUFA on training adaptations and performance examined fat oxidation, muscle metabolism, aerobic power, strength, muscle mass, muscle protein synthesis, reaction time, MVC force and neuromuscular function.

2.3.4.6 The Effect of Omega-3s on Antioxidant Status

Six trials reported the effect of N-3 PUFA supplementation on antioxidant status (Table 2). Filaire et al. [130] examined oxidative stress in judo athletes pre- and post-training training before and after 6-weeks of N-3 PUFA supplementation. N-3 PUFA supplementation increased
lipid oxidation measured from malondialdehyde concentration (p=0.03) and increased nitric oxide production with a corresponding increase in oxidative stress (p=0.04) at rest and after exercise with no change in the placebo group for either measure. In a follow-up trial, Filaire et al. [131] added two supplementation conditions to evaluate if antioxidant vitamins C and E could alter the N-3 PUFA associated increase in lipid peroxidation and oxidative stress. The addition of antioxidants to the N-3 PUFA supplement did not prevent the increase of reactive oxygen species at rest; however, there was lower malondialdehyde and reactive oxygen species after training compared to the N-3 PUFA group. McAnulty et al. [97] investigated 6-weeks of N-3 PUFA supplementation with or without antioxidant vitamins A, C and E and selenium on F2-isoprostane concentration after three consecutive days of 3-hour cycling exercise. N-3 PUFA supplementation alone increased F2-isoprostone 53% after exercise, whereas N-3 PUFA with antioxidants was not different from placebo or antioxidants alone. Martorell et al. performed two studies in elite soccer players with 8-weeks of N-3 DHA supplementation during soccer training. In the first study [132], oxidative balance in neutrophils was examined after 2–hours of soccer drills. DHA had no effect compared to placebo. In the second study [133], there was no difference in oxidative stress including lipid peroxidation; however, anti-inflammatory prostaglandin E2 (PGE2) was lower after N-3 PUFA supplementation. Poprzecki et al. [54] examined antioxidant enzyme activity and oxidative stress after acute exercise. Malondialdehyde was increased similarly in the N-3 PUFA and placebo groups after 6-weeks. The N-3 PUFA group showed higher catalase activity (p<0.05) one-hour after exercise compared to placebo. There was no different in glutathione peroxidase or superoxide dismutase activity.

2.3.4.7 The Effect of Omega-3s on Cardiovascular Function

The effect of N-3 PUFA supplementation on cardiovascular function was assessed in six trials (Table 3). Buckley et al. [127] showed that five-weeks of N-3 PUFA lowered heart rate
during submaximal running (p=0.03) but did not alter running time to exhaustion or subsequent heart rate recovery. Macartney et al. [139] found that eight-week of N-3 PUFA supplementation lowered submaximal exercise HR, HR recovery time and tended to lower HR variability (HRV) (p=0.18) after six 30-second Wingates. No change in submaximal or peak HR was observed in either group. Ninio et al. [110] examined changes in HR and HRV before and after exercise in sedentary, hypertensive, overweight males and females. Using a 2 x 2 randomized factorial design, 12-weeks of N-3 PUFA supplementation with or without exercise training and placebo were examined. HRV, resting HR and submaximal exercise HR were reduced in the N-3 PUFA groups compared to placebo. Peoples et al. [121] showed that in well-trained cyclists 8-weeks of N-3 PUFA supplementation reduced submaximal exercise HR and whole body O\textsubscript{2} consumption compared to control. Wasler et al. [111] examined the effect of 6-weeks of EPA and DHA on HR, cardiac output (CO), stroke volume (SV), mean arterial pressure (MAP). There was no effect of N-3 PUFA on HR but MAP was lower at rest. After six-weeks of supplementation stroke volume (14.1±6.3 vs. 32.3±8.7 ml) and cardiac output (8.5±1.0 vs. 10.3±1.2 L min\textsuperscript{-1}) were higher during submaximal exercise on N-3 PUFA compared to placebo.

One trial examined the effect of three-weeks of N-3 PUFA supplementation on peripheral vascular function using serum nitric oxide and flow-mediated dilation in well-trained cyclists. With N-3 PUFA supplementation, cyclists increased nitric oxide production and mean flow mediated dilation was 5.25% higher. VO\textsubscript{2}max was higher and mean maximum heart rate was 10 beats per minute lower on N-3 PUFA compared to placebo.

### 2.3.4.8 The Effect of Omega-3s on Muscle Damaging Exercise

Eleven trials examined the effect of N-3 PUFAs on post-exercise muscle damage and inflammation (Table 4). A trial conducted by Bloomer et al. [136] used treadmill walking with a weighted vest to induce muscle damage after 6-weeks of N-3 PUFA supplementation. Prior to
the exercise test, resting C-reactive protein (CRP) and tumour necrosis factor-alpha (TNF-α) were lower compared to placebo. After exercise, the N-3 PUFA groups had lower inflammation compared to placebo.

Six trials evaluated the effect of N-3 PUFA supplementation on eccentric exercise induced muscle damage. Gray et al. [140] examined the effect of 6-weeks of N-3 PUFA supplementation on markers of oxidative stress following 200 eccentric knee extensions. No difference in muscle damage, muscle soreness or MVC force was shown between the N-3 PUFA group and placebo. H₂O₂ DNA damage immediately post-exercise and plasma thiobarbituric acid reactive substances were lower at 72 hours post-exercise. Lenn et al. [112] found that 30-days of N-3 PUFA supplementation had no effect on muscle pain after 50 repetitions of eccentric bicep flexion. Lembke et al. [120] performed a similar study using 60 repetitions of eccentric bicep flexion. Participants on N-3 PUFA for 30-days had lower CRP levels 24-hours after exercise and lower muscle soreness and improved quality of life after 72-hours compared to placebo. Mickleborough et al. [135] investigated the effect of a 26-days of N-3 PUFA supplementation on muscle damage and inflammation after 20-minutes of downhill running and 4-days of recovery. TNF-α was lower from 2-96 hours after exercise. Markers of muscle creatine kinase (CK) were lower from 2-96 post-exercise, while myoglobin was lower from 24-96 hours post-exercise. Strength loss and peripheral fatigue measured from quadriceps MVC force and potentiated twitch force was attenuated in the N-3 PUFA group only. Tartibian et al. performed two studies examining 30-days of N-3 PUFA supplementation on muscle damage from bench stepping [137,141]. In the first study, the N-3 PUFA group had less inflammation measured from thigh circumference at 24 and 48-hours post-exercise and less pain and improved range of motion (ROM) at 48-hours post exercise. In the second study, N-3 PUFA supplementation attenuated the
increase of CK, myoglobin and IL-6 at 24 and 48 hours post exercise compared to placebo and control groups.

Five trials examined N-3 PUFA supplementation on muscle damage and inflammation after sport specific exercise. In paddlers Delfan et al. [128] showed an 4-week of N-3 PUFA supplementation was anti-inflammatory from lower TNF-α and IL1-β and higher IL-6 and 10 concentration compared to placebo. Also, T-helper cells were shifted towards the Th2 phenotype after N-3 PUFA supplementation. Nieman et al. [124] tested the effect of 6-weeks of N-3 PUFA supplementation on cycling performance, immune function and inflammation in trained cyclists. There was no different in 10-km time trial performance or post-exercise inflammatory markers and immune function between N-3 PUFA and placebo. Two trials examined the effect of N-3 PUFA supplementation on post-marathon muscle damage. Santos et al. showed that 60-days of N-3 PUFA supplementation increased lymphocyte proliferation and lowered IL-2, IL-10 and TNF-α concentration before the marathon. After the race, lymphocyte proliferation was still higher than control and did not alter cytokine production. Similarly, Toft et al. showed no effect of 6-weeks of N-3 PUFA supplementation on post-race acute phase proteins, inflammation or immune function compared to controls.

2.3.4.9 The Effect of Omega-3s on Pulmonary Function

The effect of N-3 PUFA supplementation on pulmonary function in athletes has been examined in two trials (Table 5). Using a randomized crossover design, Mickleborough et al. [114] showed that after 3-weeks of N-3 PUFA supplementation in athletes with exercise-induced bronchoconstriction EIB), FEV1 decreased by 3±2% post-exercise, compared to -14.5±5% on placebo. Tartibian et al. [129] showed that N-3 PUFA supplementation during 12-week of training improved FEV1 in athletes without EIB compared to placebo and active controls.
Mickleborough et al. conducted two trials examining 3-weeks of N-3 PUFA supplementation on post-exercise pulmonary function in individuals with EIB \[113\] and hyperpnea induced bronchoconstriction \[142\] (Table 5). After N-3 PUFA supplementation, post-exercise inflammatory markers were lower in both studies and the post-exercise decrease in FEV1 was attenuated compared to the placebo condition.

2.3.4.10 The Effect of Omega-3s on Adaptations to Training

Ten trials examined the effect of N-3 PUFA supplementation on adaptations to training (Table 6). Endurance capacity was examined in two trials by Boss et al. \[143\] and Raastad et al. \[134\] in sedentary and well trained individuals, respectively. N-3 PUFA supplementation had no effect on endurance capacity or VO$_2$\text{max} in either study.

One trial examined N-3 PUFA supplementation on substrate oxidation and resting energy expenditure. Gerling et al. showed 12-weeks of N-3 PUFA supplementation increased absolute resting energy expenditure by 5.3\% but this change was not significant when corrected for body mass and was not related fat metabolism.

Three trials examined N-3 PUFA supplementation on changes in muscle strength and metabolism. A trial by Cornish and Chilibeck \[116\] showed that 12-week of flax oil N-3 PUFA supplementation decreased IL-6 62±36\% in male participants but had no effect on females. There were no differences between groups for strength gains and only a minor increase in knee extensor thickness in men compared to placebo. Smith et al. performed two trials testing the effect of N-3 PUFA supplementation on muscle function. In the first trial, 8-weeks of supplementation in elderly men and women, did not alter basal muscle protein synthesis (MPS) but increased mTOR and p70s6k phosphorylation and MPS in a hyperaminoacidemia-hyperinsulinemia state \[117\]. Subsequently, 6-months of supplementation was shown to increase
thigh muscle volume 3.6% (90% CI 0.2-7.0%), handgrip strength 2.3 kg (90% CI 0.8-3.7 kg) and average 1-RM strength 4.0% (90% CI 0.8-7.3%) compared to placebo [118].

There were three trials that examined N-3 PUFA supplementation on neuromuscular function. Two trials examined motor control through reaction times tests. Guzman et al. [109] found that N-3 PUFA supplementation for 4-weeks decreased reaction time and improved task efficiency. Fontani et al. [119] also measured improved reaction time with a corresponding decrease in task EMG latency. One trial examined N-3 PUFA supplementation during exercise training. Rodacki et al. [26] showed N-3 PUFA supplementation during resistance training increased quadriceps force and rate of force development in compared to training only controls. Furthermore, the N-3 PUFA groups increased strength to a greater extent than training only controls.
Table 2 – The effect of N-3 PUFA supplementation on antioxidant status.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Malondialdehyde</th>
<th>Antioxidant Status</th>
<th>F2-isoprostanes</th>
<th>Oxidative Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filaire et al. 2010</td>
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<td>Filaire et al. 2011</td>
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<td>Martorell et al. 2013</td>
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<td>Poprzecki et al. 2009</td>
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</table>

Variable increased with N-3 PUFA supplementation (↑). Variable decreased with N-3 PUFA supplementation (↓). No change in variable (-).
Table 3 – The effect of N-3 PUFA supplementation on cardiovascular function.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Resting HR</th>
<th>Submaximal Exercise HR</th>
<th>HR Recovery</th>
<th>HRV</th>
<th>MAP</th>
<th>CO / SV</th>
<th>VO₂max</th>
<th>FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buckley et al. 2009</td>
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<tr>
<td>Macartney et al. 2041</td>
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<tr>
<td>Ninio et al. 2008</td>
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<td>Peoples et al. 2008</td>
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<td>Wasler et al. 2008</td>
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<td>Zebrowska et al. 2015</td>
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</tbody>
</table>

HR: heart rate; HRV: heart rate variability; MAP: mean arterial pressure; CO: cardiac output; SV: stroke volume; FMD: flow mediated dilation. Variable increased with N-3 PUFA supplementation (↑). Variable decreased with N-3 PUFA supplementation (↓). No change in variable (-).
Table 4 – The effect of N-3 PUFA supplementation on muscle damage and inflammation.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Inflammatory Markers at Rest</th>
<th>Post-Exercise Inflammation</th>
<th>Muscle Damage</th>
<th>Post-Damage Recovery</th>
</tr>
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<td>Bloomer et al. 2009</td>
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<td>Delfan et al. 2015</td>
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<td>Gray et al. 2014</td>
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<td>Lembke et al. 2014</td>
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<td>Lenn et al. 2002</td>
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<td>Toft et al. 2002</td>
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Variable increased with N-3 PUFA supplementation (↑). Variable decreased with N-3 PUFA supplementation (↓). No change in variable (-).
Table 5 – The effect of N-3 PUFA supplementation on pulmonary function.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Pre-Exercise FEV1</th>
<th>Post-Exercise FEV1</th>
<th>Inflammatory Markers</th>
</tr>
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<tbody>
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<td>Mickleborough et al. 2003</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
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<td>Mickleborough et al. 2013</td>
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</tbody>
</table>

FEV1: forced expiratory volume in one second. Variable increased with N-3 PUFA supplementation (▲). Variable decreased with N-3 PUFA supplementation (▼). No change in variable (-).
Table 6 – The effect of N-3 PUFA supplementation on adaptations to training.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Endurance</th>
<th>Energy</th>
<th>Strength</th>
<th>Muscle Protein Synthesis</th>
<th>Muscle Mass</th>
<th>Neuromuscular Function</th>
<th>Motor Control</th>
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</table>

Variable increased with N-3 PUFA supplementation (▲). Variable decreased with N-3 PUFA supplementation (▼). No change in variable (-).
2.3.4.11 Methodological Quality

Based on the PEDro criteria all trials included in this review were rated as moderate to high with at least scores of 5/10 (Table 7). Six trials were ranked as moderate quality with three trials scored at 5 and three at 6. Thirty trials were scored as high quality with scores of 7 (n=6), 8 (n=1), 9 (n=9) or 10 (n=14). Five trials did not compare baseline characteristics. Seven trials were conducted with participants that were not blinded. Nine trials had less than 85% of participants complete the trial and only two trials [111,118] reported adverse events to supplementation (headache and gastrointestinal distress). Eleven trials did not conceal participant allocation. Twelve trials did not blind clinicians or assessor. Twenty-five of the 36 trials included (70%) were reported as being double-blind but it was not explicitly stated how blinding was maintained. All other aspects of the PEDro criteria were adequately described.

2.3.4.12 Adverse Events

The reporting of adverse events in trials was poor. Nine of the 37 studies reported ≤85% of participants completing the trial; however, only two trials reported adverse events of headaches, nausea and gastrointestinal distress [111,118].

2.3.5 Discussion

There have been several reviews on the effects of N-3 PUFA supplementation on training adaptations and exercise performance [144-149]; however, this is the first to systematically review the literature and evaluate the quality of trials to avoid the risk of bias [150]. Findings from this systematic review provide a clear summary of the effectiveness of N-3 PUFA supplementation on performance and will guide future research in this area.

The effect of N-3 PUFA supplementation on antioxidant status and post-exercise oxidative stress was examined in six trials. Two high quality and one moderate quality studies
showed that N-3 PUFA supplementation increased lipid peroxidation measured by malondialdehyde concentration [130,151] and increased F2-isoprostanes [97]. A high-quality follow-up study by Filaire et al. showed that combining N-3 PUFAs with antioxidant vitamins C, E and beta-carotene still caused an increase in oxidative stress at rest but not after exercise. Two high quality studies by Martorell et al. [132,133] showed N-3 PUFAs had no effect on oxidative balance. Overall, it appears that N-3 PUFA supplementation has an unclear to negative affect on oxidative stress at rest and post-exercise. At this time is it unclear if N-3 PUFA associated increase in oxidative stress can impact training adaptations or exercise performance.

Cardiovascular and peripheral vascular function was examined in six trials. Four high quality studies showed a consistent effect of N-3 PUFA supplementation on lowering resting HR and HR during submaximal running and cycling exercise [110,121,127,139]. Two of these studies also showed that N-3 PUFA supplementation decreased heart rate variability [110,139]. A moderate quality study by Wasler et al. [111] examined physiologic changes in heart function and found that N-3 PUFA supplementation increased stroke volume and cardiac output, which could explain the findings of the previous studies. One high quality study tested if N-3 PUFA supplementation could enhance performance through running time to exhaustion and found no difference from placebo [127]. A moderate quality randomized crossover study in cyclists showed N-3 PUFA supplementation increased peripheral blood flow and VO$_2$max [122]. There is consistent high quality evidence demonstrating an effect of N-3 PUFA supplementation on cardiovascular function at rest and during sub-maximal exercise. At this time it is unclear if these effects translate into enhanced performance during high-intensity exercise.

Four high quality studies examined the effect of N-3 PUFA supplementation on pulmonary function. Three high-quality crossover studies examined N-3 PUFA supplementation on EIB in athletes [114] and non-athletes [113,115]. N-3 PUFA supplementation consistently
attenuated the EIB associated decrease in FEV1 compared to control and placebo conditions. This was the result of lower post-exercise inflammation and improved immune function from N-3 PUFA supplementation. In another high quality study, N-3 PUFAs were given to non-EIB athletes, FEV1 was increased with no change in the placebo group [129]. The findings of these studies demonstrate a treatment effect of N-3 PUFA supplementation on EIB and a limited but positive effect on pulmonary function in athletes.

Eleven trials examined N-3 PUFA supplementation on muscle damage and inflammation. Seven high quality studies reported lower inflammation before and after exercise compared to placebo and control conditions [120,126,128,135,136,140,141]. Of these studies Mickleborough et al. [135] reported N-3 PUFA supplementation mitigated muscle damage and strength loss in the N-3 PUFA group compared to placebo, Tartibian et al. [141] also reported lower muscle damage for the N-3 PUFA group and Lembke et al. reported lower pain in the N-3 PUFA group [120]. One moderate quality [112] and two high quality studies showed N-3 PUFA had no effect on muscle damage or inflammation compared to placebo [124,125]. Overall, evidence suggests N-3 PUFA supplementation lowers inflammation before and after exercise. Some evidence suggests an ability to attenuate induced muscle damage but further research area is required.

The effect of N-3 PUFA supplementation on training adaptations was examined in nine trials. Three trials examined adaptations related to endurance performance. Two high quality studies showed N-3 PUFA supplementation had no effect on running time to exhaustion [134] and cycling time to exhaustion and substrate oxidation [143]. Similarly, a moderate quality study also showed that N-3 PUFA supplementation did not change substrate oxidation at rest [152]. This indicates N-3 PUFA supplementation is not sufficient to increase fat oxidation and dietary macronutrient modification is necessary to promote increased fat oxidation [4].
Three high quality studies examined the effect of N-3 PUFA supplementation on strength and muscle mass. A high quality study by Smith et al. [117] found that muscle protein synthesis was increased under hyperinsulinemia-hyperaminoacidemia conditions but not at rest. When N-3 ALA supplementation was combined with resistance training in older adults, there were only minor improvements compared to placebo [116]. In contrast, Smith et al. [118] found that longer chain N-3 PUFA (EPA and DHA) supplementation increased in muscle mass and strength in older individuals. These studies suggest that N-3 PUFA supplementation with EPA and DHA but not ALA may promote muscle anabolism in older adults; however, further research in younger and previously trained individuals is needed.

Of the three trials that examined the effect of N-3 PUFA supplementation on neuromuscular function, one high and one moderate quality study examined reaction time and coordination. Fontani et al. [119] reported improved reaction time and attention from adaptations related to cortical processing. Guzman et al. [109] reported similar adaptations in reaction time and task efficiency in elite soccer players. Two high quality studies tested the effect of N-3 PUFA supplementation on neuromuscular adaptations to training. Rodacki et al. [26] reported robust improvements in central and peripheral neuromuscular function in older, previously sedentary women. These adaptations resulted in greater strength adaptations compared to controls and is consistent with Smith et al. [118].

Overall, N-3 PUFA supplementation has a mixed effect on training adaptations and exercise performance. There appears to be no effect on endurance capacity or substrate oxidation, whereas supplementation can enhance muscle anabolism and strength gains. N-3 PUFA supplementation can enhance motor control, which can impact performance, yet finding on physiologic measures of performance are mixed and require further study. Based on this systematic review, there are not enough studies in this area using common outcome measures to
develop a consensus on the effect of N-3 PUFA supplementation on training adaptations and performance at this time.

2.3.6 Practical Application

Currently there are no population based daily recommended intakes (DRIs) for N-3 PUFAs that exercisers and athletes can use to guide their nutritional intake or supplementation [80]. Several organizations have published evidence-based recommendations for daily N-3 PUFA intake based on peer-reviewed data either for prevention of specific diseases or for the maintenance of good health. Examples of such include the American Heart Association, which recommends ≥500 mg d⁻¹ of EPA+DHA for healthy patients and 2-4 g d⁻¹ for patients with high triglycerides [153]. Similarly, the American Dietetic Association and Dietitians of Canada joint position that recommends ≥500 mg d⁻¹ of N-3 PUFA and 1.3-2.7 g d⁻¹ of ALA N-3 PUFA [81]. The Institute of Medicine and FDA have set an acceptable macronutrient distribution range of 0.6-1.2% of total energy for adults and an adequate intake (AI) of <1.6 g d⁻¹ for males >18 years and <1.1 g d⁻¹ for females >18 years [82]. From the 25 positive studies identified in this systematic review, four studies used N-3 PUFA doses ≤1.2 g d⁻¹, six used a dose of 1.3-2.9 g d⁻¹ and 15 used a dose ≥3 g d⁻¹. Given that athletes have higher energy and nutrient requirements compared to the average population, they could theoretically benefit from a daily intake at the upper end daily N-3 PUFA EPA+DHA recommendations, and possibly higher doses in specific training situations that are monitored by a healthcare provider.

Of the studies reviewed, the minimum supplementation period and dose appears to be 3-weeks for promoting cardiovascular adaptations at 2 g/d of N-3 PUFA with 0.14 g EPA and 0.56 g DHA [139], 30-days for attenuating muscle damage and inflammation at 0.4 g/d of N-3 PUFA with 0.058 g EPA and 0.044 g DHA [135], 3-weeks for improving pulmonary function at 0.4 g/d of N-3 PUFA with 0.072 g EPA and 0.048 g DHA, and 8-weeks for promoting training
adaptations related to muscle mass at 4 g/d N-3 PUFA with 1.86 g EPA and 1.50 g DHA and 4-weeks at 3.5 g/d DHA for enhancing adaptation related to motor control.

McGlory et al. [154] examined the incorporation of N-3 PUFAs into red blood cells and muscle during four-weeks of fish oil supplementation (3500 mg d\(^{-1}\) EPA, 900 mg d\(^{-1}\) DHA). After 2-weeks of supplementation there was a corresponding increase in both blood and muscle N-3 PUFA percent concentration. After 4-weeks of supplementation the concentration of N-3 PUFA in both tissues was greater still. These findings along with adaptations previously discussed, suggest longer-term (>2-weeks) or chronic supplementation might confer the most benefit for adaptations to training. However, 3-weeks may be the minimum supplementation period to evoke N-3 PUFA associated adaptations, given the high treatment doses used relative to the realistic doses used in the trials in this review. Therefore, any athlete looking to achieve potential ergogenic effects of N-3 PUFAs during training or competition should begin supplementation at least 3-weeks prior to competition.

Like other macronutrients, athletes might benefit from modulating N-3 PUFA intake to support variations in training load or competition schedule [155]. Such examples could include higher dosing during: 1) periods of increased resistance training to enhance strength and neuromuscular adaptation; 2) to attenuate the inflammatory response to high volume training or muscle damaging exercise; 3) during the learning of new movement patterns or tactical training.

Limited but positive data suggests consuming N-3 PUFA supplements with high-fat foods to increases digestion and uptake into circulation [156]. Furthermore, we speculate that additional benefits of N-3 PUFA supplementation could be gained from multiple daily doses to increase physiologic exposure and uptake into target tissue such as muscle and nerves. As such, daily supplementation should be broken into two or more doses. For example, taking N-3 PUFAs with breakfast, post-training recovery nutrition and the evening meal. This would provide
multiple periods of elevated circulating N-3 PUFAs to reduce inflammation and promote training adaptations at the cellular and whole body level.

2.3.7 Future Research

Research on N-3 PUFA supplementation and exercise has used a wide variety of outcome measures to evaluate the effectiveness of this supplement. It would be valuable for researchers within a research area to use consistent outcome measures across studies to enable the comparison of findings and to support a meta-analysis on N-3 PUFA supplementation and performance. Based on the existing body of research, inflammation and muscle damage research should include markers of muscle damage (i.e. CK or myoglobin) along with at least one inflammatory marker (i.e. CRP, TNF-α) and a performance measure to gauge sport specific or physiologic recovery. Research of cardiovascular function should at least include resting and submaximal exercise heart rate. Research on pulmonary function should include FEV1. Research on performance should include fixed distance tests (e.g. cycling time trials). Neuromuscular research should include MVC force and EMG.

Placebo controlled studies should not include any source of N-3 PUFA in the placebo, including soybean oil, which is a source of ALA. ALA could contribute to a placebo effect as this source of N-3 PUFA can contribute to decreased inflammation, some muscle adaptations to exercise [116] and it can be converted into EPA and DHA[77].

Future research should be directed to determine optimal dosing strategies of N-3 PUFA triglycerides and EPA+DHA for different applications such as muscle damage recovery or pulmonary function. This could also be further separated into dosing for males and females and individuals that are recreationally active and athletes. Furthermore, there has yet to be any investigation of the effect of N-3 PUFAs consumed through the diet alone or if diet and supplementation can affect training adaptations and exercise performance.
2.3.8 Conclusion

Thirty-six moderate to high quality trials on N-3 PUFA supplementation for training adaptations and exercise performance were found from our search strategy, of which 25 support supplementation. These trials examined antioxidant status, cardiovascular function, muscle damage and inflammation, pulmonary function and adaptations to training. Moderate to high quality studies indicate N-3 PUFA supplementation does not support antioxidant status and may increase lipid peroxidation with no known detrimental effects on performance. Moderate to high quality research shows N-3 PUFA supplementation enhances cardiovascular function at rest and during submaximal exercise. Moderate to high quality research indicates that N-3 PUFA supplementation can attenuate post-exercise inflammation. High quality evidence suggests that N-3 PUFA supplementation can enhance pulmonary function in EIB and healthy individuals. There is high quality evidence that suggests N-3 PUFA supplementation increases muscle strength and mass. Moderate to high quality evidence supports N-3 PUFA supplementation for improving motor control. Given these findings, there appears to be a beneficial effect for N-3 PUFA supplementation in promoting adaptations to training. Further research is needed to determine if N-3 PUFA supplementation during training can translate into improved performance.

2.3.9 Summary for Proposed Research Direction

From this systematic review, only one study has investigated the effect of N-3 PUFAs supplementation on adaptations to resistance training in humans. Sedentary women, mean age 65 years, were randomly assigned to one of three experimental groups; strength training, three times per week for 12-weeks without any supplementation, 12-weeks of strength training with concurrent N-3 PUFAs supplementation and 60 days of pre-training ω-3 PUFA supplementation followed by 12-weeks of training with N-3 PUFA supplementation [26]. The investigators
measured changes in strength using maximal voluntary contraction force (MVC) and rate of
torque development (RTD), and electrophysiologic adaptations including electromyography
(EMG) and electromyographic delay (EMD) (the time between increased muscle electrical
activity and force production) in the knee extensors and flexors and dorsi and plantar flexors. As
a result of the training program, all groups increased MVC force and RTD. These adaptations
appear to be facilitated at least in part by improved neuromuscular function. Muscle activation,
measured by EMG was increased in all groups and muscle EMD was decreased both as a result
of training. While training was necessary to cause these adaptations, the concurrent N-3 PUFA
supplementation had an additive effect, whereby both supplementation groups had significantly
greater improvements in all measures over the training group alone. The group that took the N-3
PUFA supplement prior to and during training was not different in any measure when compared
to the training and concurrent supplementation group. This result demonstrates that N-3 PUFA
supplementation alone has no effect on neuromuscular adaptations. However, the combination of
N-3 PUFA supplementation and strength training provides a mechanism to enhance
neuromuscular adaptations, causing functional increases in muscle strength. A potential
mechanism for N-3 PUFA associated improvements in neuromuscular function could be the
increased tissue, specifically nervous tissue, concentration of N-3 DHA. Increased DHA is
thought to increase neuroprotectin D1 production, which is involved in peripheral nerve
maintenance and growth [157].

From the work of Sale [1] on neuromuscular adaptations to resistance training, there are
two aspects for which N-3 PUFA supplementation may facilitate more rapid adaptations or
enhance performance. Firstly, central adaptations based in motor learning and control play a
large role initial strength increases [28] as the brain improves coordination of prime mover
muscles and reduces co-activation of antagonist muscles. Training will create and enable the
learning of new motor programs to activate muscles. This coordination occurs from the alpha-motoneuron’s innervation of the target muscle. Patients with type 1 diabetes treated with N-3 PUFAs more than double their median nerve conduction velocity [14], which can increase stimulation and control of active muscles. Furthermore, animal studies have reported axon branching as an adaptation to training [42] to improve muscle activation, control and coordination. From research of peripheral nerve injury, N-3 PUFA supplementation has been found to promote axon branching and the increase in nerve growth factors [157], which could further increase axon branching beyond training alone thereby increasing the innervation and control of trained muscles.

Secondly, peripheral adaptations at the level of the motor unit and muscle membrane are equally involved training adaptations. Improved motor unit activation and synchronization can lead to improved rate of force development [1]. Increased acetylcholine receptor number in the motor endplate and acetylcholinesterase concentration [45] may be responsible for improved action potential transmission to the post-synaptic muscle membrane. Similarly, nerve acetylcholine concentration and release has been observed in rat small intestine after 4-weeks of fish oil supplementation [158]. These changes resulted in improved muscle contractility and were attributed to increased membrane fluidity as a result of fish oil N-3 EPA and DHA replacement of N-6 linoleic acid (LA) (18:2 N-6) and arachidonic acid (ARA) (20:5 N-6). This increase in membrane fluidity can further improve muscle action potential transmission through t-tubules to facilitate more rapid sarcoplasmic reticulum calcium release.

From this systematic review of literature, there is evidence to support further investigation into the relationship between N-3 PUFA supplementation and neuromuscular adaptations to training and exercise performance. N-3 PUFA supplementation appears to enhance both central and peripheral neuromuscular adaptations in elderly untrained women;
however, there has yet to be any investigation on the effect of supplementation on neuromuscular adaptations to exercise attenuation of neuromuscular fatigue from exercise or the effect on exercise performance in young males.
3 Objectives and hypotheses

3.1 Objectives

The objective of this thesis is to determine the effect of short-term N-3 PUFA supplementation on neuromuscular adaptations to training and neuromuscular fatigue from exercise. This will be accomplished by addressing the following sub-objectives:

1. Compare the neuromuscular fatigue between trained and recreationally-active but untrained male cyclists during a 10 km cycling time trial.
2. Describe the neuromuscular adaptations to sprint interval training (SIT) and determine the effect of N-3 PUFA supplementation can enhance the neuromuscular adaptations to two-weeks of sprint interval training in untrained young males.
3. Determine the effect of N-3 PUFA supplementation on neuromuscular adaptations to training in well-trained male athletes.

3.2 Hypothesis

Overall hypothesis

N-3 PUFA supplementation will enhance neuromuscular adaptations to exercise training.

Specific Hypothesis

1. Trained and untrained cyclists will show similar peripheral neuromuscular fatigue, while the untrained group will show greater central fatigue after a 10 km cycling time trial.
2. N-3 PUFA supplementation will enhance neuromuscular adaptations to SIT compared to placebo and attenuate training associated neuromuscular fatigue.
3. N-3 PUFA supplementation will enhance neuromuscular adaptations to athletes’ sport specific training and attenuate neuromuscular fatigue from testing.
4 A Comparative Analysis of Cycling Performance and Neuromuscular Fatigue from a 10 km time trial in Trained and Untrained Cyclists

Evan J. H. Lewis, Joy Hur, Thomas M. S. Wolever, Greg D. Wells

Contribution: As first author I designed the study, performed all of the data collection, performed all the data analysis and drafted the manuscript.

This study addressed objective 1 of this thesis by comparing the neuromuscular fatigue to a 10 km cycling time trial between training and untrained cyclists. The 10 km cycling time trial is a test that is used in the N-3 PUFA intervention studies in chapters 5 and 6. Furthermore, this study provides a comparison of performance, neuromuscular function and neuromuscular fatigue between the populations studied in the upcoming chapters.
4.1 Abstract

Previous studies have reported neuromuscular fatigue in trained cyclists after time trials (TT) of different length. This study compared cycling performance and subsequent neuromuscular fatigue during and after a 10 km TT in trained and untrained cyclists. Thirteen recreationally-active males, and five trained cyclists completed a constant work rate 10 km TT at 60% of their Watt max (mean 160 W and 210 W). Neuromuscular fatigue was assessed using quadriceps force measurements and femoral nerve stimulation before and after the TT. Central neuromuscular fatigue was measured by voluntary activation and peripheral neuromuscular fatigue by resting and potentiated twitch force. The untrained group was divided into two groups post-hoc based on TT finish time. TT time to completion was longer for the Slow-Untrained compared to Fast-Untrained and Trained groups respectively, (mean ± SD) 1059±77, 857±29 and 871±79s (p<0.001). The Trained group increased cadence over the TT, whereas the Fast-Untrained and Slow-Untrained groups did not (p=0.045). Central neuromuscular fatigue showed a main effect for time only (p=0.031). Peripheral neuromuscular fatigue was less in the Trained group than in both Untrained groups (-1 ±15% vs. -27 ± 11% and -20 ± 17%, p=0.04). These data show that central neuromuscular fatigue was not different between groups, whereas peripheral neuromuscular fatigue was more pervasive in the Untrained groups. Neuromuscular fatigue from a 10 km TT occurs in the central and peripheral neuromuscular system; however, peripheral fatigue is modulated by prior cycling training history.
4.2 Introduction

A cycling time trial (TT) requires an individual to complete a predetermined distance as quickly as possible. TTs challenge cyclists to provide a maximal effort depending on distance [159]. Therefore, TTs are an effective research tool for measuring adaptations to training using a performance orientated approach [17] and for evaluating the effect of interventions on performance [160].

During cycling exercise, neuromuscular fatigue can occur in the central and/or peripheral neuromuscular system [62,161,162] causing a decrease in muscle strength and endurance [163,164]. Fatigue is defined as a reduction in the maximal voluntary force produced by a muscle or group of muscles [163]. This can be the result of central neuromuscular fatigue from decreased neural stimulation from the brain to the alpha-motoneurons that innervate groups of muscle fibres in a targeted muscle and altered activity of muscle afferents (central neuromuscular fatigue). Peripheral neuromuscular fatigue occurs from altered action potential transmission along muscle fibres to stimulate the force-generating contractile mechanism, metabolic stress and other factors distal to the neuromuscular junction.

In trained cyclists, peripheral neuromuscular fatigue is more pervasive after short TTs (4-5 km) from reliance on anaerobic metabolism [159,165]. Peripheral neuromuscular fatigue is thought to occur up to an individual critical threshold related to the ability to buffer lactate, which provides feedback to alter central motor output and reduce cycling power production [60,165,166]. Central neuromuscular fatigue is more pervasive after longer TTs (20-40 km) [159] where blood lactate is stable. These findings suggest a time- and intensity-dependent relationship for TT-induced neuromuscular fatigue in trained cyclists. It is unknown if this relationship applies to untrained individual given differences in cycling training history and training associated adaptations that increase muscle buffering capacity [167,168]. Therefore,
untrained individuals might be more susceptible to central fatigue during short TTs as a result of lower muscle buffering capacity resulting in afferent feedback.

Differences in cycling performance and neuromuscular fatigue have yet to be compared between trained and untrained cyclists. The 10 km TT is of short enough duration that both trained cyclists and untrained yet recreationally active individuals can complete the test in <30 minutes. Therefore, the purpose of this study was to compare cycling performance and neuromuscular fatigue from a 10 km TT between trained and untrained cyclists and relate these finding to neuromuscular fatigue measurements in trained cyclists [60,159,165,166]. We hypothesized the Trained cyclists would complete the TT faster than the Untrained group and both groups would experience similar levels of peripheral neuromuscular fatigue, whereas the Untrained group would experience central neuromuscular fatigue and the Trained group would not.

4.3 Methods

4.3.1 Participants

Recreationally-active males (n=13) (≤ 5 h exercise·wk⁻¹) who had no training in cycling and trained competitive male cyclists and triathletes (n=5) (≥ 12 h exercise·wk⁻¹) were recruited from the Toronto area. Participant’s descriptive data are shown in Table 1. After inspecting our data, the Untrained group showed a wide variance in finish times (Figure 1) despite being recruited from the same population that impaired the ability to compare neuromuscular fatigue from the TT. A finish time of 930 seconds (15:30) divided the Untrained group into a Fast-Untrained (n=7) and Slow-Untrained (n=6) of nearly equal size and allowed for the examination of the neuromuscular functions and fatigue that contributed to this performance. The University
of Toronto Research Ethics Board approved the study, and all participants gave their written informed consent to participate.

Table 2 – Participant descriptive data.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Slow- Untrained</th>
<th>Fast-Untrained</th>
<th>Trained</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>26.8 ± 4.8</td>
<td>25.7 ± 2.6</td>
<td>43 ± 9.6*****</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.8 ± 5.6</td>
<td>175.8 ± 6.1</td>
<td>175.3 ± 2.9</td>
<td>0.94</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.6 ± 13.6</td>
<td>71.6 ± 6.4</td>
<td>76.1 ± 10.7</td>
<td>0.65</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>17.4 ± 5.2</td>
<td>11.8 ± 3.3</td>
<td>13.7 ± 3.6</td>
<td>0.091</td>
</tr>
<tr>
<td>VO₂ max (L·min⁻¹)</td>
<td>2.7 ± 0.4</td>
<td>3.3 ± 0.7</td>
<td>4.1 ± 0.6*****</td>
<td>0.002</td>
</tr>
<tr>
<td>VO₂ max (mL·kg·min⁻¹)</td>
<td>35.8 ± 2.1</td>
<td>46.0 ± 6.6*</td>
<td>54.4 ± 5.4****</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Watt max</td>
<td>300 ± 40</td>
<td>320 ± 50</td>
<td>410 ± 30***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Max Heart Rate (b·min⁻¹)</td>
<td>189.0 ± 7.8</td>
<td>189.3 ± 10.1</td>
<td>171.5 ± 7.0****</td>
<td>0.017</td>
</tr>
<tr>
<td>Training (h·wk⁻¹)</td>
<td>3.7 ± 1.5</td>
<td>4.6 ± 1.5</td>
<td>13.3 ± 3.4****</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data is shown as mean ± SD. * Different from Untrained (p<0.05). ** Different from Fast-Untrained (p<0.05).
**Figure 1** - Distribution of participant finish times. A – shows the distribution of finish times with 930 seconds indicated as a dashed line. B is a box and wisker plot of the untrained participants as one group or separated in Slow- and Fast-Untrained.
4.3.2 Study Design

A parallel study design was used to examine the difference in performance and neuromuscular fatigue during and after a laboratory-based 10 km cycling TT. Participants came to the laboratory for two visits at the same time of day, separated by 48 hours (range 2-7 days). Participants were required to refrain from exercise for at least 24 hours before each visit and were encouraged to arrive fully rested and hydrated.

4.3.3 Descriptive Characteristics

At the first visit, participants height, weight and body fat percentage (Omron Fat Loss Monitor, model HBF-306CAN, Omron Healthcare, Bannockburn, Illinois) were measured wearing lightweight shorts. Participants were then familiarized with the neuromuscular testing described below.

Participants’ VO\textsubscript{2max} was determined using breath-by-breath gas-analysis (MetaMax 3B, CORTEX, Leipzig, Germany) during an incremental cycling test on an ergometer (Monark 839E, Vansboro, Sweden). Work rate was set at 50 W, 100 W and 150 W for 2 minutes each before increasing by 25 W min\textsuperscript{-1} thereafter. Participants were encouraged to keep their cadence above 80 RPM throughout. VO\textsubscript{2max} was determined as the highest value achieved over a 20-second period. Watt max (W\textsubscript{max}) was determined from the stage at which participants’ cadence dropped below 50 rpm. Maximum heart rate was the highest recorded during the test (Polar Electro, Finland).

4.3.4 10 km Time Trial

The competitive cyclists were not familiarized with the TT as they had extensive experience in performing TTs and maximal-effort cycling. The untrained participants had all performed 10 km TTs as part of a larger study in our laboratory within four weeks of this study.
and this is an analysis of a subset of data. Participants cycled on an electronically braked ergometer (Monark 839E, Vansboro, Sweden) with no feedback other than distance completed displayed on a computer monitor. TT cycling work rate was set at 60% of $W_{\text{max}}$ as determined from the VO$_2$max test with the ergometer in hyperbolic mode. The TT work rate was set at 60% of $W_{\text{max}}$ from preliminary testing so untrained participants could complete the TT without stopping. This fixed work rate allowed participants to vary their cadence to adjust cycling speed. As cycling work rate was held constant, speed and time to completion were determined by cadence. The work rate and intensity was 160 W (range 120-210) and 64% VO$_2$max (range 61-70%) for the Untrained and 220 W (range 200-240 and 62% VO$_2$max (range 56-70%) for the Trained groups.

TT time, cadence, heart rate and speed were averaged over 2.5 km sections. Breath-by-breath measurements of minute ventilate (VE), tidal volume (VT), volume of oxygen (VO$_2$) and carbon dioxide (VCO$_2$), respiratory exchange ratio (RER) and breath frequency (B$_F$) were averaged over 2.5 km sections. A finger prick blood lactate sample was taken prior to the TT and at 3 minutes post TT (Lactate Scout, Cardiff, UK).

The untrained participants all wore running shoes and used the manufacturer's pedals, whereas the cyclists used their own pedals and cycling shoes.

4.3.5 Neuromuscular Testing

Neuromuscular fatigue was assessed before and 3-minutes after the TT. Participants were seated in a custom built isometric dynamometer with their hip and knee angle fixed at 90° throughout. Testing involved a train of 5 maximal stimulations of the femoral nerve to measure twitch characteristics, followed by 3 MVCs described below. Force was measured using a load cell (SM-500-I9; Durham Instruments, Pickering, Canada) adjusted to each participant. The load cell was connected in a direct line to the right ankle at the level of the malleoli. Change in
neuromuscular variables from pre to post-TT was considered as neuromuscular fatigue. Data were collected using PowerLab 8/35 (ADInstruments, Colorado Springs, CO, USA). EMG signal was amplified with a Dual BioAmp amplifier (ADInstruments, Colorado Springs, CO, USA; bandwidth frequency 10-500 Hz input impedance 200 MΩ, common mode rejection ratio = 85 dB, gain = ±1%). The placement of the EMG and stimulating electrodes were outlined in indelible ink to ensure similar placement after the TT. All data were transmitted to a PC and analyzed using a custom analysis program (Matlab 6.0; Mathworks Inc.).

4.3.5.1 Measurement of Global Fatigue

Quadriceps peak force was measured during three 5-second maximal voluntary contractions (MVC), each separated by 60-seconds rest. The best MVC was used for measurement of peak muscle force.

4.3.5.2 Measurement of Central Neuromuscular Fatigue

Quadriceps voluntary activation (VA) was used to measure central neuromuscular fatigue. A high-frequency doublet (100 Hz; 10 ms inter-stimulus interval) was given at 2.5-seconds into the MVC and at 2-seconds after the contraction with the muscle in a potentiated state[169]. Stimulation intensity was set at 120% of optimal intensity. The ratio of the amplitude of the superimposed twitch during the MVC over the potentiated twitch ($Q_{TW}$) was used to calculate voluntary activation as follows:

$$VA(\%) = (1 – \text{superimposed twitch} / Q_{TW}) \times 100$$

Stimulations of the femoral nerve were delivered from a high-voltage (400 V) constant current stimulator (Biopac, BSLSTMA, Santa Barbara, California), controlled by a custom designed program (LabChart 7, ADInstruments, Colorado Springs, CO, USA). A square wave, 1-ms stimulation was delivered from a cathode (10 mm diameter) (Kendall 100, Covidien, Saint-Laurant, Quebec, Canada) placed over the femoral nerve at the femoral triangle beneath the
inguinal ligament. The anode (5 x 10 cm, DJO, Vista, CA, USA) was placed on the lower portion of the gluteal fold opposite to the cathode. Determination of optimal stimulation intensity (77.9 ± 19.6 mA) was achieved by increasing stimulation intensity until a further 5 mA increase did not alter peak quadriceps twitch force and vastus lateralis M-wave amplitude and was reassessed at each visit.

4.3.5.3 Measurement of Peripheral Neuromuscular Fatigue

The femoral nerve was stimulated with a train of 5 stimulations, each separated by 5-seconds rest at 100% of optimal intensity. Twitch peak force (tPF), rate of force development (tRFD), time to peak (tTP) and time to half relaxation (tHRT) and total twitch area under the curve (tAUC) were measured and averaged. Corresponding M-wave data from the lateralis (VL) were measured for amplitude (mA), duration (mD) and total area under the curve (mAUC).

The rate of force development (RFD) from 20% and 80% of peak force was calculated for each MVC and averaged. The electromechanical delay (EMD) was calculated from the time difference from the increase in quadriceps EMG activity and force >10 standard deviations from resting baseline.

Changes in QTW is a sensitive method for detecting low levels of neuromuscular fatigue[170], especially when post-activation potentiation is different between groups as a result of different exercise durations[166], as we expected in the present study.

Skin on the vastus lateralis (VL), vastus medialis (VM) and the patella were shaved and cleaned with alcohol wipes to ensure low signal impedance (Z <5 kΩ). EMG signals were recorded using Ag-AgCl electrodes (Kendall 100, Covidien, Saint-Laurant, Quebec, Canada; inter-electrode distance 25 mm) placed lengthwise over the mid-belly of the muscle with the reference electrode over the patella[171]. Muscle activation was determined by calculating the EMG root mean square (RMS) averaged over 0.2-second periods from when muscle force began
to plateau until immediately before the superimposed stimulation[50]. Mean EMG RMS from three MVC was used for determining change. Post-TT EMG values were normalized to mean pre-TT values.

4.3.6 Statistics

All data are presented as means ± SD (SPSS v22; IBM Corp., Armonk, N.Y., USA). Descriptive data were analyzed using a one-way ANOVA. Cycling variables were analyzed using a group by distance (2.5, 5.0, 7.5 and 10.0 km) repeated measured ANOVA. Neuromuscular data were analyzed using repeated measured ANOVA. Mauchley’s Test of Sphericity was used to assess homogeneity of data. Violations for heart rate, VE, VO$_2$, RER data were corrected using Greenhouse-Geiser adjustment. The changes in cycling variables over distance were compared using Bonferroni pair-wise procedure. Group interaction effects were assessed using Tukey’s honestly significant differences post-hoc analysis. Effect sizes were calculated as partial eta squared ($\eta^2$). Pearson’s correlation coefficient was used to identify significant associations within cycling variables and between cycling and neuromuscular variables. Significant correlations were then tested using stepwise linear regression to evaluate the strength of the observed relationships. Significance for all tests was set at 2-tailed $p< 0.05$.

4.4 Results

All participants completed the protocol with the following exceptions. One participant in the Trained group was uncomfortable with femoral nerve stimulations and only completed the MVCs. TT heart rate values for one Slow-Untrained and one Fast-Untrained participant of <145 b/min$^{-1}$ were excluded as sampling errors. Respiratory gas exchange were not collected from one Fast-Untrained (n=6) and one Trained (n=4) participant because they felt claustrophobic wearing the gas collection mask.
4.4.1 Descriptive Characteristics

The Trained group (n=5) was older (p = 0.015), had higher $W_{\text{max}}$ (p<0.001) and lower maximum heart rate (p=0.017) and exercised more hours per week (p<0.001) than participants in the Slow- (n=6) and Fast- (n=7) Untrained Groups. The Trained Group had a higher absolute and relative VO$_{2\text{max}}$ compared to the Slow- and Fast-Untrained groups (p<0.01) and the Fast-Untrained group had a higher relative VO$_{2\text{max}}$ compared to the Slow-Untrained group (p<0.01) (Table 1).

4.4.2 10 km Time Trial

There were main effects of group ($F_{[2,15]=18.32}$, p<0.001, $\eta^2=0.71$) for time to complete each 2.5 km segment of the TT ($F_{[3,45]=3.28}$, p=0.029, $\eta^2=0.18$) (Figure 1A). The Fast-Untrained and Trained both completed each 2.5 km section faster than the Slow-Untrained group (p<0.01).

Heart rate showed main effects for group ($F_{[2,13]=5.42}$, p<0.019, $\eta^2=0.45$) and TT distance ($F_{[3,39]=98.99}$, p<0.001, $\eta^2=0.88$). The Fast-Untrained group had a higher HR compared to the Trained group (p=0.016). HR increased during the TT with each time point differing from each other (p<0.001) (Figure 1B).

There was a significant group × distance interaction for RPM ($F_{[6,45]=2.52}$, p=0.045, $\eta^2=0.25$) (Figure 1C) and for speed ($F_{[6,45]=2.73}$, p=0.024, $\eta^2=0.27$) (Figure 1D). The Trained group increased RPM and speed throughout the TT, whereas the Fast-Untrained group decreased both throughout the TT. The Slow-Untrained group maintained a similar RPM and speed from 0-7.5 km and then increased from 7.5-10.0 km.

Blood lactate was higher post-TT, 8.4 ± 2.4 mM·L$^{-1}$, than pre-TT, 1.9 ± 0.4 mM·L$^{-1}$, (main effect of time, $F_{[1,15]=94.32}$, p<0.001, $\eta^2=0.90$). The change in the Slow-Untrained, Fast-Untrained and Trained groups, 6.1±2.5, 7.5±1.8 and 6.1±2.5 mM·L$^{-1}$, did not differ significantly.
4.4.3 Global Fatigue

MVC force was not different between groups ($F_{(2,15)}=0.61, p=0.56$) (Table 2) but was lower after the TT ($F_{(2,15)}=10.08, p=0.005$). There was no group x time interaction ($F_{(2,15)}=1.09, p=0.36$).

4.4.4 Central Fatigue

Quadriceps VA showed a main effect for time was present ($F_{(2,14)}=5.73, p=0.031, \eta^2=0.26$) with the Slow-Untrained, Fast-Untrained and Trained groups changing -6%±6, -8%±12 and 1%±4, but the group x time interaction was not significant ($F_{(2,14)}=1.47, p=0.26$).

4.4.5 Peripheral Fatigue

A group x time interaction for tPF revealed Slow-Untrained and Fast-Untrained groups had higher fatigue than the Trained group after the TT, decreasing -27%±11, -20%±17 and -1%±15 respectively ($F_{(2,14)}=4.10, p=0.040, \eta^2=0.37$) (Table 2).

A group x time interaction showed tRFD in the Trained group was higher after the TT while the Slow-Untrained and Fast-Untrained groups both decreased ($F_{(2,14)}=3.801, p=0.048, \eta^2=0.35$) (Table 2).

A group x time interaction for tAUC showed the Trained group AUC was higher after the TT while the Slow-Untrained and Fast-Untrained groups were both lower ($F_{(2,14)}=9.90, p=0.002, \eta^2=0.59$) (Table 2).

A group x time interaction was found for $Q_{TW}$ force ($F_{(2,14)}=35.71, p<0.001, \eta^2=0.38$) with the Slow-Untrained and Fast-Untrained groups showing greater decrease in force compared to the Trained group (-37%±7, -26%±18 and -12%±13) (Table 2).

No group, time or interaction effects were observed for tTP, tHRT or MVC RFD and EMD (Table 2).
4.4.6 Surface EMG

mA tended to be higher in the Trained group following the TT ($F_{[2,15]}=3.40$, $p=0.075$, $\eta^2=0.40$), but was not different between groups or by time (Table 2). No group, time or interaction effects were observed for mD, mAUC VL or VM EMG (Table 2).

4.4.7 Respiratory Data

VE showed a main effect for group ($F_{[2,13]}=6.55$, $p=0.011$, $\eta^2=0.50$) and time ($F_{[3,39]}=64.37$, $p<0.001$, $\eta^2=0.83$) (Table 3). The Slow-Untrained Group had a lower VE than the Fast-Untrained ($p=0.036$) and Trained group ($p=0.015$). Over the duration of the TT, VE increased with all time points different from each other ($p<0.05$).

VT revealed a main effect for time only ($F_{[3,39]}=8.74$, $p<0.001$, $\eta^2=0.40$), with 0.0-2.5 km lower than 2.5-5.0 km only ($p<0.02$) (Table 3).

$B_F$ was not different between groups ($F_{[2,13]}=1.92$, $p=0.19$, $\eta^2=0.23$) there was a main effect for time ($F_{[3,39]}=52.98$, $p<0.001$, $\eta^2=0.86$) with $B_F$ increasing over time and all time points different from each other (Table 3). In the Trained group, $B_F$ increased in line with cadence (Figure 1C). The Fast-Untrained group increased $B_F$ and cadence declined over the TT. The Slow-Untrained group’s $B_F$ increased over distance while cadence was unchanged until increasing in the last 2.5 km.

$VO_2$ and $VCO_2$ showed main effects for group ($F_{[2,13]}=12.11$, $p=0.001$, $\eta^2=0.65$), ($F_{[2,13]}=5.20$, $p=0.022$, $\eta^2=0.44$) and time ($F_{[3,39]}=22.29$, $p<0.001$, $\eta^2=0.63$), ($F_{[1.4,39]}=17.25$, $p<0.001$, $\eta^2=0.57$) respectively (Table 3). The Trained group had a higher $VO_2$ and $VCO_2$ compared to the Slow-Untrained ($p<0.01$) but only higher $VO_2$ compared to the Fast-Untrained ($p=0.027$). $VO_2$ and $VCO_2$ increased over the duration of the TT with all time points higher than 0.0-2.5 km ($p<0.003$). Mean $VO_2$ for the TT was $90\pm9\%$, $89\pm13\%$ and $87\pm12\%$ for the Slow-Untrained, Fast-Untrained and Trained groups.
RER was not different between groups or time and no interactions were observed (Table 3).

4.4.8 Correlation and Regression

TT overall time was negatively correlated with average RPM ($r = -0.98, p<0.001$) and relative VO$_2$max ($r = -0.57, p=0.014$). Regression analysis of neuromuscular fatigue and cycling data revealed that change in tPF was related to change in VA and TT total time ($r^2=0.71$, $p<0.001$).
Figure 2 – Cycling time trial variables (A) time, (B) heart rate, (C) cadence (revolutions per minute (RPM)), (D) Speed, averaged over 2.5 km. + Indicates time points are different (p<0.05). * Trained and Fast-Untrained groups different from Untrained group (p<0.05). ** Fast-Untrained group different from Trained group (p<0.05).
Table 2 – Pre and post 10 km time trial neuromuscular measurements of central and peripheral neuromuscular fatigue.

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Fast Untrained</th>
<th>Trained</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global Fatigue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVC (N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>540 ± 135</td>
<td>520 ± 102</td>
<td>565 ± 126</td>
<td></td>
</tr>
<tr>
<td>Post+</td>
<td>465 ± 130</td>
<td>454 ± 73</td>
<td>547 ± 119</td>
<td>0.36</td>
</tr>
<tr>
<td>% Change</td>
<td>-13 ± 9</td>
<td>-12 ± 15</td>
<td>-2 ± 13</td>
<td></td>
</tr>
<tr>
<td><strong>Central Fatigue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VA (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>92 ± 5</td>
<td>87 ± 8</td>
<td>91 ± 1</td>
<td></td>
</tr>
<tr>
<td>Post+</td>
<td>86 ± 6</td>
<td>79 ± 7</td>
<td>91 ± 3</td>
<td>0.26</td>
</tr>
<tr>
<td>% Change</td>
<td>-6 ± 6</td>
<td>-8 ± 12</td>
<td>1 ± 4</td>
<td></td>
</tr>
<tr>
<td><strong>Peripheral Fatigue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPF (N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>131 ± 24</td>
<td>110 ± 19</td>
<td>106 ± 10</td>
<td>0.040</td>
</tr>
<tr>
<td>Post</td>
<td>94 ± 22</td>
<td>88 ± 20</td>
<td>103 ± 12</td>
<td></td>
</tr>
<tr>
<td>% Change</td>
<td>-27 ± 11</td>
<td>-20 ± 17</td>
<td>-1 ± 15</td>
<td></td>
</tr>
<tr>
<td>tAUC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>14 ± 5</td>
<td>9 ± 2</td>
<td>11 ± 6</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>9 ± 4</td>
<td>9 ± 1</td>
<td>13 ± 6</td>
<td>0.024</td>
</tr>
<tr>
<td>% Change</td>
<td>-34 ± 17</td>
<td>-7 ± 28</td>
<td>18 ± 42</td>
<td></td>
</tr>
<tr>
<td>tRFD (Ns⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2783 ± 589</td>
<td>2246 ± 554</td>
<td>1857 ± 597</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>2222 ± 664</td>
<td>1629 ± 479</td>
<td>2096 ± 229</td>
<td>0.048</td>
</tr>
<tr>
<td>% Change</td>
<td>-19 ± 18</td>
<td>-29 ± 24</td>
<td>14 ± 31</td>
<td></td>
</tr>
<tr>
<td>tTP (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>6.7 ± 3.1</td>
<td>5.3 ± 1.1</td>
<td>5.6 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>5.2 ± 0.90</td>
<td>5.3 ± 0.70</td>
<td>6.7 ± 2.4</td>
<td>0.35</td>
</tr>
<tr>
<td>% Change</td>
<td>-20 ± 34</td>
<td>1 ± 25</td>
<td>21 ± 40</td>
<td></td>
</tr>
<tr>
<td>tHRT (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>5.5 ± 2.3</td>
<td>5.0 ± 0.90</td>
<td>4.6 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>5.3 ± 1.3</td>
<td>4.7 ± 0.90</td>
<td>4.4 ± 0.70</td>
<td>0.98</td>
</tr>
<tr>
<td>% Change</td>
<td>-3 ± 26</td>
<td>-5 ± 15</td>
<td>-3± 14</td>
<td></td>
</tr>
<tr>
<td>RFD (Ns⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1638 ± 916</td>
<td>1753 ± 849</td>
<td>1864 ± 840</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>1365 ± 631</td>
<td>1432 ± 761</td>
<td>1864 ± 975</td>
<td>0.62</td>
</tr>
<tr>
<td>% Change</td>
<td>-16 ± 41</td>
<td>-17 ± 19</td>
<td>1 ± 54</td>
<td></td>
</tr>
<tr>
<td>EMD (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>5.4 ± 2.2</td>
<td>5.8 ± 1.5</td>
<td>5.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>4.9 ± 1.5</td>
<td>4.0 ± 2.0</td>
<td>5.8 ± 2.2</td>
<td>0.12</td>
</tr>
<tr>
<td>% Change</td>
<td>-8 ± 45</td>
<td>-26 ± 40</td>
<td>26 ± 58</td>
<td></td>
</tr>
<tr>
<td>Qtw (N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>225 ± 75</td>
<td>183 ± 32</td>
<td>157 ± 28</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>140 ± 42</td>
<td>135 ± 26</td>
<td>137 ± 8</td>
<td>0.034</td>
</tr>
<tr>
<td>% Change</td>
<td>-37 ± 7</td>
<td>-26 ± 18</td>
<td>-12 ± 13</td>
<td></td>
</tr>
</tbody>
</table>
## Surface EMG Evoked Response

### mA (mV)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>% Change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mA</strong></td>
<td>5.7 ± 1.9</td>
<td>7.9 ± 1.8</td>
<td>6.4 ± 1.2</td>
<td>0.075</td>
</tr>
<tr>
<td><strong>% Change</strong></td>
<td>-6 ± 12</td>
<td>-12 ± 13</td>
<td>15 ± 18</td>
<td></td>
</tr>
<tr>
<td><strong>mD (ms)</strong></td>
<td>10.3 ± 3.1</td>
<td>10.1 ± 3.0</td>
<td>7.1 ± 2.1</td>
<td></td>
</tr>
<tr>
<td><strong>% Change</strong></td>
<td>1 ± 20</td>
<td>4 ± 13</td>
<td>-5 ± 11</td>
<td></td>
</tr>
<tr>
<td><strong>mAUC (μV·s⁻¹)</strong></td>
<td>3.3 ± 1.2</td>
<td>4.6 ± 1.0</td>
<td>3.5 ± 0.3</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>% Change</strong></td>
<td>-14 ± 9</td>
<td>-10 ± 21</td>
<td>-11 ± 7</td>
<td></td>
</tr>
</tbody>
</table>

### MVC Response

#### VL EMG (rms)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>% Change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre</strong></td>
<td>0.51 ± 0.20</td>
<td>0.53 ± 0.22</td>
<td>0.56 ± 0.15</td>
<td></td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>0.49 ± 0.26</td>
<td>0.53 ± 0.20</td>
<td>0.53 ± 0.23</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>% Change</strong></td>
<td>-3 ± 38</td>
<td>1 ± 27</td>
<td>-5 ± 20</td>
<td></td>
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</tbody>
</table>

#### VM EMG (rms)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>% Change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre</strong></td>
<td>0.56 ± 0.17</td>
<td>0.49 ± 0.18</td>
<td>0.78 ± 0.27</td>
<td></td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>0.48 ± 0.21</td>
<td>0.51 ± 0.19</td>
<td>0.71 ± 0.45</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>% Change</strong></td>
<td>-14 ± 21</td>
<td>5 ± 17</td>
<td>-9 ± 37</td>
<td></td>
</tr>
</tbody>
</table>

Data is shown as mean ± SD. P value for interaction. + Indicates main effect for time (p<0.05). * Different from Untrained (p<0.05). ** Different from Fast-Untrained (p<0.05). tPF, twitch peak force; tAUC, twitch area under the curve; tRFD, twitch rate of force development; tTP, twitch time to peak force; tHRT, time to half relaxation; RFD, rate of force development; EMD, electromechanical delay; QTW, potentiated twitch force; mA, M-wave amplitude; mD, M-wave duration; mAUC M-wave area under the curve. VL EMG, vastus lateralis EMG; VM EMG, vastus medialis EMG.
Table 3 – Respiratory data measured during the 10 km time trial.

<table>
<thead>
<tr>
<th></th>
<th>km</th>
<th>Untrained</th>
<th>Fast Untrained</th>
<th>Trained</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VE (L min⁻¹)</td>
<td>0.0 - 2.5</td>
<td>59.8 ± 17.8</td>
<td>92.6 ± 14.2</td>
<td>93.3 ± 14.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 - 5.0</td>
<td>83.3 ± 19.0</td>
<td>114.6 ± 21.5</td>
<td>117.1 ± 18.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 - 7.5</td>
<td>88.9 ± 15.6</td>
<td>117.5 ± 23.3</td>
<td>130.4 ± 24.2</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>7.5 – 10.0+</td>
<td>93.8 ± 15.5</td>
<td>124.2 ± 24.0*</td>
<td>145.1 ± 25.6*</td>
<td></td>
</tr>
<tr>
<td>VT (L)</td>
<td>0.0 - 2.5</td>
<td>1.8 ± 0.4</td>
<td>2.3 ± 0.8</td>
<td>2.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 - 5.0</td>
<td>2.3 ± 0.7</td>
<td>2.5 ± 0.8</td>
<td>3.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 - 7.5</td>
<td>2.2 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>3.1 ± 0.4</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>7.5 – 10.0+</td>
<td>2.2 ± 0.7</td>
<td>2.3 ± 0.7</td>
<td>3.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>VO₂ (L min⁻¹)</td>
<td>0.0 - 2.5</td>
<td>2.0 ± 0.5</td>
<td>2.7 ± 0.3</td>
<td>3.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 - 5.0</td>
<td>2.5 ± 0.5</td>
<td>3.0 ± 0.5</td>
<td>3.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 - 7.5</td>
<td>2.6 ± 0.4</td>
<td>3.0 ± 0.5</td>
<td>3.8 ± 0.3</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>7.5 – 10.0+</td>
<td>2.6 ± 0.4</td>
<td>3.0 ± 0.5</td>
<td>3.9 ± 0.4***</td>
<td></td>
</tr>
<tr>
<td>VCO₂ (L min⁻¹)</td>
<td>0.0 - 2.5</td>
<td>2.2 ± 0.7</td>
<td>3.1 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 - 5.0</td>
<td>2.9 ± 0.6</td>
<td>3.5 ± 0.5</td>
<td>3.8 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 - 7.5</td>
<td>2.9 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>3.9 ± 0.5</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>7.5 – 10.0</td>
<td>3.0 ± 0.4</td>
<td>3.5 ± 0.6</td>
<td>3.9 ± 0.7*</td>
<td></td>
</tr>
<tr>
<td>RER</td>
<td>0.0 - 2.5</td>
<td>1.04 ± 0.11</td>
<td>1.13 ± 0.11</td>
<td>1.01 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 - 5.0</td>
<td>1.13 ± 0.04</td>
<td>1.16 ± 0.05</td>
<td>1.04 ± 0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 - 7.5</td>
<td>1.12 ± 0.04</td>
<td>1.12 ± 0.04</td>
<td>1.03 ± 0.16</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>7.5 – 10.0</td>
<td>1.14 ± 0.05</td>
<td>1.15 ± 0.02</td>
<td>1.00 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>B F (1 min⁻¹)</td>
<td>0.0 - 2.5</td>
<td>33 ± 6</td>
<td>41 ± 7</td>
<td>34 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 - 5.0</td>
<td>39 ± 8</td>
<td>48 ± 10</td>
<td>39 ± 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 - 7.5</td>
<td>42 ± 8</td>
<td>51 ± 11</td>
<td>43 ± 10</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>7.5 – 10.0+</td>
<td>45 ± 8</td>
<td>56 ± 12</td>
<td>47 ± 11</td>
<td></td>
</tr>
</tbody>
</table>

Data is shown as mean ± SD. P value for interaction. + Indicates main effect for time (p<0.05). * Different from Untrained (p<0.05). ** Different from Fast-Untrained (p<0.05). VE, minute ventilation; VT, tidal volume, VO₂, volume of oxygen consumed, VCO₂, volume of carbon dioxide exhaled; RER, ratio of carbon dioxide exhaled in oxygen inhaled; B F, breath frequency.
4.5 Discussion

This study compared the cycling performance and central and peripheral neuromuscular fatigue after a constant work rate 10 km cycling TT between untrained and trained cyclists. Previous studies have investigated neuromuscular fatigue after TT exercise of varying distances in trained cyclists [60,159,165,166]. Our data demonstrate that some recreationally-active but untrained cyclists can complete a 10 km TT in the same time as trained cyclists by using high cadence. Contrary to our hypothesis the Trained group experienced less peripheral neuromuscular fatigue than the Slow- and Fast-Untrained groups. These data provide evidence of differences in cycling and neuromuscular capacity between trained and untrained cyclists.

4.5.1 Central Neuromuscular Fatigue was Related to Cycling Performance

The constant work rate TT used in this study emphasized neuromuscular control of cycling, as speed was directly related to voluntary control of cycling cadence. The decrease in cadence measured in this Fast-Untrained group is representative of the onset of central neuromuscular fatigue observed in the first half of TT type exercise in untrained individuals [172] and possibly in response to the increase in muscle H+. Subsequent quadriceps muscle feedback to the central nervous system (CNS) from group III/IV afferents might be responsible for slowing cadence as a protective mechanism to limit peripheral neuromuscular fatigue muscle stress [60]. During a constant work rate TT, a change of cadence results in a shift on the cycling force-frequency curve [173]. While the Trained group decreased force by increasing cadence, the Fast-Untrained group decreased cadence, further stressing the locomotor muscles.

The selection of cycling cadence and subsequent force-frequency relationship influences muscle fibre recruitment patterns and neuromuscular fatigue. Constant work rate cycling at 80
and 90 RPM requires lower muscle activation in trained and untrained cyclists compared to 50, 60 or 70 RPM, thereby reducing neuromuscular fatigue [174,175]. High cadence cycling (120 RPM) is thought to elicit fatigue from the recruitment of fast-fatiguing type II fibres [176]. In contrast, low cycling cadence (50 RPM) elicits fatigue as a result of glycogen depletion in type II fibres required to generate muscle force [177]. In the present study, the average cycling cadence was 95, 117 and 117 RPM for the Slow-Untrained, Fast-Untrained and Trained groups. The Slow-Untrained group may have selected a lower cadence in attempts to delay fatigue and prepare for a ‘sprint to the finish’. The high cadence selected by the Fast-Untrained group potentially fatigued the fast twitch motor units [176] and caused a rapid increase in muscle H+ that decreased cadence and contributed to the measured neuromuscular fatigue.

In the present study, all groups cycled at a similar VO$_2$, RER and had similar post-TT blood lactate. This indicates overall intensity was similar between groups, but the rate of lactate increase over the TT is unknown. The Slow-Untrained group TT time was longer than the Fast-Untrained and Trained groups as a result of lower cadence, yet there was no difference in central neuromuscular fatigue between the groups. As such, a 10 km TT may elicit differences in neuromuscular control of cycling between groups of different cycling experience; however, these differences do not contribute to significant changes in central neuromuscular fatigue.

4.5.2 Peripheral Fatigue was Different Between Groups

Recent data shows peripheral neuromuscular fatigue is the primary contributor to neuromuscular fatigue after a short (<30min) TTs [159], potentially as a feed forward mechanism through group III/IV afferents to prevent exercising beyond a critical threshold of fatigue. Peripheral neuromuscular fatigue in the Slow- and Fast-Untrained groups measured from Q$_{TW}$ force, was similar (-37% and -26%) to previously reported data on the critical threshold of peripheral neuromuscular fatigue (~35%) after TTs of different lengths [60,159,165]. This
suggests the Slow- and Fast-Untrained groups’ self-selected cadence was adjusted to not exceed this critical threshold. Exercising above an individual critical threshold results in the recruitment of type II fibres to maintain muscle force [175,178] and metabolic stress (i.e. lactate, H\(^+\)) that cannot be regulated by the muscle [179,180]. The Trained group completed the TT under their critical threshold, as Q\(_{TW}\) force was -12%, providing evidence of the advanced muscle buffering capacity and metabolic efficiency of trained cyclists [167,181].

Peripheral fatigue was also measured from impaired contractile function from lower tPF, tAUC and tRFD in the Slow- and Fast-Untrained groups only. No change in M-wave duration or AUC was measured, indicating muscle membrane excitability was unchanged. We interpret these data as peripheral neuromuscular fatigue occurred from altered excitation-contraction coupling from impaired Ca\(^{2+}\) kinetics [182,183]. Metabolic perturbations associated with muscle fatigue (H\(^+\)), can impact the development of muscle force through lower Ca\(^{2+}\) release from the sarcoplasmic reticulum [182]. Specifically, ryanodine receptor channels open less frequently and for a shorter duration when intramyocellular pH decreases [69].

The Trained group did not approach the proposed critical threshold of peripheral neuromuscular fatigue as the cycling work rate was fixed at 60% of W\(_{max}\) where other studies used self-selected power [159,166]. This decision was made in attempts to isolate neuromuscular control of cycling.

### 4.6 Conclusion

In conclusion, the Trained and Untrained groups showed a similar manifestation of neuromuscular fatigue to data previously reported in trained cyclists [60,159,165,166]. Peripheral neuromuscular fatigue was the primary source of fatigue in all groups and was more pervasive in the Slow- and Fast-Untrained groups from excitation-contraction coupling failure from increased blood lactate. Central fatigue was observed in the Fast-Untrained group during
the TT as a progressive decrease in cadence and post-TT decrease in VA. The Trained group was likely able to mitigate neuromuscular fatigue from cycling training history and associated metabolic and neuromuscular adaptations. These findings illustrate the neuromuscular and cycling differences between trained and untrained cyclists completing a standard laboratory test and provide a basis for investigating interventions to alter neuromuscular fatigue and cycling performance.
5 Neuromuscular Adaptations to Sprint Interval Training and the Modulating effect of Seal Oil Omega-3 Supplementation

Evan J. H. Lewis, Frédéric Stucky, Peter W. Radonic, Adam H. Metherel, Thomas M. S. Wolever, Greg D. Wells

Contribution: As first author I designed the study, performed 80% of the data collection, performed 90% the data analysis and drafted the manuscript.

The results from chapter 4 showed a clear difference in performance and neuromuscular fatigue between the trained and untrained groups. This study addressed objective 2 of this thesis by examining the neuromuscular adaptations to sprint interval training in study 1. In study 2, N-3 PUFA supplementation was compared against a placebo to determine its effects on neuromuscular adaptations. Currently the neuromuscular adaptations to sprint interval training are unknown. The 10 km cycling time trial was used as a performance-orientated measure of adaptation to training.
5.1 Abstract

Sprint interval training (SIT) stimulates rapid metabolic adaptations within skeletal muscle but the nature of neuromuscular adaptations is unknown. Omega-3 polyunsaturated fatty acids (N-3 PUFA) are suggested to enhance neuromuscular adaptations to exercise. Therefore, we measured the neuromuscular adaptations to SIT (Study-1) and conducted a placebo controlled randomized double blinded study to determine the effect of N-3 PUFA supplementation on neuromuscular adaptations to SIT (Study-2). In Study-1, seven active men (24.4±2.6 y, VO₂ peak 43.8±8.7 ml·kg·min⁻¹) completed two-weeks of SIT with pre and post-training 10 km cycling time trials (TT). In Study-2, 30 active men (24.5±4.2 y, VO₂ peak 41.0±5.1 ml·kg·min⁻¹) were randomly assigned to receive N-3 PUFA (2330 mg·d⁻¹)(n=14) or olive oil (n=16) during two-weeks of SIT with pre and post-training TTs. Four-week post-training a SIT session and TT were also performed. Change in neuromuscular function was assessed from resting twitches, quadriceps maximal voluntary contraction (MVC) force and potentiated twitch force (Qtw). Study-1 showed that SIT did not elicit significant neuromuscular adaptations. Study-2 showed that N-3 PUFA supplementation had no significant effect on neuromuscular adaptations. Training caused lower MVC force (mean ± SD; N-3 PUFA -9±11%, placebo -9±13% (p<0.05 time)) and Qtw peripheral fatigue (N-3 PUFA -10±19%, placebo -14±13% (p<0.05 time)). TT time was lower after training in all groups (Study-1 -10%, Study-2 N-3 PUFA -8%, placebo -12% (p<0.05 time)). Two-weeks of SIT improved TT performance in the absence of measurable neuromuscular adaptations. N-3 PUFA supplementation had no significant effect on SIT training adaptations.
5.2 INTRODUCTION

Sprint interval training (SIT) consists of repeated periods of brief maximal exercise interspersed with longer rest periods. Metabolic adaptations within skeletal muscle have been suggested as the primary contributor to increased sprint peak power, fatigue resistance and exercise capacity [18] and cycling performance [17]. Reported metabolic adaptations include increased muscle glycogen storage, greater muscle mitochondrial number and enzyme concentration, and increased membrane glucose transporters and monocarboxylate transporter concentrations [18,21,184].

It is well established that SIT can cause metabolic adaptations; however, it is unknown if neuromuscular adaptations contribute to previously reported improvements in exercise capacity [18] and performance [17]. Previous work has shown that the neuromuscular system is directly involved in the initial adaptations to training [1,33]. When muscles are challenged with a novel stimulus, the neuromuscular system rapidly adapts, leading to improvements in muscle coordination, activation and ultimately force generating capacity [1]. Furthermore, the neuromuscular system has been identified as a primary contributor to increased muscle strength in new exercisers when adopting a new training technique [32,33].

There have been few investigations of nutritional interventions on adaptations to SIT [185,186]. Omega-3 polyunsaturated fatty acid (N-3 PUFA) supplementation has been identified as a potential ergogenic aid for neuromuscular adaptations to exercise [26,138] and therefore might enhance neuromuscular adaptations to SIT.

Two experiments were performed to investigate: 1) the neuromuscular adaptations to SIT and; 2) the effect of N-3 PUFA supplementation on neuromuscular adaptations to SIT. The purpose of Study 1 was to describe the neuromuscular adaptations to two-weeks of SIT. We hypothesized that neuromuscular function as measured by quadriceps maximal voluntary
contraction (MVC) force would improve following SIT and fatigue would be lower on the last training session compared to the first.

The purpose of Study 2 was to determine the effect of N-3 PUFA supplementation combined with SIT on neuromuscular adaptations compared to placebo. We hypothesized that N-3 PUFA supplementation would enhance neuromuscular adaptations, such that the increase in MVC force would be greater in the N-3 PUFA group compared to placebo.

5.3 METHODS

5.3.1 Ethics Statement

The protocol and consent procedures were approved by the University of Toronto Research Ethics Board. All participants were informed of study procedure and gave written informed consent.

5.3.2 Participants

Males ≥18 y from the University of Toronto area, that were recreationally active (<5 h·wk⁻¹) but not following a structured exercise program were recruited for these studies. All prospective participants were screened for eligibility by measuring BMI (within 18.5 to 29.9) and assessing cardiac function using 12-lead electrocardiography (CardioPerfect Workstation, Welych-Allyn, Skaneateles Falls, New York, USA) that was reviewed by a physician. The PAR-Q exercise readiness questionnaire was used for additional physical activity readiness screening (www.csep.ca/english/view.asp?x=698).

Participants in Study 2 were further screened for HIV and hepatitis C to prevent any potential adverse events with the study supplement and exercise training. A dietary screen was used to ensure these participants were not consuming any form of N-3 PUFA supplement or consuming fish ≥ 3 times per week for 4-weeks prior to beginning the study.
Seven healthy males were recruited for Study 1. This was exploratory in nature and used a sample size similar to training groups in previous SIT studies [17,18,21]. 30 healthy males were recruited for Study 2 separately from Study 1. This sample size was determined from the post-training difference in maximal voluntary contraction force between groups receiving placebo or fish oil N-3 PUFA during resistance training [26]. Using a difference in means of 25 N (SD 15), $\alpha=0.05$ and $\beta=0.8$, 14 participants per group are required to detect a significant difference between the placebo and N-3 PUFA group.

The descriptive data for the participants is shown in Table 1. Figure 1 shows an overview of the progression of each study. Study 1 used a one-way, repeated measures, within-participant design. Study 2 was conducted as a parallel design, placebo controlled, randomized control trial.

5.3.3 Descriptive Characteristics

At the first visit, participants height, weight and body fat percentage (Omron Fat Loss Monitor, model HBF-306CAN, Omron Healthcare, Bannockburn, Illinois, USA) were measured. Participants’ VO$_2$max was determined using breath-by-breath gas-analysis (MetaMax 3B, CORTEX, Leipzig, Germany) during an incremental cycling test on an ergometer (Monark 839E, Vansboro, Sweden) as previously described [138]. Work rate was set at 50 W, 100 W and 150 W for 2 minutes each before increasing by 25 W min$^{-1}$ thereafter. VO$_2$peak was determined as the highest value achieved over a 20-second period. Watt max ($W_{\text{max}}$) was determined from the stage at which participants’ cadence dropped below 50 rpm. Maximum heart rate was the highest recorded during the test (Polar Electro, Finland).
Table 1 – Participant descriptive data.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Study 1</th>
<th>Study 2 Placebo</th>
<th>Study 2 N-3 PUFA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>24.4 ± 2.6</td>
<td>24.8 ± 4.7</td>
<td>24.1 ± 3.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>182.4 ± 4.8</td>
<td>176.1 ± 6.2</td>
<td>176.9 ± 7.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.1 ± 12.4</td>
<td>77.7 ± 13.3</td>
<td>75.5 ± 10.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>17.4 ± 5.2</td>
<td>17.4 ± 6.0</td>
<td>15.4 ± 4.6</td>
<td>0.4</td>
</tr>
<tr>
<td>VO₂ peak (L min⁻¹)</td>
<td>3.6 ± 0.8</td>
<td>3.1 ± 0.6</td>
<td>3.2 ± 0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>VO₂ peak (mL·kg⁻¹·min⁻¹)</td>
<td>43.8 ± 8.7</td>
<td>40.0 ± 3.6</td>
<td>42.3 ± 6.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Watt max</td>
<td>310 ± 50</td>
<td>260 ± 40</td>
<td>275 ± 40</td>
<td>0.4</td>
</tr>
<tr>
<td>Max Heart Rate (b·min⁻¹)</td>
<td>192.7 ± 7.3</td>
<td>187.1 ± 14.8</td>
<td>187.6 ± 8.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Training (h·wk⁻¹)</td>
<td>4.3 ± 1.0</td>
<td>2.6 ± 2.1</td>
<td>3.9 ± 1.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data is shown as mean ± SD. P value for independent samples t-test between groups in Study 2.

5.3.4 10 km Time Trial Performance Test

Participants cycled on an electronically braked ergometer (Monark 839E, Vansboro, Sweden) with no feedback other than distance completed displayed on a computer monitor. Cycling work rate was set at 60% of W_max as determined from the VO₂ max test with the ergometer in hyperbolic mode. This fixed work rate allowed participants to vary their cadence to adjust cycling speed. TT cadence and heart rate were averaged over the test.

5.3.5 Sprint Interval Training Protocol

Training started at least three days after visit (V) V2. Six SIT sessions were completed over two-weeks as previously described [18]. Participants increased the number of sprints from four to seven and completed four on the final day of training (Figure 1). Training occurred on alternating days (i.e. Monday, Wednesday, Friday) with two days rest between V5 and V6. Each visit started with a five-minute self-paced warm-up on a stationary cycle ergometer. Participants were then fitted to a mechanically braked cycle ergometer (Monark 894E, Vansboro, Sweden) and repeated 30-second maximal effort sprints against 0.075 kg·kg⁻¹ body weight with four-minutes of recovery between bouts. Participants were instructed to begin sprinting ~5-seconds before each interval to achieve a high cadence before the load was automatically applied by the manufacturers software (Anaerobic Test Software version 3.2.1.0, Monark). Participants were
strongly encouraged by the research team to maintain maximal effort throughout the 30-second intervals. Light pedaling was encouraged between sprints to promote recovery. Peak, mean and minimum power and percent power drop (fatigue index) were recorded for each interval.

**Figure 1** – Flow diagram showing the experimental overview of participants in Study 1 and 2. Studies occurred separately. Black arrows indicate blood sampling. White arrows indicate neuromuscular testing. SIT-4 indicates the number of sprints intervals performed by participants. TT, 10 km time trial.

### 5.3.6 Neuromuscular Testing

Neuromuscular function was assessed before and 3-minutes after SIT on V3, V8 and V10. Testing involved a train of 5 maximal stimulations of the femoral nerve to measure twitch characteristics, followed by 3 MVCs described below. Participants were seated in a custom built isometric dynamometer with their hip and knee angle fixed at 90° throughout. Force was measured using a load cell (SM-500-I9; Durham Instruments, Pickering, Ontario, Canada) adjusted to each participant. The load cell was connected in a direct line to the right ankle at the level of the malleoli. Change in neuromuscular variables from pre to post-training was considered as neuromuscular fatigue. Data were collected using PowerLab 8/35 (ADInstruments, Colorado Springs, Colorado, USA). The EMG signals were amplified with a Dual BioAmp
amplifier (ADInstruments, Colorado Springs, Colorado, USA; bandwidth frequency 10-500 Hz input impedance 200 MΩ, common mode rejection ratio = 85 dB, gain = ±1%). The placement of the EMG and stimulating electrodes were outlined in indelible ink to ensure similar placement after training. All data were transmitted to a laptop and analyzed using a custom analysis program (Matlab 6.0; Mathworks Inc.).

5.3.6.1 Measurement of Global Fatigue

During neuromuscular testing, quadriceps peak force was measured during three 5-second maximal voluntary contractions (MVC), each separated by 60-seconds rest. The maximum force achieved was used MVC as the measurement of peak force. Change in MVC force was considered as global neuromuscular fatigue.

5.3.6.2 Measurement of Central Neuromuscular Fatigue

Quadriceps voluntary activation (VA) was used to measure central neuromuscular fatigue. A high-frequency doublet (100 Hz; 10 ms inter-stimulus interval) was given at 2.5-seconds into the MVC and at 2-seconds after the contraction with the muscle in a potentiated state [169]. Stimulation intensity was set at 120% of optimal intensity. The ratio of the amplitude of the superimposed twitch during the MVC over the potentiated twitch (Qtw) was used to calculate voluntary activation as follows:

\[
VA(\%) = (1 – \text{superimposed twitch / Qtw}) \times 100
\]

Stimulations of the femoral nerve were delivered from a high-voltage (400 V) constant current stimulator (Biopac, BSLSTMA, Santa Barbara, California, USA), controlled by a custom designed program (LabChart 7, ADInstruments, Colorado Springs, Colorado, USA). A square wave, 1-ms stimulation was delivered from a cathode (10 mm diameter) (Kendall 100, Covidien, Saint-Laurant, Quebec, Canada) placed over the femoral nerve at the femoral triangle beneath the inguinal ligament. The anode (5 x 10 cm, DJO, Vista, California, USA) was placed on the
lower portion of the gluteal fold opposite to the cathode. Determination of optimal stimulation intensity was achieved by increasing stimulation intensity until a further 5 mA increase did not alter peak quadriceps twitch force and vastus lateralis M-wave amplitude and was reassessed at each visit.

5.3.6.3 Measurement of Peripheral Neuromuscular Fatigue

The femoral nerve was stimulated with a train of 5 stimulations, each separated by 5-seconds rest at 100% of optimal intensity. Twitch peak force (tPF), rate of force development (tRFD), time to peak (tTP) and time to half relaxation (tHRT) and total twitch area under the curve (tAUC) were measured and averaged. Corresponding M-wave data from the lateralis (VL) were measured for amplitude (mA), duration (mD) and total area under the curve (mAUC).

The rate of force development (RFD) from 20% and 80% of peak force was calculated for each MVC and averaged. The electromechanical delay (EMD) was calculated from the time difference from the increase in quadriceps EMG activity and force >10 standard deviations from resting baseline.

Changes in Qtw is a sensitive method for detecting neuromuscular fatigue from cycling [166] and provides a method to quantify peripheral fatigue compared to the previously described critical threshold of peripheral neuromuscular fatigue [159,165]. Five seconds after Qtw a low frequency doublet (10 Hz; 100 ms inter-stimulus interval) was given. The ratio of this stimulation to Qtw was used to measure low force frequency fatigue (LFF) as previously described [187].

Skin on the vastus lateralis (VL), vastus medialis (VM) and the patella were shaved and cleaned with alcohol wipes to ensure low signal impedance (Z <5 kΩ). EMG signals were recorded using Ag-AgCl electrodes (Kendall 100, Covidien, Saint-Laurant, Quebec, Canada; inter-electrode distance 25 mm) placed lengthwise over the mid-belly of the muscle with the
reference electrode over the patella [171]. Muscle activation was determined by calculating the EMG root mean square (RMS) averaged over 0.2-second periods from when muscle force began to plateau until immediately before the superimposed stimulation [50]. Mean EMG RMS from the three MVCs was used for determining change.

5.3.7 Training and Dietary Controls

All participants were asked to refrain from any strenuous activity for 24 hours prior to testing and were instructed not to participate in any exercise beyond activities of daily living during the two-weeks of training. In Study 2, participants were allowed to return to their pre-study exercise habits during the 4-week washout period.

Participants were asked to complete a three-day food record culminating on V2. During the study, participants were encouraged to eat similar foods and not alter their diet with the exception of the provided supplement in Study 2. Prior to the second and third TT (Study 2), participants were given a copy of their food record for the day prior to the TT and asked to follow the food choices and quantities as closely as possible.

5.3.8 Study 2 Experimental and Placebo Supplements

Participants in Study 2 were randomized to either the experimental N-3 PUFA group or placebo. The supplement was given to the participant in a sealed opaque envelope at the end of V3. Participants consumed their supplement for the duration of training (12-days). Participants consumed 5 mL of seal oil containing 1115 mg N-3 PUFA (375 mg EPA, 230 mg DPA, 510 mg DHA and with 5000 IU retinyl palmitate and vitamin D3) (Auum Inc., Timmons, Canada) (NPN 80021190) or 5 mL olive oil (Bertolli, Mississauga, Canada) matched with 5000 IU retinyl palmitate and vitamin D (Auum Inc., Timmons, Canada). Participants were instructed to take 1 – 2.5 mL servings orally twice daily, and to let the oil remain in the mouth for 1-minute before swallowing to allow for potential sublingual absorption [87].
Seal oil was chosen as the experimental N-3 supplement for this study because seal oil triacylglycerol molecules have N-3 PUFA fats primarily in the sn-1 and sN-3 positions, as opposed to the sn-2 position of fish oil N-3 PUFA [10,86]. Fats in the sn-3 position are preferentially cleaved by sublingual lipases, and the sn-1 fat is cleaved in the small intestine, while the sn-2 fatty acid is left for later esterification [87,88]. These structural differences enable chylomicrons and chylomicron remnants containing seal oil N-3 PUFA to have a higher rate of clearance from the blood compared to fish oil intake in animal studies [89,188].

5.3.9 Randomization and concealment

Participants were assigned a sequential study ID based on their enrollment. Treatment assignment (N-3 PUFA or placebo) was determined from a computer generated random number sequence using variable block sizes to enhance concealment. Supplement bottles were then sealed in opaque envelopes and labeled with participant ID by a researcher not involved in the study. Participants received their supplement at the end of the first SIT session. Study researchers were unblinded after neuromuscular and plasma N-3 PUFA analysis was completed.

5.3.10 Blood Sampling

A resting 8 mL blood sample from the antecubital vein was collected into K3-EDTA Vacutainer tubes (BD Vacutainer, Mississauga, Canada) at the beginning of Visit 3, 8 and 10. Samples were centrifuged (3000 rpm for 15 minutes at 4°C). Plasma samples were collected and stored at -80°C for later analysis of plasma N-3 PUFA concentration.

At visit 3, 8 and 10 a finger prick blood lactate sample was taken prior to training and 3-minutes after sprint 4 (Lactate Scout, Cardiff, UK).
5.3.11 Lipid Extraction and Gas Chromatography-Flame Ionization Detection

All lipid extraction techniques have been previously described by Chen et al. [189]. To extract total lipids, Folch, Lees, and Sloane Stanley’s method was applied by use of chloroform : methanol : 0.88% KCl (2 : 1 : 0.75 by vol.) with a known quantity of heptadecanoic acid (17:0) as internal standard. Following extraction, total lipid extracts (TLE) were heated for 1h at 100°C with 14% boron trifluoridemethanol in methanol to transesterify lipids to fatty acid methyl esters (FAME; ester-linked fatty acids). Gas chromatography-flame ionization detection (GC-FID) was used to quantify FAME.

A Varian-430 gas chromatograph (Varian, Lake Forest, California, USA) with an Agilent capillary column (DB-23ms; 30 m · 0.25 mm i.d. · 0.25 μm film thickness) and an FID was used to analyze FAME injected in splitless mode. Injector and detector ports were set at 250°C and helium carrier gas was set at a constant flow rate of 0.7 mL/min. A specific temperature program was used during FAME elution, it was set at 50°C for 2 min, slowly increasing by 20°C/min, and held at 170°C for 1 min, then at 3°C/min and held at 212°C for 5 min to complete the run at 28 min. Retention times of authentic FAME standards (Nu-Chek Prep, Inc., Elysian, MN, USA) were used to identify peaks. Internal standard (17:0) peaks were compared to FAME peaks to calculate fatty acid concentrations, with final values expressed percent concentration in plasma.

5.3.12 Statistics

All data are presented as means ± SD (SPSS v22; IBM Corp., Armonk, N.Y., USA). Mauchley’s test of sphericity was used to assess the variance of data and where violations occurred, corrections were made using the Greenhouse-Geiser adjustment to prevent inflation of F-ratios.
Study 1 Wingate and TT data were analyzed using a paired samples t-test. Neuromuscular data were analyzed using a one-way repeated measures analysis of variance (ANOVA) with Bonferroni’s pair-wise comparison to determine differences between times. Two analyses were performed in Study 2 to examine effect of N-3 PUFA supplementation on adaptations to training (V2/3 vs. V8/9) and the subsequent effect of N-3 PUFA washout and detraining compared to baseline (V2/3 vs. V10/11). This analysis plan was selected as V10 and V11 data were exploratory and to account for the number of participants lost to follow-up after V9 (Figure 1). Wingate, plasma fatty acid composition, TT and within visit neuromuscular percent change data were analyzed using a group (2) by time (2) repeated measures ANOVA. Neuromuscular data were analyzed with a group (2) by time (4) repeated measures ANOVA. Where there was a main-effect for time, Bonferroni’s pair-wise comparison to determine differences between times. Study 2 descriptive data were analyzed using an independent samples t-test. Significance was set at p≤0.05.

5.4 Study 1 Results

5.4.1 Adaptations to SIT

In response to training, participant’s body weight decreased from 82.1 ± 12.4 kg to 81.0 ± 13.0 kg (p=0.007) with no change in body fat (13.4 ± 7.8 vs. 12.9 ± 7.8% (p=0.2).

After 2-weeks of SIT, first sprint peak power increased 21% from 710 ± 210 W to 860 ± 230 W (p=0.05) and peak power in the fourth sprint tended to be higher as well, 670 ± 160 W compared to 790 ± 180 W (p=0.08). Average power in the first sprint was unchanged at 645 ± 161 W compared to 655 ± 140 W (p=0.4), whereas average power in the fourth sprint increased 12.5% from 480 ± 100 W to 540 ± 110 W (p=0.03). There was no change in the percent power drop on the first or fourth sprint.
5.4.2 Global Fatigue

MVC force was not significantly different between pre-V3 and pre-V8 (Table 2).

5.4.3 Central Neuromuscular Fatigue

Quadriceps VA was not different within or between V3 and V8.

5.4.4 Peripheral Neuromuscular Fatigue

\[ t_{PT} \] was not different between pre-V3 and pre-V8, but was significantly lower after each training session \((p<0.001)\). \[ t_{RFD} \] was lower after V3 training \((p=0.02)\) but not after V8 \((p=0.1)\). \[ t_{AUC} \] was not different between pre-V3 and pre-V8 \((p=0.7)\), but was significantly lower after each training session \((p=0.001)\). There were no differences within or between V3 and V8 for \[ t_{TP} \] \((p=0.6)\) or \[ t_{HRT} \] \((p=0.6)\) (data not shown). \[ q_{tw} \] was not different between pre-V3 and pre-V8 \((p=0.8)\), but was significantly lower after each training session \((p<0.02)\). There was a significant increase in LFF after V3 and V8 training sessions \((p<0.05)\). There were no differences between or within V3 and V8 for MVC RFD \((p=0.1)\) or EMD \((p=0.8)\).

5.4.5 Surface EMG

No differences were observed within or between V3 and V8 for VL EMG \((p=0.1)\) or VM EMG \((p=0.3)\) measured during MVCs. No differences were found within or between V3 and V8 for M-wave mA \((p=0.2)\), mD \((p=0.2)\) or mAUC \((p=1.0)\) (data not shown).

5.4.6 10 km TT Performance

TT time was improved by 10% after training, decreasing from 1263 ± 244 s to 1137 ± 248 s \((p<0.001)\). Mean RPM tended to increase in response training from 95 ± 10 to 100 ± 13 \((p=0.1)\), while heart rate was not different between tests \((p=0.8)\).
Table 2 – Neuromuscular changes in response to a single SIT session and 2-weeks of training.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Visit 3</th>
<th>% Change</th>
<th>Pre</th>
<th>Visit 8</th>
<th>% Change</th>
<th>Abs</th>
<th>%</th>
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<tr>
<td>MVC Force (N)</td>
<td>592±147</td>
<td>499±128</td>
<td>-14±15</td>
<td>634±212</td>
<td>559±191</td>
<td>-11±11</td>
<td>0.04</td>
<td>0.4</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>VA (%)</td>
<td>88±9</td>
<td>82±10</td>
<td>-7±4</td>
<td>87±5</td>
<td>86±5</td>
<td>-1±4</td>
<td>0.2</td>
<td>0.04</td>
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<tr>
<td><strong>Peripheral Fatigue</strong></td>
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<tr>
<td>tPF (N)</td>
<td>123±25</td>
<td>71±28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-41±22</td>
<td>121±36</td>
<td>71±17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-38±19</td>
<td>&lt;0.001</td>
<td>0.5</td>
</tr>
<tr>
<td>tAUC (AU)</td>
<td>13±4</td>
<td>7±3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-40±25</td>
<td>12±5</td>
<td>7±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-37±23</td>
<td>0.001</td>
<td>0.7</td>
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<tr>
<td>tRFD (N s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2715±578</td>
<td>1163±791&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2481±718</td>
<td>1704±749</td>
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<td>0.2</td>
</tr>
<tr>
<td>RFD (N s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2043±872</td>
<td>1212±494</td>
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<td>1553±826</td>
<td>-13±42</td>
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<td>0.1</td>
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<tr>
<td>Qtw (N)</td>
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<td>116±32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-36±18</td>
<td>199±43</td>
<td>122±26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-38±9</td>
<td>&lt;0.001</td>
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</tr>
<tr>
<td>LFF (AU)</td>
<td>0.80±0.11</td>
<td>1.0±0.22</td>
<td>31±22</td>
<td>0.78±0.11</td>
<td>0.95±0.09</td>
<td>24±17</td>
<td>0.003</td>
<td>0.4</td>
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<tr>
<td><strong>Surface EMG</strong></td>
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<tr>
<td>VL (rms)</td>
<td>0.71±0.20</td>
<td>0.78±0.31</td>
<td>6±18</td>
<td>0.66±0.20</td>
<td>0.69±0.21</td>
<td>4±13</td>
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<td>0.7</td>
</tr>
<tr>
<td>VM (rms)</td>
<td>0.59±0.21</td>
<td>0.70±0.21</td>
<td>15±40</td>
<td>0.53±0.21</td>
<td>0.61±0.21</td>
<td>20±20</td>
<td>0.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Study 1 was analyzed using a one-way ANOVA. Data is shown as mean ± SD. P-value for absolute (Abs) and percent change (%) interaction effect. ‘<sup>a</sup>’ Indicates different from Pre values (p<0.05); ‘b’ Indicates different from Pre V3; ‘c’ Indicates different from Post V3; ‘**’ Indicates different from V3 percent change. MVC, maximal voluntary contraction force; VA, voluntary activation; tPF, twitch peak force; tAUC, twitch area under the curve; tRFD, twitch rate of force development; RFD, rate of force development; Q<sub>TW</sub>, potentiated twitch force; VL EMG, vastus lateralis EMG; VM EMG, vastus medialis EMG.
5.5 Study 2 Results

Of the 30 participants that started the study, two did not complete the training protocol and one participant’s data was excluded for inconsistent effort (n=27) (Figure 1). Seven participants were lost to follow-up for visit 10 (n=20).

5.5.1 Adaptations to SIT

Neither SIT nor placebo and N-3 PUFA supplementation had any significant effect on body mass for either group; however, body composition showed a main effect for time with lower percent body fat (p=0.05).

After two weeks of SIT there were no interaction effects, main effects for group or time in first sprint peak power (p=0.2), average power (p=0.3), or minimum power (p=0.4) (Figure 2). First sprint percent power drop (fatigue index) showed a main effect for time from V3 to V8 and decreased from 59 ± 12% to 53 ± 11% (p=0.04). Fourth sprint average power showed a main effect for time from V3 to V8 and increased from 366 ± 71 to 390 ± 89 W (p=0.02).

After 4-weeks of washout there were no interaction effects, main effects for group or time in first sprint peak power (p=0.4), average power (p=0.4), minimum power (p=0.2), percent power drop (p=0.8).

5.5.2 Global Fatigue

Quadriceps MVC force showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.001) (Table 3). Pairwise comparisons showed MVC force was also lower at pre-V8 compared to pre-V3 (p=0.006) and that post-SIT MVC force was lower at after all SIT session (p<0.001). MVC force percent change from V3 to V8 showed an interaction effect as the placebo group decreased 11% and the N-3 PUFA group was unchanged (p=0.04). A main effect for time showed V10 percent change was lower than V3 (p=0.03).
5.5.3 Central Neuromuscular Fatigue

VA showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.02) (Table 3). Pairwise comparisons showed that pre-V8 tended to be lower than pre-V3 (p=0.055). VA percent change showed no difference between V3 and V8 or V3 and V10 (p>0.05).

5.5.4 Peripheral Neuromuscular Fatigue

Peripheral neuromuscular data is shown in Table 3. tPF showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.001). Pairwise comparisons showed that post-SIT tPF was lower after all SIT session (p<0.01). tPF percent change showed a main effect for time and was lower at V8 and V10 compared to V3 (p<0.02).

tRFD showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.001). Pairwise comparisons showed that post-SIT tRFD was lower at after all SIT session (p<0.01). Pairwise comparison showed that post V10 was higher than post V3 (p=0.03). tRFD percent change showed no difference between V3 and V8 but V10 was lower compared to V3 (p<0.02).

tAUC showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.03). Pairwise comparisons showed that post-SIT tAUC was lower at after all SIT session (p<0.01). tAUC percent change showed no difference between V3 and V8 or V3 and V10 (p>0.05). tTP and tHRT were not significantly different at V8 or V10 compared to V3.

MVC RFD showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.01). Pairwise comparisons showed that post-SIT Qtw was lower at after all SIT V3 and V8 only (p<0.01). RFD percent change at V8 or V10 was not significantly different from V3.

Qtw showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.001). Pairwise comparisons showed that pre-V8 was lower than pre-V3 and post-SIT Qtw was lower after all SIT session (p<0.01). Qtw percent change showed a main effect for time and was lower at V8 and V10 compared to V3 (p<0.02).
LFF showed a main effect for time from V3 vs. V8 and V3 vs. V10 (p<0.001). Pairwise comparisons showed that post-SIT LFF was higher after all SIT session (p<0.03). LFF percent change at V8 and V10 was not significantly different from V3.

5.5.5 Surface EMG

VL EMG showed a main effect for time between V3 and V10 only (p=0.001) (Table 3). Pairwise comparisons showed pre-V10 was lower than pre-V3 (p=0.02). Percent change showed no difference between groups or times. VM EMG and percent change were not significantly different between groups or times (p>0.05).

There was no interactions, time or group effects for mA and mD. mAUC showed a main effect for time between V3 and V8 (p=0.04) and V3 and V11 for percent change (p=0.02) (data not shown).

5.5.6 Blood Lactate

Blood lactate showed no difference between groups but a main effect for time, increasing at V3 from 1.9 ± 0.8 mmol L⁻¹ to 15.1 ± 2.7 mmol L⁻¹ and at V8 from 1.9 ± 0.5 mmol L⁻¹ to 14.5 ±3.3 mmol L⁻¹ (p<0.001). At V10 lactate increased from 2.0 ± 0.4 mmol L⁻¹ to 13.7 ± 5.5 mmol L⁻¹ (p<0.001).
Figure 2 – Study 2 peak, average and percent power drop in the first sprint of each training session. Placebo and N-3 PUFA groups are combined. Values are mean ± SD. Main effect for time for percent power drop (* p<0.05).
**Table 3** – Neuromuscular changes in response to a single SIT session, 2-weeks of training with placebo or N-3 PUFA supplementation and follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>% Change</th>
<th>Pre</th>
<th>Post</th>
<th>% Change</th>
<th>P-Interaction</th>
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<tr>
<td></td>
<td>N-3 PUFA</td>
<td></td>
<td></td>
<td>Placebo</td>
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<td>Global Fatigue</td>
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<tr>
<td>MVC Force (N)+</td>
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<tr>
<td>V3</td>
<td>579 ± 124</td>
<td>415 ± 129</td>
<td>-29 ± 16</td>
<td>598 ± 137</td>
<td>440 ± 86</td>
<td>-24 ± 12</td>
<td>0.9 0.03</td>
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<tr>
<td>V8</td>
<td>516 ± 94</td>
<td>408 ± 10a</td>
<td>-18 ± 12</td>
<td>536 ± 94</td>
<td>425 ± 83</td>
<td>-24 ± 11*</td>
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<tr>
<td>V10</td>
<td>544 ± 75</td>
<td>453 ± 103</td>
<td>-18 ± 15</td>
<td>519 ± 101</td>
<td>418 ± 126</td>
<td>-20 ± 16*</td>
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<tr>
<td>Central Fatigue</td>
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<tr>
<td>VA (%)+</td>
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<tr>
<td>V3</td>
<td>91 ± 4</td>
<td>89 ± 6</td>
<td>-2 ± 8</td>
<td>89 ± 7</td>
<td>85 ± 7</td>
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<td>V8</td>
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<tr>
<td>V10</td>
<td>87 ± 8</td>
<td>83 ± 7</td>
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<td>79 ± 10</td>
<td>-8 ± 8</td>
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<td>Peripheral Fatigue</td>
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<tr>
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<tr>
<td>V3</td>
<td>106 ± 25</td>
<td>52 ± 23a</td>
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<td>122 ± 32</td>
<td>54 ± 23a</td>
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<td>101 ± 38</td>
<td>57 ± 28a</td>
<td>-48 ± 24</td>
<td>106 ± 32</td>
<td>65 ± 34a</td>
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<td>14 ± 7</td>
<td>7 ± 5a</td>
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<td>V10</td>
<td>12 ± 5</td>
<td>8 ± 6a</td>
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<td>13 ± 5</td>
<td>7 ± 4a</td>
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<td>tRFD (N s⁻¹)+</td>
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<tr>
<td>V3</td>
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<tr>
<td></td>
<td>V3</td>
<td>V8</td>
<td>V10</td>
<td>Qtw (N)+</td>
<td>V3</td>
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<tr>
<td>V3</td>
<td>1622 ± 596</td>
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<tr>
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<td>1544 ± 636</td>
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<tr>
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<td>1628 ± 746</td>
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<td>1391 ± 643</td>
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<tr>
<td>V3</td>
<td>184 ± 47</td>
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<td>200 ± 34</td>
<td>101 ± 25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-49 ± 13</td>
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</tr>
<tr>
<td>V8</td>
<td>156 ± 46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99 ± 36&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>176 ± 38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107 ± 38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-36 ± 13&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.7</td>
</tr>
<tr>
<td>V10</td>
<td>179 ± 34</td>
<td>118 ± 52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-38 ± 21&lt;sup&gt;*&lt;/sup&gt;</td>
<td>180 ± 33</td>
<td>113 ± 39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-38 ± 13&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>LFF (AU)+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3</td>
<td>0.78 ± 0.10</td>
<td>1.10 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39 ± 35</td>
<td>0.77 ± 0.12</td>
<td>1.00 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32 ± 26</td>
<td>0.7</td>
</tr>
<tr>
<td>V8</td>
<td>0.82 ± 0.13</td>
<td>0.97 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20 ± 17</td>
<td>0.79 ± 0.11</td>
<td>0.95 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24 ± 31</td>
<td>0.7</td>
</tr>
<tr>
<td>V10</td>
<td>0.74 ± 0.05</td>
<td>0.96 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30 ± 15</td>
<td>0.77 ± 0.09</td>
<td>0.96 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24 ± 13</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Surface EMG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VL (rms)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3</td>
<td>0.70 ± 0.22</td>
<td>0.69 ± 0.22</td>
<td>-7 ± 15</td>
<td>0.70 ± 0.25</td>
<td>0.70 ± 0.25</td>
<td>3 ± 12</td>
<td></td>
</tr>
<tr>
<td>V8</td>
<td>0.63 ± 0.19</td>
<td>0.63 ± 0.22</td>
<td>4 ± 25</td>
<td>0.69 ± 0.29</td>
<td>0.65 ± 0.28</td>
<td>-2 ± 24</td>
<td>0.4</td>
</tr>
<tr>
<td>V10+</td>
<td>0.60 ± 0.18</td>
<td>0.55 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5 ± 16</td>
<td>0.43 ± 0.26</td>
<td>0.48 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2 ± 33</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>VM (rms)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3</td>
<td>0.49 ± 0.13</td>
<td>0.55 ± 0.19</td>
<td>13 ± 18</td>
<td>0.64 ± 0.24</td>
<td>0.70 ± 0.25</td>
<td>36 ± 97</td>
<td></td>
</tr>
<tr>
<td>V8</td>
<td>0.54 ± 0.10</td>
<td>0.58 ± 0.58</td>
<td>1 ± 19</td>
<td>0.52 ± 0.12</td>
<td>0.58 ± 0.23</td>
<td>20 ± 31</td>
<td>0.1</td>
</tr>
<tr>
<td>V10</td>
<td>0.65 ± 0.29</td>
<td>0.68 ± 0.30</td>
<td>5 ± 11</td>
<td>0.53 ± 0.24</td>
<td>0.52 ± 0.22</td>
<td>3 ± 24</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Data were analyzed using a group (2) by time (4) repeated measures ANOVA comparing visit 3 vs. visit 8 and visit 3 vs. visit 10. Percent change was analyzed using a group (2) by time (2) repeated measures ANOVA. Data is shown as mean ± SD. P-value for absolute (Abs) and percent change (%) interaction effect. ‘+’ Indicates main effect for time (p<0.05); ‘a’ Indicates different from Pre values (p<0.05); ‘b’ Indicates different from Pre V3; ‘c’ Indicates different from Post V3; ‘**’ Indicates different from V3 percent change. MVC, maximal voluntary contraction force; VA, voluntary activation; tPF, twitch peak force; tAUC, twitch area under the curve; tRFD, twitch rate of force development; RFD, rate of force development; Q<sub>TW</sub>, potentiated twitch force; VL EMG, vastus lateralis EMG; VM EMG, vastus medialis EMG.
5.5.7 Plasma N-3 PUFA

Plasma results are shown in Table 4, absolute concentrations are shown in Appendix I. Between V3 and V8, there was an interaction effect for total plasma N-3 PUFA and total HUFA concentration with an increase in the N-3 PUFA group and no change in placebo (p=0.04, p=0.05). Eicosapentaenoic acid (EPA) showed an interaction effect with the N-3 PUFA group increasing 80% and placebo group decreasing 11% at visit 8 (p=0.003). Docosapentaenoic acid (DPA) did not quite show an interaction effect (p=0.06) but a main effect for time was present with the N-3 PUFA and placebo groups increasing 18% and 6%, respectively (p=0.001). There was an interaction effect for docosahexaenoic acid (DHA) with the N-3 PUFA group increasing 50% compared to no change in the placebo group (p<0.001). There was no change in total N-6 PUFA or arachidonic acid (ARA) concentration; however, N-6 PUFA:N-3 PUFA showed an interaction effect with a 21% decrease in the N-3 PUFA group.

At V10, no plasma N-3 or N-6 PUFA measures were different from V3 except a main effect for time was still present for DPA as N-3 PUFA and placebo were elevated 20% and 10% respectively (p=0.03).
Table 4 – Study 2 fatty acid composition (%) of plasma.

<table>
<thead>
<tr>
<th></th>
<th>V3</th>
<th>V8</th>
<th>V10</th>
<th>P Interaction</th>
<th>Placebo</th>
<th>N-3 PUFA</th>
<th>P Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.9 ± 0.4</td>
<td>0.003</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>DPA</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1*</td>
<td>0.06</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td>DHA</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>1.5 ± 0.4</td>
<td>&lt;0.001</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Total N-3</td>
<td>3.2 ± 0.5</td>
<td>3.2 ± 0.6</td>
<td>3.2 ± 0.6</td>
<td>3.9 ± 0.8</td>
<td>0.04</td>
<td>3.2 ± 0.7</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>ARA</td>
<td>5.8 ± 0.9</td>
<td>5.7 ± 1.1</td>
<td>5.7 ± 1.2</td>
<td>6.0 ± 1.1</td>
<td>0.3</td>
<td>5.6 ± 1.5</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>Total N-6</td>
<td>36.9 ± 3.9</td>
<td>37.3 ± 3.9</td>
<td>35.5 ± 3.0</td>
<td>36.5 ± 3.3</td>
<td>0.7</td>
<td>37.2 ± 3.6</td>
<td>35.6 ± 4.0</td>
</tr>
<tr>
<td>Total HUFA</td>
<td>9.4 ± 1.2</td>
<td>9.6 ± 1.8</td>
<td>9.2 ± 1.4</td>
<td>10.5 ± 1.6</td>
<td>0.05</td>
<td>9.2 ± 1.9</td>
<td>9.3 ± 1.7</td>
</tr>
<tr>
<td>N-6 : N-3</td>
<td>11.9 ± 2.5</td>
<td>11.8 ± 2.3</td>
<td>11.5 ± 2.1</td>
<td>9.3 ± 1.2</td>
<td>0.01</td>
<td>11.9 ± 2.1</td>
<td>10.9 ± 2.7</td>
</tr>
</tbody>
</table>

Change in plasma fatty acid percent composition was analyzed using a group (2) by time (2) repeated measures ANOVA comparing visit 3 vs. visit 8 and visit 3 vs. visit 10. Data is shown as mean ± SD. * indicates main effect for time (p<0.05).
5.5.8 10 km TT Performance

There was a main effect for time as TT time decreased from V2 to V9 by -8% and for the N-3 PUFA and placebo groups respectively (p<0.001) (Figure 4). Comparison of V2 and V11 showed a main effect for time with a decrease of -10% for both groups (p=0.001).

Cycling cadence showed a main effect for time as mean RPM increased from V2 to V9 (p=0.02) (Figure 4). At V11 RPM tended to be higher than V2 (p=0.06). Mean heart rate was not different between groups or time at V9 (p=0.4) or V11 (p=0.2).

Figure 3 – Study 2 10 km time trial performance. Overall time is displayed on the primary axis. Mean cycling cadence (RPM) is displayed on the secondary axis. * Cycling time different from Visit 2 (p<0.05). + Cadence different from Visit 2 (p<0.05).
5.6 Discussion

These studies are the first to report changes in neuromuscular function following acute Wingate-based SIT and investigate the role of neuromuscular adaptations in overall adaptations to SIT. Findings from Study 1 and 2 demonstrate that acute SIT causes neuromuscular fatigue primarily from peripheral factors. Two-weeks of SIT does not appear to promote measurable neuromuscular adaptations, and in fact this type of training appears to cause residual neuromuscular fatigue. However, we did observe cycling specific training adaptations measured from Wingate variables and improved 10 km TT performance in both studies that might have been the result of previously described metabolic adaptations [18,21,184].

5.6.1 Neuromuscular Adaptations to SIT

Findings from Study 1 and 2 indicate that two-weeks of SIT, with or without N-3 PUFA supplementation, does not stimulate neuromuscular adaptations as measured my quadriceps MVC force, contrary to our hypotheses. A single session of SIT was a potent stimulator of neuromuscular fatigue, eliciting a -14-29% change in MVC force, which is similar to the -18% change reported by Fernandez et al. [61] after two Wingates. Surprisingly there was no evidence of central neuromuscular fatigue in the present studies; however, peripheral neuromuscular fatigue was pervasive as evident from lower resting twitch characteristics including tPF (-41-56%) and tAUC (-40-60%), and Qtw (-36-49%). Here we report Qtw peripheral fatigue beyond the previously suggested critical threshold of ~35% after constant cycling exercise [60,165,166].

It is possible that the intermittent nature of SIT training, compared to constant cycling, allowed the metabolic stress within the muscles to partially resolve between each bout. Thus enabling participants to continue training at maximal effort while allowing fatigue to progressively increase after each sprint. We measured a significant increase in blood lactate in
response to training; however, it is likely that metabolic stress from multiple factors including the accumulation of H+ and inorganic phosphates, altered calcium balance and glycogen depletion were involved in the manifestation such high peripheral neuromuscular fatigue.

Despite the intense nature of the SIT protocol and acute neuromuscular fatigue measured during the first training session, neuromuscular adaptations to SIT at V8 were not apparent in either study. Study 2 participants showed residual training induced neuromuscular fatigue in pre-V8 neuromuscular testing. Residual peripheral neuromuscular fatigue was also present as measured from Qtw. Given this residual fatigue it is surprising that there was no low force frequency fatigue from training. Neuromuscular measurement after V8 training showed similar global and peripheral neuromuscular fatigue as measured after V3 training. Percent change in Qtw was significantly less than that measured at V3; however, we do not interpret this as a training adaptation given the pre-training fatigue.

The exploratory endpoint of V10 showed that participants’ neuromuscular function returned to pre-training baseline. Training induced global and peripheral fatigue was attenuated in both groups as demonstrated by MVC and Qtw suggesting SIT induced neuromuscular adaptations might have been realized during the post-training period. Previously observed increases in glucose transporter 4 and cytochrome C oxidase subunit-4 during six-weeks of SIT are maintained up to six-weeks post-training [21]; however, the observed increase in MCT transporters that regulate muscle lactate and H+ are only maintained one-week post-training [21]. Therefore the attenuated neuromuscular fatigue observed at V10 might be the result of both neural and metabolic adaptations to SIT.
5.6.2 N-3 PUFA Supplementation Does Not Alter Neuromuscular Adaptations to SIT

Plasma N-3 PUFA concentrations increased in response to two-weeks of supplementation and subsequently decreased over the four-weeks after supplementation (Table 4). Previous reports have suggested N-3 PUFA supplementation can enhance neuromuscular adaptations to exercise training in well-trained males [138] and previously sedentary elderly females [26]. From these studies and others, N-3 PUFAs have been suggested to enhance adaptations to exercise through a variety of mechanisms including increased acetylcholine concentration and acetylcholinesterase activity at the neuromuscular junction [158]; altered membrane dynamics from increased N-3 PUFA incorporation that could increase nerve conduction velocity [14]; or improved peripheral blood flow [190]. In the present study, N-3 PUFA supplementation had no effect on adaptations to SIT or detraining. This might have been the result of the supplementation duration used in the present study. Previous findings have suggested that three-weeks of N-3 PUFA supplementation is the minimum duration to promote any neuromuscular ergogenic effects [138]. Alternatively, the intensity of training could have overwhelmed the potential influence of N-3 PUFA as was observed in recent evaluation of beta-alanine on adaptations to SIT [185]. As such, future SIT investigations could evaluate a higher N-3 PUFA supplementation dose during a longer period of training.

5.6.3 SIT Adaptations are Cycling Specific

SIT training data indicates participants in Study 1 improved cycling force generating capacity (peak power) whereas Study 2 participants showed improvements in muscle endurance (percent power drop). Previous investigation of SIT has demonstrated an improvement in cycling performance more consistent with Study 1 as a result of increased muscle glycogen
storage and metabolic capacity [18]; however these metabolic adaptations along with increased muscle MCT concentration could also enhance cycling performance as observed in Study 2.

Participants in both studies decreased 10 km TT time (-8-12%) after two-weeks of SIT, which is consistent with previous findings [3]. After four-weeks of detraining TT time was still improved (-10%) compared to V2, which is consistent with SIT-associated maintenance of glucose transporter 4 and cytochrome C oxidase subunit-4 during detraining [21]. Improved TT performance was related to higher cycling cadence, which provides some evidence of cycling specific neuromuscular adaptations. However, the metabolic cost of higher cadence cycling is fueled by carbohydrate-based metabolism [176]. Thus suggesting that cycling specific neuromuscular and metabolic adaptations might have occurred in concert to improve TT performance.

5.7 Conclusions

Findings from these studies show that a single SIT session causes peripheral neuromuscular fatigue. Furthermore, two-weeks of SIT with or without N-3 PUFA supplementation does not enhance neuromuscular function measured by change quadriceps MVC force, contrary to our hypothesis. The residual neuromuscular fatigue observed from training in Study 2 suggests neuromuscular adaptations may not have been realized without a post-training recovery period. N-3 PUFA supplementation increased plasma concentration of EPA, DPA and DHA but did not significantly enhance or impair adaptations to training from similar improvements in 10 km TT performance between studies and groups. It appears that the stress and neuromuscular fatigue from training were more pervasive than the previously reported ergogenic effect of N-3 PUFA supplementation during prolong training [26]. As such, two-weeks of SIT training does improved 10 km cycling TT performance; however, previously
reported metabolic adaptations to SIT appear to have been the primary contributor to these cycling adaptations with neuromuscular adaptations potentially playing a secondary role.
6 21 Days of Seal Oil Omega-3 Fatty Acid Supplementation Improves Aspects of Neuromuscular Function and Performance in Male Athletes Compared to Olive Oil Placebo


Contribution: As first author I designed the study, performed 90% of the data collection, performed all the data analysis and drafted the manuscript.

This study addressed objective 3 of the thesis and determined the effect of short-term N-3 PUFA supplementation on neuromuscular adaptations to training in well-trained males. Based on the findings from chapter 5, this study provided participants with a longer supplementation period. Participants recruited for the study were all athletes actively training in and competing in a sport. As such, they continued with their individual training throughout the study.
6.1 Abstract

Omega-3 polyunsaturated fatty acids (N-3) are essential nutrients for human health and integral components of neural tissues. There is evidence that N-3 supplementation may benefit exercise performance, however, no study has investigated the ergogenic potential of N-3 supplementation. Our objective was to determine the effect of short-term N-3 supplementation on neuromuscular-function and physical-performance in well-trained athletes. Male athletes (n=30), 25 years (SD 4.6), training 17 h wk\(^{-1}\) (SD 5) completed this randomized, placebo-controlled, parallel-design study. At baseline a blood sample was collected, maximal voluntary isometric contractions (MVC) with electromyography (EMG) recordings were measured, and participants underwent various performance tests including a Wingate test and 250 kJ time trial (TT) followed by repeated MVC and EMG measurement. Participants were then randomly assigned to receive N-3 (5ml seal oil, 375 mg EPA, 230 mg DPA, 510 mg DHA) or placebo (5ml olive oil) for 21-days after which baseline testing was repeated. The magnitude-based inference approach was used to estimate the probability that N-3 had a beneficial effect on neuromuscular-function and performance of at least ±1%. Data are shown as mean ± 90% confidence-interval. Plasma EPA was higher on N-3 than placebo (p=0.004) but the increases in DPA and DHA were not significant (p=0.087, p=0.058). N-3 supplementation had an unclear effect on MVC force (4.1 ± 6.6%) but increased vastus lateralis EMG by 20 ± 18% vs. placebo (very likely beneficial). N-3 supplementation reduced Wingate percent power drop by 4.76 ± 3.4% vs. placebo (very likely beneficial), but the difference in TT performance was unclear (-1.9 ± 4.8%). Our data indicate that N-3 PUFA supplementation improved peripheral neuromuscular function and aspects of fatigue with an unclear effect on central neuromuscular function. Clinical trial registration NCT02014233.
6.2 Background

The ability of skeletal muscles to generate force and resist fatigue is essential to sport performance. Training adaptations within the neuromuscular and skeletal muscle systems modulate muscle force generating capacity and the resistance to fatigue. Much investigation has occurred to determine the effect of nutritional supplements on the adaptations of skeletal muscle to strength training (e.g. protein supplementation) and methods to enhance endurance performance (e.g. carbohydrate loading). To date, however, there has been limited investigation on the effect of nutritional supplements on the neuromuscular system.

Central and peripheral nerves are comprised of fatty acids which are predominantly polyunsaturated [11]. Omega-3 (N-3) polyunsaturated fatty acids (PUFAs) are an integral component of neurons, nerve endings, myelin and muscle membranes [11]. N-3 PUFAs are essential nutrients which must be provided by the diet due to the inability of the body to synthesize them endogenously. The shortest N-3 PUFA is alpha linoleic acid (ALA; 18:3) found in seeds and nuts. Eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22-6) are longer chain N-3 PUFAs found in marine sources (e.g. fatty fish, seal). Studies on the effects of N-3 PUFA supplementation have predominantly focused on their potential ability to reduce cardiovascular risk factors [16]; however, there is growing evidence that N-3 PUFAs might enhance neural function [14,157] and adaptations to exercise [26,141]. Deficiencies in dietary N-3 PUFAs lead to reduced Na⁺/K⁺ ATPase activity and higher stimulation intensity for signal conduction [11]. In contrast, N-3 PUFA supplementation in different clinical and applied settings has enhanced nerve conduction velocity [14], membrane fluidity, sensitivity to acetylcholine [158] and also reduced post-exercise inflammation [137,141].

The effect of N-3 PUFA supplementation on neuromuscular and physical adaptations to resistance training was studied in postmenopausal sedentary women who were randomly
assigned to one of 3 groups: N-3 PUFA supplementation for 60-days followed by 90-days resistance training with N-3 PUFA supplementation (2 g d\(^{-1}\) of fish oil), resistance training with N-3 PUFA supplementation or resistance training with no supplementation [26]. Supplementation without training over 60-days had no effect on quadriceps maximal voluntary contraction force or EMG. After training all groups showed improvements in neuromuscular function and physical test; however, the N-3 PUFA groups showed significantly greater quadriceps peak torque and muscle EMG activation and the time delay between increased muscle electrical activity and contraction onset was decreased. These findings provide evidence of the ergogenic potential of this nutritional supplement. Since Lui et al. [191] found that N-3 PUFA were incorporated into muscle membrane after 21-days of supplementation, we hypothesized that 21-days of N-3 PUFA supplementation would enhance neuromuscular function and physical performance.

Therefore, our objective was to determine if short-term N-3 PUFA supplementation has an ergogenic effect through adaptations in the neuromuscular system. The primary endpoint of this study was change in quadriceps maximal voluntary contraction force as this directly affects performance. The secondary endpoints were change in muscle activation during quadriceps maximal voluntary contractions and a series of performance tests.

### 6.3 Methods

Thirty-one healthy male athletes were recruited for this parallel design, placebo controlled study and 30 completed the study. The change in maximal voluntary contraction force between the N-3 PUFA and control group from Rodacki et al. [26] were used to calculate the necessary sample of the 30 participants. A study overview is shown in Figure 1. Participant’s descriptive characteristics are given in Table 1. All competed in summer Olympic sports (e.g. rowing, sailing, triathlon, running) that require well developed strength-endurance and
endurance. Participants were screened for eligibility based on sport specific competitive history (>2 years), training hours per week (>12h), performing back squats as part of their regular training and not consuming any form of N-3 PUFA supplement nor consuming fish ≥ 3 times per week for 4-weeks prior to beginning the study. The PAR-Q exercise readiness questionnaire was used for medical screening (www.csep.ca/english/view.asp?x=698). All participants were informed of the study procedure and provided written consent. Approval for this project was obtained from the University of Toronto Research Ethics Board.

6.3.1 Pre-experimental Procedures

Height, weight and body fat percentage (Omron Fat Loss Monitor, model HBF-306CAN, Omron Healthcare, Bannockburn, Illinois) were measured wearing lightweight shorts. After a 10-minute cycling warm-up at 80 watts, participants were fitted to a custom-made isometric dynamometer. Velcro straps were used to restrain the chest and hips. Quadriceps muscle force was measured from a load cell (SM-500-I9; Durham Instruments, Pickering, Canada) attached in series to the malleolus of the right ankle. The knee and trunk-thigh angle was fixed at 90°. Force measurements were recorded using a PowerLab 8/35 (ADInstruments, Colorado Springs, CO).

6.3.2 Neuromuscular Testing

6.3.2.1 Electrical Stimations

Stimulations of the femoral nerve were delivered from a high-voltage (400 V) constant current stimulator (Biopac, BSLSTMA, Santa Barbara, California), controlled by a custom designed program (LabChart 7, ADInstruments, Colorado Springs, CO, USA). A square wave, 1-ms stimulation was delivered from a cathode (10 mm diameter) (Kendall 100, Covidien, Saint-Laurant, Quebec, Canada) placed over the femoral nerve at the femoral triangle beneath the inguinal ligament. The anode (5 x 10 cm, DJO, Vista, CA, USA) was on the lower portion of the
gluteal fold opposite to the cathode. Determination of optimal stimulation intensity (67.6 ± 14.4 mA; range 41.5 – 89.6) was achieved by increasing stimulation intensity until a further 5 mA increase did not alter peak quadriceps twitch force and vastus lateralis M-wave amplitude.

6.3.2.2 Maximal Voluntary Contractions

Maximal voluntary isometric contraction (MVC) was used to determine peak muscle force from the dominant quadriceps. During each neuromuscular testing session, three 5-second MVCs were performed, each separated by 1-minute rest. Participants were strongly encouraged during each trial. The best MVC was used for measurement of peak muscle force. The rate of force development (RFD) was calculated for each MVC and averaged. This was performed using a custom analysis program (Matlab 6.0; Mathworks Inc.) and defined as the slope of the force time curve between 20% and 80% of peak force [26].

6.3.2.3 Maximal Quadriceps Activation

A superimposed high-frequency doublet (100 Hz; 10 ms inter-stimulus interval) was given at 2.5-seconds into the MVC and at 5-seconds after the contraction with the muscle in a potentiated state. Stimulation intensity was 120% of the measured optimal stimulation intensity to ensure supra-maximal stimulation during the MVCs. The ratio of the amplitude of the superimposed twitch during the MVC over the potentiated twitch was used to calculate voluntary activation as follows:

\[ VA(\%) = \left(1 - \frac{\text{superimposed twitch}}{\text{potentiated twitch}}\right) \times 100 \]

6.3.2.4 Electromyographic Recordings

Skin on the vastus lateralis (VL) and the patella was shaved free of hair, lightly abraded with sandpaper and cleaned with alcohol to ensure low impedance \((Z < 5 \, \text{k}\Omega)\). VL EMG signal was recorded using Ag-AgCl electrodes (Kendall 100, Covidien, Saint-Laurant, Quebec, Canada;
inter-electrode distance of 25 mm) place lengthwise over the mid-belly of the muscle with the reference electrode placed over the patella [171]. EMG measurements were recorded at 2 kHz, using PowerLab 8/35 (ADInstruments, Colorado Springs, CO, USA). EMG signal was amplified with a Dual BioAmp amplifier (ADInstruments, Colorado Springs, CO, USA; bandwidth frequency 10-500 Hz input impedance 200 MΩ, common mode rejection ratio = 85 dB, gain = ±1%), transmitted to a PC and analyzed using a custom analysis program (Matlab 6.0; Mathworks Inc.).

Muscle activation was determined by calculating the EMG root mean square (RMS) from when muscle force began to plateau until immediately before the superimposed stimulation. RMS was averaged over 0.2-second periods. The mean of all three MVC was used for determining change. All RMS values were normalized to the pre-Visit 1.

The electromechanical delay (EMD) was calculated from the time difference from the increase in quadriceps EMG activity and force >10 standard deviations from resting baseline. This calculation was performed in our custom MatLab analysis program.

6.3.3 Determination of Back Squat 10 Repetition Maximum

After a series of submaximal warm-up sets, participants selected a weight thought to be their 10RM max based on current training weights. If the participant thought the weight was not maximal while lifting, they were encouraged to stop and additional weight was added using the methods of McLester et al. [192]. This process continued until a weight the participant could lift for 10 repetitions was determined. To standardize squat depth, an elastic band was tied across the back of the squat rack such that participants’ gluteal muscles would touch the band when the femur was parallel to the ground. Repetition only counted when contact was made with the band and band height was measured and kept constant for all visits.
6.3.4 Maximal Oxygen Uptake

Participants’ aerobic capacity was determined using breath-by-breath gas-analysis (MetaMax 3B, CORTEX, Leipzig, Germany) during an incremental cycling test on an ergometer (Lode Excalibur, Groningen, Netherlands). Resistance was set at 50 W, 100 W and 150 W for two minutes each before increasing by 25 W per minute thereafter. VO$_2$max was determined as the highest value achieved over a 20-second period. Watt max was determined from the stage at which participants’ cadence dropped below 50 rpm.

6.3.5 Experimental Protocol

The experimental protocol consisted of two identical testing sessions separated by 21-days of supplementation. Neuromuscular testing occurred before and after a series of performance tests to examine changes in neuromuscular function, performance and neuromuscular fatigue respectively.

6.3.5.1 Visit 1

Participants returned to the lab at least 48 hours after completing the pre-experimental procedures. Participants were then weighed to determine Wingate test resistance, before warming-up on a cycling ergometer (Lode Excalibur, Groningen, Netherlands) for 10-minutes at 80 W.

Neuromuscular testing (pre-Visit 1) was then performed as described above. Next participants were allowed to stretch before performing 3 maximal squat jumps and countermovement jumps on a force plate (AccuPower ACP, AMTI, Watertown, MA, USA) to measure jump height using to the manufacturers software (AccuPower v1.6.3, AMTI, Waterdown, MA, USA).
After 5-minutes rest, participants completed as many pushups as possible in 1-minute, were given 1-minute rest and then repeated the test a second time. Participants entered a pushup position with elbows at 90° flexion and an elastic band was placed in contact with chest to denote minimum depth. 1-minute rest was given before repeating the protocol. This assessment was interpreted as a measure of upper-body strength and strength endurance.

After a 5 minute break participants began to warm-up for the back squats by performing 4-6 reps at 20%, 40%, 60%, 80% of their 10RM max before performing the maximum number of repetitions at 100% of the predetermined 10RM max squat weight.

Participants cycled against light resistance for 10 minutes before performing a 30 second Wingate test (Monark 894, Vansbro, Sweden) at 7.5% of bodyweight [193]. Peak power, average power and power drop (%) were recorded.

Participants recovered for 20-minutes with low intensity cycling before completing, a 250 kJ cycling time trial (Lode Excalibur, Groningen, Netherlands). The bike was set in a pedaling-dependent with power varied by cadence [194]. Resistance was set using 75% of maximum power reached in the VO$_2$max test according to the methods of Jeukendrup et al. [194]. Participants received no verbal feedback during the time trial regarding the distance they had covered and could only see work completed as an indication of distance travelled. A standing fan was set at the same speed to reduce thermal stress.

As soon as reasonably possible after the time trial, participants returned to the isometric dynamometer to repeat neuromuscular testing (post-Visit 1). At the completion of testing, participants were given their randomly assigned supplement in a sealed opaque envelope.

6.3.5.2 Visit 2

Participants were asked to refrain from training for 24 hours prior, to control for athletic recovery. All procedures were identical to Visit 1. Participants were not informed of their
performance scores from visit 1 to ensure that their performance was not artificially enhanced through mental self-competition.

**Figure 2** - Experimental design.
6.3.5.3  Training and Dietary Controls

Participants were recruited during their regular training season to ensure constant levels of activity. For the duration of the study, participants were instructed to maintain normal level of training, with the exception of resting for 24-h prior to each testing session. This was confirmed during the second visit and changes recorded. Participants were instructed to refrain from consuming >3 servings of fish per week, in addition to any non-supplied omega-3 supplements. At the beginning of Visit 1, participants completed a 24-h diet record. This was scanned and sent back to each participant prior to Visit 2 to ensure the same foods were eaten prior to each test. All testing took place at the same time of day to avoid temporal variation.

6.3.5.4  Omega-3 Supplementations

For 21-days, participants consumed 5 mL of seal oil N-3 PUFA (5000 mg N-3 PUFA, 375 mg EPA, 230 mg DPA, 510 mg DHA and with 1000 IU vitamin D3) (Auum Inc., Timmons, On) or 5 mL olive oil (Bertolli, Mississauga, Canada) with 1000 IU vitamin D added. Participants were instructed to take 2 – 2.5 mL servings orally twice daily, and to let the oil remain in the mouth for 1-minute before swallowing to allow for sublingual absorption [87].

Seal oil was chosen as the source of experimental N-3 supplement for this study because mammalian (seal) triacylglycerol molecules have N-3 PUFA fats primarily in the sn-1 and sn-3 positions, as opposed to the sn-2 position of fish oil N-3 PUFA [10,86]. Fats in the sn-3 position are preferentially cleaved by sublingual lipases, and the sn-1 fat is cleaved in the small intestine, while the sn-2 fatty acid is left for later esterification [87,88]. These structural differences are thought to enable chylomicrons and chylomicron remnants containing seal oil N-3 PUFA to have a higher rate of clearance from the blood compared to fish oil intake [89], potentially increasing bioavailability.
6.3.5.5 Randomization and Concealment

Participants were assigned a sequential study ID based on their enrollment. Treatment assignment (N-3 PUFA or placebo) was determined from a computer generated random number sequence. Supplement bottles were then sealed in opaque envelopes and labeled with participant ID by a researcher not involved in the study. Participants received their supplement at the end of visit 1. Study researchers were unblinded after plasma N-3 analysis was completed.

6.3.5.6 Blood Sampling

A resting 8 mL blood sample from the antecubital vein was collected into K3-EDTA Vacutainer tubes (BD Vacutainer, Mississauga, Canada) at the beginning of Visit 1 and 2. Samples were centrifuged (3000 rpm for 15 minutes at 4°C). Plasma samples were collected and stored at -80°C for later analysis of plasma N-3 PUFA concentration.

6.3.5.7 Lipid Extraction and Gas Chromatography-Flame Ionization Detection

All lipid extraction techniques have been previously described by Chen et al. [189]. To extract total lipids, Folch, Lees, and Sloane Stanley’s method was applied by use of chloroform : methanol : 0.88% KCl (2 : 1 : 0.75 by vol.) Total lipid extraction (TLE) with known quantity of heptadecanoic acid (17:0) was heated for 1h at 100°C with 14% boron trifluoridemethanol to convert to fatty acid methyl esters (FAME; ester-linked fatty acids). Gas chromatography-flame ionization detection (GC-FID) was used to quantify FAME.

A Varian-430 gas chromatograph (Varian, Lake Forest, CA, USA) with a Varian FactorFour capillary column (VF-23ms; 30 m · 0.25 mm i.d. · 0.25 lm film thickness) and an FID was used to analyze FAME injected in splitless mode. Injector and detector ports were set at 250°C and helium carrier gas was set at a constant flow rate of 0.7 mL/min. A specific
temperature program was used during FAME elution, it was set at 50°C for 2 min, slowly
increasing by 20°C/min, and held at 170°C for 1 min, then at 3°C/min and held at 212°C for 5
min to complete the run at 28 min. Retention times of authentic FAME standards (Nu-Chek
Prep, Inc., Elysian, MN, USA) were used to identify peaks. Final values are expressed percent
concentration in plasma. Protocol for GC-FID was adopted from Chen et al. [189] where it is
well described.

6.3.6 Statistical Analyses

This trial was registered at clinicaltrials.gov (NCT02014233) using a planned statistical
analysis of repeated measures ANOVAs for all neuromuscular and performance measures. After
analysis had begun we became aware of the magnitude-based inferences analysis approach
[195]. The authors determined this approach was more appropriate given our sample size and
population of interest.

The standardized difference in means of neuromuscular measures was compared as
follows: pre-Visit 1 to pre-Visit 2 and pre-Visit 2 to post-Visit 2. As well the standardized
difference in means of performance tests from Visit-1 and Visit-2 were compared using pooled
standard deviation as previously described by our lab [196]. The difference between time points
was examined quantitatively using magnitude based inferences as described by Hopkins et al.
[195].

A threshold of ±1.0% was set as threshold for beneficial or harmful effects on
performance as previously described [197]. The effect of supplementation was interpreted as
unclear when the odds ratio (OR) of benefit ≥ harm was less than 66 as determined by >25%
possible beneficial effect and <0.5% unacceptable risk of harm according to the following
formula [197].

\[
\text{OR} = \frac{\%\text{benefit}}{100 - \%\text{benefit}} / \frac{\%\text{harmful}}{100 - \%\text{harmful}}
\]
The inference was generated from the confidence limits derived from the p-value associated with the effect and the magnitude of the difference in means such that a probability that the true value falls within the 90% confidence interval corresponds with a qualitative inference of a 0% “most unlikely” effect; 0.5% “very unlikely”; 5% “unlikely”; 25% possibly; 75% likely; 95% likely; 99.5% very likely; 100% most likely. Where confidence intervals included values that were both positive and negative, OR are used to interpret if findings are meaningful and discussed further. Raw data is presented as mean ± SD for N-3 PUFA and PLA groups respectively. Interpreted data is presented as mean ± 90% CI with corresponding qualitative inference and OR where appropriate.

Changes in blood N-3 PUFA concentration were analyzed using a repeated measures-ANOVA (Group x Time) and differences in descriptive statistics were analyzed using an unpaired t-test (SPSS v21, Armonk, NY). Significance was set at $p < 0.05$.

6.4 Results

One participant was unable to complete Visit 2 due to competition related travel. His data was removed from analysis and final analysis was performed with an n = 30.

When baseline characteristics were compared, the experimental N-3 PUFA and placebo groups were not different from each other.
Table 3 – Descriptive data for study participants in the omega-3 supplementation group (N-3 PUFA) and placebo group. Independent t-test determined no difference between groups.

<table>
<thead>
<tr>
<th>Measure</th>
<th>N-3 PUFA (n=18)</th>
<th>Placebo (n=12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>180.3 ± 7.5</td>
<td>180.0 ± 7.7</td>
<td>0.91</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.8 ± 8.0</td>
<td>80.2 ± 10.8</td>
<td>0.69</td>
</tr>
<tr>
<td>Age (y)</td>
<td>23.7 ± 4.9</td>
<td>26.0 ± 3.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>12.4 ± 3.3</td>
<td>12.9 ± 4.4</td>
<td>0.74</td>
</tr>
<tr>
<td>VO2max (ml·kg⁻¹·min⁻¹)</td>
<td>49.3 ± 14.2</td>
<td>48.9 ± 3.4</td>
<td>0.93</td>
</tr>
<tr>
<td>10RM Squat Max (kg)</td>
<td>92.7 ± 23.2</td>
<td>97.7 ± 16.8</td>
<td>0.40</td>
</tr>
<tr>
<td>Watt max</td>
<td>350 ± 47.4</td>
<td>345 ± 35.2</td>
<td>0.86</td>
</tr>
<tr>
<td>Training (h·wk⁻¹)</td>
<td>17 ± 6</td>
<td>16 ± 5</td>
<td>0.64</td>
</tr>
</tbody>
</table>
6.4.1 Neuromuscular Measurements

6.4.1.1 MVC Force

On N-3 PUFA supplement quadriceps MVC force increased from 643 ± 144 N to 670 ± 175 N (4.1%), while on placebo force increased from 677 ± 107 to 683 ± 154 (0.03%) (Figure 2). This represents an unclear change of (4.1% ± 6.6; OR = 30). When MVC force was compared from pre-Visit 2 to post-Visit 2, there was a similar decrease in force of -20.3% and -21.2% representing an unclear difference (0.95% ± 7.5; OR = 2). Similarly, there was an unclear difference in force between post-Visit 1 and post-Visit 2 (0.90% ± 8.6; OR = 2).

6.4.1.2 Rate of Force Development

Quadriceps RFD was measured during each MVC and averaged. On N-3 PUFA supplement MVC force increased from 2376 ± 709 N s\(^{-1}\) to 2522 ± 929 (7.6%), while on placebo 2101 ± 608 N s\(^{-1}\) to 2354 ± 700 (17.1%). This represents an unclear difference (9.5% ± 22; OR = 11). Comparison of RFD from pre-Visit 2 to post-Visit 2 showed a similar change of -16.2% ± 3.9 and -22.9% ± 29.6 representing an unclear difference between groups (-6.7% ± 14; OR = 15). When post-Visit 1 and post-Visit 2 were compared the N-3 PUFA group was -1.4% ± 23.6 lower and PLA was 14.6% ± 37.5 higher, representing a likely harmful effect (-16.0% ± 19.0; OR = 0).

6.4.1.3 Electromyography and Electromyography Delay

Quadriceps muscle activation was calculated from the RMS EMG signal collected from the VL and normalized to pre-Visit 1 (Figure 3). Participants on N-3 PUFA supplement increased muscle activation 9.0% ± 37.8, while on placebo EMG decreased 12.9% ± 19.4. This represents a very likely benefit (22.0% ± 20.0; OR = 763). When RMS was compared from pre-Visit 2 to post-Visit 2, N-3 PUFAs had a likely harmful effect compared to placebo (-11.3% ±
12; OR = 0), while the change between post-Visit 1 and post-Visit 2 represented an unclear difference (5.9% ± 3.9; OR = 2).

On N-3 PUFA participants’ EMD increased from 0.053 ± 0.014 s to 0.055 ± 0.019 (16.5%), while on placebo EMD was lower 0.050 ± 0.020 s to 0.042 ± 0.018 (-0.85%). This represents an unclear difference (17.3% ± 39.0; OR = 11). Comparison of EMD change between pre-Visit 2 to post-Visit 2 showed a likely beneficial effect of N-3 PUFA (-35.3% ± 35.1; OR = 361), while there was an unclear difference between post-Visit 1 and post-Visit 2 (3.6% ± 28.0; OR = 2).

6.4.1.4 Voluntary Activation

Maximal quadriceps activation was tested using the interpolated twitch technique during each MVC and averaged. On N-3 PUFA, voluntary activation increased from 91.1% ± 7.5 to 91.8% ± 5.6 (1.3%), while on placebo increased from 89.5% ± 8.9 to 92.2% ± 3.7 (4.0%). This represents an unclear change between time points (2.7% ± 6.8). Comparison of voluntary activation from pre-Visit 2 to post-Visit 2 was unclear (0.26% ± 4.7), as was post-Visit 1 to post-Visit 2 (0.050% ± 3.4).

6.4.2 Performance Measures

Changes from Visit 1 to Visit 2 are shown in Table 2 to examine both raw differences and interpreted percent changes. Both groups have similar performances in Visit 1, showing homogeneity of the sample population.

Change in the number of repetitions of back squat was compared before and after supplementation. From pre-testing 10RM determination to Visit 1, there was an increase of 3 ± 4 and 2 ± 2 repetitions performed by the N-3 PUFA and placebo group respectfully.
A change in performance was measured in the Wingate test. The N-3 PUFA group Wingate power drop was lower from Visit 1 to Visit 2, while the PLA group showed an increase in fatigue. This was very likely beneficial (4.76 ± 3.4%; OR = 6870).

In the 250 kJ TT the N-3 PUFA group mean was slower than the PLA group by 39.4 seconds. At Visit 2, the N-3 PUFA group decreased their time by 6.2 ± 131.6 seconds while the PLA group increased by 22.9 ± 65.1 seconds. With N-3 PUFA supplementation 9 participants improved time trial time by 105.7 ± 106.0 seconds, whereas 4 PLA participants improved by 39.2 ± 75.8 seconds.

6.4.3 Plasma Fatty Acid Analysis

Gas-flame chromatography performed to determine change in the percent composition of plasma fatty acids between groups (Table 3). Plasma EPA increased in the N-3 PUFA group with no change in PLA after supplementation (p=0.004). There was a trend for higher DPA and DHA after supplementation (p=0.087, p=0.058). Analysis of absolute change in plasma is shown in Appendix II.
Figure 2 – Change in quadriceps maximal voluntary contraction (MVC) force is shown as percent change from pre-visit 1. Data are shown as mean ± SD.
**Figure 3** – Change in vastus lateralis (VL) EMG RMS is shown as percent change from pre-Visit 1. * Very likely beneficial increase compared to placebo. + Very likely harmful effect compared to placebo. Data are shown as mean ± SD.
The measures Visit 1 and Visit 2 for the omega-3 supplementation (N-3 PUFA) and placebo groups. The differences are given as a percent ± 90% confidence limits and corresponding inference.

<table>
<thead>
<tr>
<th>N-3 PUFA</th>
<th>Placebo</th>
<th>ΔN-3 vs. Δ PLA (% ±90% CL)</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td>Visit 2</td>
<td>Visit 1</td>
<td>Visit 2</td>
</tr>
<tr>
<td>34.6 ± 7.7</td>
<td>34.7 ± 6.7</td>
<td>36.6 ± 3.1</td>
<td>38.0 ± 7.1</td>
</tr>
<tr>
<td>Counter Movement Jump (cm)</td>
<td>43.5 ± 9.4</td>
<td>43.6 ± 7.2</td>
<td>45.5 ± 4.7</td>
</tr>
<tr>
<td>50 ± 14</td>
<td>51 ± 13</td>
<td>49 ± 13</td>
<td>51 ± 14</td>
</tr>
<tr>
<td>23 ± 11</td>
<td>24 ± 10</td>
<td>25 ± 10</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>13 ± 4</td>
<td>15 ± 4</td>
<td>12 ± 2</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>Wingate Average Power (W)</td>
<td>841.30 ± 160.50</td>
<td>838 ± 176.84</td>
<td>835 ± 136.65</td>
</tr>
<tr>
<td>593.1 ± 95.78</td>
<td>599.7 ± 92.65</td>
<td>611.3 ± 90.69</td>
<td>616.1 ± 104.44</td>
</tr>
<tr>
<td>54.8 ± 9.54</td>
<td>54.6 ± 10.33</td>
<td>49.1 ± 5.82</td>
<td>53.0 ± 5.46</td>
</tr>
<tr>
<td>1133.2 ± 296.1</td>
<td>1126.9 ± 265.7</td>
<td>1093.8 ± 269.8</td>
<td>1116.7 ± 269.6</td>
</tr>
</tbody>
</table>
Table 3 - Fatty acid composition (%) of plasma for the omega-3 supplementation (N-3 PUFA) and placebo groups. * Significant group by time interaction. All values are mean ± SD percentages for the omega-3 supplementation group (N-3 PUFA) and placebo group (PLA). Pre, data collected prior to 21-days of supplementation. Post, data collect after 21-day of supplementation. Data are shown as mean ± SD.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>N-3 PUFA</th>
<th></th>
<th>Placebo</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>EPA (20:5 N-3)</td>
<td>0.41 ± 0.16</td>
<td>0.68 ± 0.27*</td>
<td>0.59 ± 0.34</td>
<td>0.49 ± 0.24</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>DPA (22:5 N-3)</td>
<td>0.29 ± 0.092</td>
<td>0.35 ± 0.091</td>
<td>0.31 ± 0.095</td>
<td>0.30 ± 0.055</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td>DHA (22:6 N-3)</td>
<td>1.61 ± 0.32</td>
<td>1.81 ± 0.43</td>
<td>1.65 ± 0.45</td>
<td>1.56 ± 0.042</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>α-Linolenic (18:3 N-3)</td>
<td>0.39 ± 0.12</td>
<td>0.38 ± 0.18</td>
<td>0.41 ± 0.10</td>
<td>0.36 ± 0.11</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Linoleic (18:2 n-6)</td>
<td>21.68 ± 2.85</td>
<td>21.01 ± 1.89</td>
<td>22.75 ± 3.67</td>
<td>21.93 ± 3.16</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Arachidonic (20:4 n-6)</td>
<td>8.00 ± 2.07</td>
<td>7.43 ± 1.69</td>
<td>7.42 ± 1.93</td>
<td>7.03 ± 1.97</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>
6.5 DISCUSSION

This present study sought to elucidate the effect of 21-days of N-3 PUFA supplementation on neuromuscular function and performance measures in well-trained male athletes. To our knowledge this is the first study to evaluate the effects of a N-3 PUFA supplement on athletes while measuring changes in both neuromuscular function and performance. This study found that N-3 PUFA supplementation increased muscle activation and attenuated fatigue as assessed during a Wingate test after maximal back squat exercise by reducing percent power drop.

6.5.1 Neuromuscular Function

The primary outcome of this study was change in MVC force from pre-Visit -1 to pre-Visit 2. The N-3 PUFA group had an unclear 4.1% ± 6.6 increase in MVC force, indicating that individuals can experience a beneficial or harmful change. However, VL EMG activation averaged over the three EMGs was increased 9% in the N-3 PUFA group compared to baseline, resulting in a very likely beneficial 22.0 ± 20.0 increase in muscle activation. These data suggest N-3 PUFA supplementation enhanced peripheral (neuromuscular junction to contractile apparatus) but not central (brain to neuromuscular junction) neuromuscular adaptations. In comparison, Rodacki et al. [26] showed that 2 g d⁻¹ fish oil N-3 PUFA supplementation for 90 days during resistance training in sedentary women increased muscle activation and MVC force by ~50% over training only controls. Three differences between this work and the present study was supplement duration and dose of 90 days compared to 21 days and 2 g d⁻¹ compared to 1.1 g d⁻¹, and training status of the populations. However, a longer supplementation period and/or a higher dose might be necessary for the observed increase in muscle activation to translate into a beneficial increase in muscle force in this highly trained population. These results along with
Rodacki et al. [26] provide evidence of the effect N-3 PUFA supplementation on neuromuscular function across the training spectrum and suggest a beneficial relationship between N-3 PUFA supplementation and neuromuscular adaptations to training.

6.5.2 Neuromuscular Fatigue

When we examined the neuromuscular fatigue assessed by change from pre to post Visit 2, N-3 PUFA had a mixed effect. Neuromuscular fatigue was apparent but not different between groups as MVC force was ~20% and central fatigue measured by voluntary activation was unchanged in both groups. Peripheral measures showed no difference in quadriceps rate of force development, while EMD was lower, indicating a faster activation of the contractile apparatus. Interestingly, N-3 PUFA VL EMG activation in was 10% lower compared to pre-Visit 2 testing suggesting peripheral neuromuscular fatigue. We suggest that the N-3 group performed more work during visit 2 as demonstrated by attenuated fatigue in the Wingate test and the unclear 1.96% ± 4.8 difference in 250 kJ TT performance. Regardless, VL EMG at post-Visit 2 returned to baseline levels, therefore we interpret any potential harm with caution.

6.5.3 Performance Changes

Over the 21-day intervention, all participants were instructed to continue with their normal daily training. This was an important aspect of the study intervention, as results from Rodacki et al. [26] revealed N-3 PUFA supplementation only enhanced neuromuscular function during resistance training and not during normal activities of daily living. As expected, both groups showed evidence of training adaptations during the supplementation period as the participants were all in off-season base training. In the neuromuscular assessment, MVC RFD increased similarly between groups, while the number of back squat repetitions with 10RM weight was increased ~20% from familiarization to Visit 1 and 2 respectively. The improvement in 10RM back squat repetitions caused 4.76% ± 3.4 higher fatigue during the Wingate test in the
placebo group. We did not observe any change in peak or mean Wingate power between visits, suggesting no change in central neuromuscular function; however, the attenuation of fatigue observed in the N-3 group suggests maintained peripheral neuromuscular function. This finding is consistent with the observed increase in muscle activation. Performance in the 250 kJ time trial showed an unclear difference of 1.96% ± 4.8 between N-3 PUFA and placebo. This measure was the most variable of the protocol as the participants were fatigued from previous tests despite competing in strength-endurance and endurance sports (i.e. rowing, sailing, cycling, triathlon). This was an inherent challenge of our protocol, as we wanted to measure the effect of N-3s on all tests requiring all energy systems from power (ATP) to aerobic endurance. Nevertheless, 50% of participants on N-3 PUFA improved time trial performance while only 33% on placebo improved. Future evaluation of N-3 PUFA supplementation should focus on one or two of these areas to tease out the effects, as the combination clearly increases variability of results.

6.5.4 Mechanism of Action

Our data suggests N-3 PUFA supplementation improved peripheral neuromuscular function with an unclear effect on MVC force. Animal model research has shown N-3 PUFAs can increase acetylcholine concentration and acetylcholinesterase activity at the neuromuscular junction [158]. This could increase the rate of action potential transmission across the neuromuscular junction, thereby increasing muscle activation and EMG.

N-3 PUFA supplementation is suggested to alter cellular membrane composition and fluidity [158]. This may enhance nerve conduction through lower nerve resistance or improved ion channel function from the regulation of mitogen activated kinase transcription factors [198]. In this study, N-3 PUFA supplementation may have altered muscle membrane dynamics as demonstrated by increased EMG activation. This could have enhanced muscle action potential conduction through the working muscle. Altered membrane dynamics could have mitigated
muscle damage resulting from the 10RM squat test. A reduction in damage might explain the attenuated Wingate test performance observed in the N-3 PUFA group. Similarly, reduced muscle damage could maintain muscle action potential conduction, thereby maintaining muscle excitation contraction coupling and ultimately muscle force generating capacity [199].

6.5.5 Supplement Duration

This study used a short-term supplementation period (21-days), as findings could be applied to support athlete adaptations during training camps or a pre-competition taper. Other studies with ergogenic application have demonstrated positive results in terms of reducing post-exercise inflammation [137], increasing muscle protein synthesis [117] or adaptations to training [26] using supplement periods of 4-12 weeks. While our supplementation period was shorter than other studies, it has been shown that 7 days is sufficient to increase plasma EPA concentrations [200] and attenuate muscle soreness from eccentric exercise [102]. Furthermore, 14 and 21-days of supplementation in humans [154] and pigs [191], was sufficient to increase muscle N-3 PUFA concentration.

Our supplementation protocol used a realistic N-3 PUFA supplement dose of 1115 mg d⁻¹ essential fatty acids, compared to previous N-3 PUFA intervention studies using arguably super-physiologic doses of 3000 – 8000 mg d⁻¹ [117,121,154,191]. Therefore, we feel these results have a high level of applicability to athletes and the training population, as high doses of N-3 PUFA may not be tolerable by individuals over the long term and also can be expensive.

This is one of the first exercise studies to use a seal oil N-3 PUFA supplement in contrast to the more commonly used fish oil N-3 PUFA. Seal oil N-3 PUFAs are located the sn-1/3 position of triglycerides whereas fish oil N-3 PUFAs exist in the sn-2 position. This difference has been shown to promote more rapid digestion of seal oil N-3 PUFAs leading to subsequent higher incorporation into non-hepatic tissues in rats [201]. Both placebo and experimental N-3
PUFA supplements used in this study were administered orally to allow for potential sublingual absorption of the sn-3 N-3 [87]. This method of digestion and absorption might have increased the amount of available N-3 PUFAs for incorporation into muscle and nervous tissue, as transit of N-3 PUFAs through the stomach can cause oxidation [137]. However, there has yet to be a direct comparison digestion and metabolism of fish and seal N-3 PUFA to determine if sublingual administration is superior.

6.5.6 Strengths and Limitations

Several limitations became apparent throughout this study. Using participants from an endurance training background but different sports, yielded differences in athletic abilities. While triathletes excelled at the endurance portions of the experiment (time trial), they had more variability in the 10 RM squat test. It would be advisable that future studies of a similar nature recruit athletes from the same, or similar, athletic backgrounds and focus on a specific energy system.

6.6 Conclusion

In conclusion, 21-days of N-3 PUFA supplementation showed no effect on quadriceps MVC force, the primary outcome of this trial. However, N-3 PUFA supplementation did enhance neuromuscular adaptations to training through increased quadriceps EMG activation during the MVC contraction and attenuated fatigue during a 30-second Wingate test compared to the placebo group. These data provide a basis for further investigation of the effects of N-3 PUFA supplementation on the neuromuscular system and as an ergogenic aid for trained individuals.
7 Further Discussion and Limitations

7.1 Discussion

The research findings presented in this thesis partially supports the hypothesis that N-3 PUFA supplementation will enhance neuromuscular adaptations to exercise training. Data from chapter 4 indicate that individuals of all training backgrounds experience neuromuscular fatigue during exercise; however, the extent of fatigue varies with training history. This observed impairment in neuromuscular function provides an opportunity for intervention to enhance neuromuscular function in both untrained and trained individuals.

When untrained males performed two-weeks of SIT, in chapter 5, neither the placebo nor the experimental N-3 PUFA group showed any measurable neuromuscular adaptations, specifically from increased MVC force. Given the high-intensity nature of the protocol, any potential adaptations may have been masked by residual neuromuscular fatigue from training or the supplementation period was too short. In contrast, well-trained males showed improved peripheral neuromuscular function and fatigue resistance after three-weeks of N-3 PUFA supplementation compared to placebo, in chapter 6.

When the plasma N-3 PUFA concentrations are examined between chapter 5 and 6 it is interesting to note that EPA, DPA and DHA concentrations showed a higher absolute change in the SIT study (chapter 5, table 4) after two-weeks of supplementation than in the well-trained athlete study (chapter 6, table 3). This could be the result of different baseline diets or the SIT study participants had higher compliance with supplementation than those in the well-trained athlete study because of more frequent contact with the investigators. Alternatively, the N-3 PUFAs could have been taken up by peripheral tissues, the dose and duration were not sufficient to cause an increase in blood N-3 PUFAs.
Seal oil N-3 PUFAs were used for the experiments in this thesis because of the different structure and proposed differences in bioavailability compared to fish oil [188,202]. While seal oil contains EPA and DHA in similar concentrations to fish oil, seal oil contains a higher amount of DPA. There has been limited research on the effect of DPA supplementation alone; however, it is thought that that DPA acts as a reservoir for EPA and DHA [203]. When DPA alone was given as a supplement, there was a significant increase in DPA triacylglycerol and phospholipid fractions [203], which could be incorporated directly into neural tissue or act as a precursor for the incorporation of DHA into neural membranes.

From the research presented in this thesis, N-3 PUFA supplementation has been shown to affect adaptations to training and exercise performance systemically through changes in cardiovascular, pulmonary and neuromuscular function, and within the muscle from changes in muscle protein synthesis and attenuation of muscle damage and inflammation. Figure 1 provides a summary of identified and proposed N-3 PUFA mechanisms of action.

With regards to the findings from the experiments within this thesis, the most relevant mechanism of action for N-3 PUFAs supplementation to enhance neuromuscular function appears to occur through [97,130-132] incorporation into muscle and nerve membranes. N-3 PUFA supplementation, specifically EPA, has been shown to replace N-6 PUFA AA in tissue membranes [77,158]. This replacement of AA and the incorporation of N-3 PUFAs has been shown to improve membrane dynamics [158].

In nerve tissue, improved membrane dynamics might lower conduction resistance and subsequently increase conduction velocity in the central neuromuscular system [14] possibly through enhanced Na⁺/K⁺ handling [204]. Increased nerve conduction velocity could also enhance central fatigue from the feedback of group III/IV afferents back to the brain. At the NMJ, N-3 PUFA supplementation has been shown to increase acetylcholine kinetics in an animal
model [158]. Increased acetylcholine kinetics could increase the transmission of action potentials across the NMJ, especially in a fatigued state. At the level of skeletal muscle, N-3 PUFA incorporation might increase the speed of action potential propagation along the sarcolemma and thereby increase muscle activation and maintain muscle function or mitigate fatigue [138].
Figure 1 - Proposed mechanism of action for N-3 PUFA supplementation on adaptations to training and exercise performance. a-MN, alpha motor neuron; NCV, nerve conduction velocity; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AchR, acetylcholine receptor; AchE, acetylcholinesterase; MAP, muscle action potential, SR Ca\(^{2+}\), sarcoplasmic reticulum calcium; MPS, muscle protein synthesis; mTOR, mammalian target of rapamycin; AA, amino acid; FEV1, forced expiratory volume in 1-second; CO, cardiac output; SV, stroke volume; PGI, ; SVR, systemic vascular resistance; NO, nitric oxide; ARA, arachidonic acid; COX2, cyclooxygenase 2; TNFa, tumor necrosis factor-alpha; PGE\(_2\), prostaglandin E2; CK, creatine kinase; Mb, myoglobin.
7.2 Significance of Findings

The experiments within this thesis provide a novel examination of the role of N-3 PUFA supplementation on adaptations to exercise. Initial adaptations to new exercise occur largely within the neuromuscular system and result in a rapid increase in strength [1]. As training progresses, neuromuscular adaptations begin to plateau while metabolic adaptations become the leading driver of adaptations [1]. The N-3 PUFA intervention studies within this thesis provide an examination of the effect of N-3 PUFAs during different types of training in different populations.

The SIT study presented in chapter 5 provides a novel examination of neuromuscular adaptations combined with the investigation of N-3 PUFA supplementation. There has only been one other study to investigate nutritional interventions with Wingate based SIT. Beta-alanine supplementation was found to have no effect on training adaptations or TT performance after six-weeks of training [185]. Based on the findings from the N-3 PUFA supplementation trial along with the beta-alanine findings, it appears that SIT alone might be a maximal training stimulus.

The findings presented in chapter 6 contribute to the growing body of evidence of the positive effects of N-3 PUFA supplementation for adaptations to training summarized in the systematic review in chapter 2.2. In this review N-3 PUFA supplementation was shown to improve cardiovascular function during exercise, pulmonary function in those with or without bronchoconstriction, attenuate exercise induced muscle damage and inflammation, increase muscle protein synthesis and aspects of neuromuscular function. For the first time N-3 PUFAs have been investigated as an ergogenic aid in well-trained individuals. The data indicate that after three-weeks of supplementation, well-trained individuals increased muscle activation and attenuated fatigue during sprint cycling compared to the placebo group. This is the first study to
show that individuals with highly developed neuromuscular systems can improve function with an N-3 PUFA supplement. These ergogenic effects were measured using a realistic dose of N-3 PUFA supplementation, not a supra-physiologic dose, making these findings directly translatable to all male athletes. Furthermore, these findings support the further investigation and refinement of N-3 PUFA supplementation in well-trained individuals.

7.3 Limitations

There were several limitations of the studies performed in chapters 4-6 of this thesis. A general limitation is that all experiments were conducted with male participants. This decision was made as males and females have different neuromuscular fatigue profiles. In response to cycling exercise, men show both central and peripheral neuromuscular fatigue whereas women show central fatigue [205]. Given the effect of N-3 PUFAs on peripheral neuromuscular function from chapter 6, men might benefit more from N-3 PUFA supplementation. However, the use of only men in these studies limits the application of the findings to half of the population. Future studies could examine the effect of N-3 PUFA supplementation in females or compare the effect on neuromuscular adaptations between males and females.

Nutritional supplementation studies have an inherent limitation in that participants must consume their assigned supplement per protocol and maintain a consistent diet throughout the study. The different changes in plasma N-3 PUFA concentrations between chapter 5 and 6 discussed in chapter 7.1 highlight this limitation. These studies attempted to control for participants’ habitual diet by instructing them to consume the same foods before baseline and end-point testing using a three-day diet log. While habitual intake of long chain N-3 PUFAs were screened for during enrollment, all diets contain a varying level of ALA N-3 PUFAs from various plant, nut and seed sources. This ALA can be converted to EPA and DHA N-3 PUFAs that could impact the effect of supplementation by resulting in a lower absolute change in N-3
PUFA concentration. Dietary N-6 PUFA intake was not controlled for in these studies. Dietary N-6 PUFA intake can vary and some research indicates a high intake of N-6 PUFA : N-3 PUFA might negatively impact exercise performance and health [8,144]. As such, dietary N-6 PUFA intake could act as a confounding variable.

Another limitation is the use of different experimental protocols between studies. The studies in chapter 5 and 6 investigated the effect of N-3 PUFA supplementation on neuromuscular adaptations to training over two and three-weeks respectively. The different study durations were chosen for their success in promoting adaptations to training in the respective population. SIT has been shown to elicit training adaptations after two-weeks [17,18], whereas well-trained athlete require longer training periods to achieve adaptations given their training history. It is important to note that the purpose of this thesis was not to compare effect of N-3 PUFA supplementation on the rate or absolute change of neuromuscular adaptations to training between populations, it was to examine if N-3 PUFA supplementation could enhance adaptations to training using different populations.

Another limitation to this work was the lack of consistency in testing protocols. Neuromuscular function measured in chapter 6 did not assess peripheral neuromuscular function using resting and potentiated twitches. The neuromuscular testing protocol was improved in chapters 4 and 5 to provide a more comprehensive analysis. Similarly, the cycling TT in chapter 6 used a Lode cycle ergometer to complete a 250 kJ TT whereas a Monark 839E ergometer was used in chapters 4 and 5 to complete a 10 km TT. The 250 kJ TT has been reported to be similar in duration to a 10 km TT [17]. An important difference to note between these two ergometers is that the Lode allows participants to vary their power output to change cycling speed similar to cycling on the road, whereas participants using the Monark must vary their cadence to change speed. This technical limitation is important to note as result from the Lode would better reflect
adaptations in ‘real-world cycling’; however, both tests required participants to complete a fixed amount of work or distance in the shortest time possible.

In chapter 6, nine different exercise tests were used to investigate the effect of N3 PUFA supplementation on performance. While the protocol was designed allow recovery between tests (5-minutes of recovery between jump tests, push-ups and squats, 10-minutes after squats and 20-minutes after the Wingate), it is possible that the length of this protocol masked the ergogenic effect of N-3 PUFA supplementation. The testing order was selected based on the energy systems used in each exercise (i.e. ATP/PCr to aerobic) and rest intervals were selected according to current athlete testing [206]. Given the endurance sport background of most of the participants, the potential ergogenic effects of N-3 PUFA supplementation on cycling specific testing could have been lost. This is evident from the attenuation of fatigue during the Wingate test and the non-significant 2% improvement in time trial time.

7.4 Future Direction

The N-3 PUFA supplementation source used in these experiments was seal oil N-3 PUFAs. Stereochemical analysis shows that seal oil N-3 PUFAs are structurally different from fish oil N-3 PUFAs [10]. Animal model data suggests that seal oil is digested and metabolized more quickly than fish oil N-3 PUFA [89,201]. Since the majority of studies included in the systematic review in chapter 2.2 used fish oil, it would be important to investigate the difference in digestion and metabolism of fish and seal oil N-3 PUFA in humans. Depending on the differences found form such a comparison study; it would then be interesting to compare the effect of seal oil compared to fish oil on neuromuscular adaptations to exercise.

In the SIT experiments described in chapter 5, N-3 PUFA supplementation did not appear to promote significant neuromuscular adaptations as the training itself caused neuromuscular fatigue. To build on this research, it would be important to examine a longer duration of training
and supplementation to enable the neuromuscular system to adapt to the training. Based on the findings from the systematic review of literature (chapter 2.3) the only study outside of this thesis to investigate the effect of N-3 PUFA supplementation on neuromuscular adaptations to training used a 12-week duration [26]. Based on the results of chapter 6 a minimum duration for a future study would be 3-weeks [138]. Furthermore, performing post-training neuromuscular testing 2-9 days after training would also allow participants’ neuromuscular system time to recover and adapt [169] and therefore provide a more complete description of the neuromuscular adaptations to SIT.

In chapters 6, the effect of N-3 PUFA supplementation was examined on adaptations to training and a series of performance tests. A follow-up study could measure the effects of N-3 PUFA supplementation on isolated aspects of training adaptations or sport. Such studies could examine the change in muscle function using an isolated exercise such as leg press or a sport specific exercise such as Olympic weightlifting snatch. Alternatively, endurance performance could be tested by measuring changes in cycling TT time. This approach would provide a clear indication of the effect of N-3 PUFA supplementation on sport specific outcomes.

Given the limitations of nutritional supplementation studies discussed in chapter 7.2 and the unclear relationship between N-6 PUFA : N-3 PUFA, an important study would be to investigate the dietary manipulation of N-3 PUFA intake through whole foods alone or in combination with N-3 PUFA supplementation. MacIntosh et al. [207] have recently developed and validated a high N-3 PUFA and a low N-6 PUFA diet that could be used for such an experiment.
7.5 Conclusion

The overall hypothesis of this thesis was to determine if N-3 PUFA supplementation would enhance neuromuscular adaptations to exercise training. From the systematic review and laboratory experiments performed for this thesis it appears that N-3 PUFA supplementation enhances adaptations to exercise; however, the experiments performed within this thesis only partially support the hypothesis that N-3 PUFA supplementation can enhance neuromuscular adaptations to exercise.

Hypothesis 1 – Trained and untrained cyclists will show similar peripheral neuromuscular fatigue, while the untrained cyclists will show greater central fatigue after a 10 km cycling TT.

Conclusions 1 – In response to a 10 km TT, peripheral neuromuscular fatigue was the primary source of fatigue in all trained and untrained cyclists. Untrained cyclists showed higher neuromuscular fatigue than trained cyclists. There was no difference in central neuromuscular fatigue between groups. Prior cycling training experience is related to the accumulation of neuromuscular fatigue.

Hypothesis 2 – N-3 PUFA supplementation will enhance neuromuscular adaptations to SIT compared to placebo and attenuate training associated neuromuscular fatigue.

Conclusion 2 - Two-week of SIT did not stimulate any measurable neuromuscular adaptations. N-3 PUFA supplementation did not enhance or impair neuromuscular adaptations to 2-weeks of SIT and to not affect training associated neuromuscular fatigue.
Hypothesis 3 – N-3 PUFA supplementation will enhance neuromuscular adaptations to training and attenuate neuromuscular fatigue from testing.

Conclusion 3 – N-3 PUFA supplementation improved neuromuscular function in well-trained males from increased EMG muscle activation and attenuated sprint cycling induced fatigue. N-3 PUFA supplementation did not alter neuromuscular fatigue from testing compared to placebo.
8 References


### Appendices I

Concentration of fatty acids in plasma (mg/100mL) from sprint interval training study, Chapter 5.

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<th>Visit 11</th>
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SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acids; N-6, omega-6 fatty acid; N-3, omega-3 fatty acids; PUFAs, poly-unsaturated fatty acids; HUFAs, highly-unsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Differences from Visit 4 to Visit 9 and Visit 4 to Visit 11 were determined using a repeated measures ANOVA. ‘+’ shows main effect for time (p<0.05); ‘¶’ shows main effect for group (p<0.05); ‘*’ shows interaction effect (p<0.05).
Appendices II

Absolute concentration of fatty acids in plasma (mg/100mL) from Chapter 6.

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<td>17.89±6.72</td>
<td>19.82±3.30</td>
<td>16.34±8.09</td>
<td>17.34±7.76</td>
</tr>
<tr>
<td>C 18:1n-9</td>
<td>1.94±0.55</td>
<td>1.87±0.39</td>
<td>1.70±0.48</td>
<td>3.44±6.98</td>
</tr>
<tr>
<td>C 20:1n-9</td>
<td>0.25±0.09</td>
<td>0.25±0.06</td>
<td>0.25±0.11</td>
<td>0.26±0.12</td>
</tr>
<tr>
<td>C 22:1n-9</td>
<td>0.13±0.06</td>
<td>0.11±0.04</td>
<td>0.13±0.10</td>
<td>0.11±0.04</td>
</tr>
<tr>
<td>C 24:1n-9</td>
<td>1.23±0.40</td>
<td>1.15±0.26</td>
<td>0.98±0.31+</td>
<td>1.04±0.40+</td>
</tr>
<tr>
<td>MUFAs</td>
<td>22.81±7.91</td>
<td>24.78±3.66</td>
<td>20.65±9.17</td>
<td>23.65±8.66</td>
</tr>
<tr>
<td>C 18:2n-6</td>
<td>28.84±7.87</td>
<td>28.96±5.22</td>
<td>24.47±8.87+</td>
<td>22.73±9.32+</td>
</tr>
<tr>
<td>C 18:3n-6</td>
<td>0.35±0.28</td>
<td>0.35±0.17</td>
<td>0.31±0.14</td>
<td>0.30±0.15</td>
</tr>
<tr>
<td>C 20:2n-6</td>
<td>0.55±0.23</td>
<td>0.72±0.19</td>
<td>0.47±0.19¶</td>
<td>0.63±0.39¶</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td><strong>C 20:4n-6</strong></td>
<td>10.74±4.79</td>
<td>11.37±3.16</td>
<td>8.24±2.95+</td>
<td>8.58±3.47+</td>
</tr>
<tr>
<td><strong>C 22:4n-6</strong></td>
<td>0.24±0.08</td>
<td>0.24±0.07</td>
<td>0.20±0.08+</td>
<td>0.19±0.07+</td>
</tr>
<tr>
<td><strong>C 22:5n-6</strong></td>
<td>0.14±0.07</td>
<td>0.15±0.06</td>
<td>0.11±0.05+</td>
<td>0.11±0.04+</td>
</tr>
<tr>
<td><strong>N-6</strong></td>
<td>40.86±12.17</td>
<td>41.80±6.60</td>
<td>33.81±10.23+</td>
<td>32.55±12.05+</td>
</tr>
<tr>
<td><strong>C 18:3n-3</strong></td>
<td>0.52±0.19</td>
<td>0.51±0.15</td>
<td>0.38±0.12</td>
<td>0.46±0.27</td>
</tr>
<tr>
<td><strong>C 20:3n-3</strong></td>
<td>1.89±0.74</td>
<td>2.07±0.56</td>
<td>1.66±0.58</td>
<td>2.19±2.14</td>
</tr>
<tr>
<td><strong>C 20:5n-3</strong></td>
<td>0.90±0.66</td>
<td>0.58±0.24</td>
<td>0.59±0.34*</td>
<td>0.85±0.43*</td>
</tr>
<tr>
<td><strong>C 22:5n-3</strong></td>
<td>0.49±0.22</td>
<td>0.45±0.14</td>
<td>0.39±0.14</td>
<td>0.46±0.18</td>
</tr>
<tr>
<td><strong>C 22:6n-3</strong></td>
<td>2.58±1.17</td>
<td>2.48±0.60</td>
<td>2.06±0.90</td>
<td>2.44±0.99</td>
</tr>
<tr>
<td><strong>N-3</strong></td>
<td>6.37±2.46</td>
<td>6.08±1.03</td>
<td>5.09±2.09</td>
<td>6.40±4.00</td>
</tr>
<tr>
<td><strong>PUFAs</strong></td>
<td>47.23±14.05</td>
<td>47.88±7.09</td>
<td>38.90±12.32+</td>
<td>38.96±16.05+</td>
</tr>
<tr>
<td><strong>HUFAs</strong></td>
<td>16.96±6.99</td>
<td>17.33±3.88</td>
<td>13.26±5.05+</td>
<td>14.83±7.32+</td>
</tr>
<tr>
<td><strong>EPA+DHA</strong></td>
<td>3.47±1.73</td>
<td>3.05±0.66</td>
<td>2.65±1.24*</td>
<td>3.28±1.41*</td>
</tr>
<tr>
<td><strong>N-6/N-3</strong></td>
<td>6.84±1.61</td>
<td>6.99±1.25</td>
<td>6.64±4.91</td>
<td>5.08±3.01</td>
</tr>
<tr>
<td>% N-3 HUFA in</td>
<td>35.01±4.50</td>
<td>32.74±5.26</td>
<td>35.47±38.98</td>
<td>40.07±51.00</td>
</tr>
<tr>
<td>total HUFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Fatty</strong></td>
<td><strong>Acids</strong></td>
<td>126.99±34.42</td>
<td>131.18±15.39</td>
<td>108.21±36.36+</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acids; N-6, omega-6 fatty acid; N-3, omega-3 fatty acids; PUFAs, poly-unsaturated fatty acids; HUFAs, highly-unsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Differences from Pre to Post were determined using a repeated measures ANOVA. ‘+’ shows main effect for time (p<0.05); ‘¶’ shows main effect for group (p<0.05); ‘*' shows interaction effect (p<0.05).