

CYCLING ENDURANCE PERFORMANCE AND PERIPHERAL MUSCLE FATIGUE
FOLLOWING MARINE LIPID FRACTION PCSO-524™ SUPPLEMENTATION AND
ECCENTRIC EXERCISE IN UNTRAINED MALES

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ABSTRACT

Jacob Sinex

CYCLING ENDURANCE PERFORMANCE AND PERIPHERAL MUSCLE FATIGUE FOLLOWING MARINE LIPID FRACTION PCSO-524™ SUPPLEMENTATION AND ECCENTRIC EXERCISE IN UNTRAINED MALES

INTRODUCTION: Intensive or unaccustomed eccentric exercise is known to cause exercise-induced muscle damage (EIMD) commonly resulting in delayed onset muscle soreness (DOMS). EIMD/DOMS can result in decrements in endurance and resistance exercise performance. Although many treatments have been investigated for the prevention and alleviation of EIMD/DOMS, success has been limited. PCSO-524™ marine lipid fraction has been found to attenuate symptoms of inflammatory diseases such as arthritis and asthma, and thus may be a viable intervention to reduce inflammation-related EIMD/DOMS.

PURPOSE: The purpose of this study was to evaluate the effects of PCSO-524™ supplementation on cycling endurance performance, peripheral muscle fatigue, muscle soreness and muscle damage following eccentric exercise. **METHODS:** Subjects were thirty-two healthy, untrained men aged 18 – 26 who had not previously exercised for more than three times per week for 30 minutes each session for the past 90 days and had not participated in resistance training during the previous 60 days. Subjects were randomly assigned to consume 8 capsules PCSO-524™ (800 mg olive oil, 400 mg lipid extract) daily or olive oil (1200 mg) placebo for 30 days. Subjects completed three 20 minute cycling time trials (TT), thirty days of PCSO-524™ or placebo supplementation, a 20 minute downhill run (DHR) at -16% grade after 26 days of supplementation and potentiated quadriceps twitch force ($Q_{tw,pot}$) measures before and after the latter two TT. Each subject completed a familiarization TT, a pre-supplementation TT and a TT 24 hours following DHR. DOMS was measured via 11 point pain scale at baseline, following supplementation and 24, 48, 72 and 96 hours following

DHR. Slow skeletal troponin I (sTnI) was measured pre-supplementation, post-supplementation and following DHR at 0, 2, 24, 48, 72 and 96 hours. **RESULTS:** Cycling time trial performance was not significantly affected by DHR for PCSO-524™ ($F = .106, p = .749$) or placebo ($F = .122, p = .732$) groups. No significant differences were found in mean power output between PCSO-524™ and placebo groups. PCSO-524™ attenuated DOMS significantly at 72 hours ($T = 2.48, p < .05; 95\% \text{ CI} = 0.3 - 2.6$), and 96 hours post-run ($T = 2.1, p < .05; 95\% \text{ CI} = 0.04 - 2.7$). $Q_{\text{tw,pot}}$ decreased for the placebo group following DHR ($T = 2.095, p < .05$), but did not change significantly for the PCSO-524™ group ($T = .38, p > .05$). Serum sTnI was significantly ($p < .05$) lower in the PCSO-524™ group at 2, 24, 48, 72 and 96 hours post-DHR. **CONCLUSIONS:** Thirty days of PCSO-524™ supplementation does not improve cycling endurance performance in untrained men after eccentric exercise compared to placebo. Thirty days of PCSO-524™ supplementation reduces muscle soreness following eccentric exercise, peripheral quadriceps fatigue in EIMD-affected muscles after endurance exercise and muscle damage following eccentric exercise.

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CHAPTER 1

INTRODUCTION

Intensive or unaccustomed eccentric exercise is known to cause acute exercise-induced muscle damage (EIMD) commonly resulting in delayed onset muscle soreness (DOMS). After an intensive or unaccustomed bout of eccentric exercise, a dull, aching pain combined with stiffness peaks around 24 – 48 hours and typically is resolved in 5 – 7 days (Tiidus, 2008). Typical types of exercise that may be expected to induce DOMS include any exercise with an eccentric component, such as repeatedly lowering a heavy weight, running downhill, repeated vertical jumping or bench stepping exercises. Several hypotheses have been proposed to explain the cause of DOMS including lactic acid, muscle spasm, connective tissue damage, muscle damage, inflammation and enzyme efflux theory (Cheung, Hume, & Maxwell, 2003). It is clear that none of these hypotheses alone is sufficient to fully explain DOMS. The current consensus is that these factors may each contribute to DOMS to some degree (Armstrong, 1984; Lewis, Ruby, & Bush-Joseph, 2012).

DOMS symptoms generally occur 24-48 hours following an initiating event causing exercise-induced muscle damage and resolve in about 5 – 7 days post-exercise (Cheung et al., 2003). Several stages of EIMD contribute to the symptoms felt during DOMS including the initiating event, inflammatory response and repair processes. EIMD is characterized by disruptions of the muscle fiber including “(a) disruption of the sarcolemma, (b) swelling or disruption of the sacrotubular system, (c) distortion of the contractile components of the myofibrils, (d) cytoskeleton damage, and (e) extracellular muscle fiber matrix abnormalities” (Beck et al., 2007). EIMD is a process that begins with the initiating event and proceeds through a series of inflammatory responses, cellular restructuring and eventual repair.

DOMS has been shown to be related to changes in muscle and joint function, characterized by altered functional perception, decreases in strength and power, and altered muscle recruitment patterns (Cheung et al., 2003). Subjects experiencing DOMS after performing 50 maximal eccentric repetitions using their forearm flexors had impaired force perception and joint angle perception using the exercised arm (Saxton et al., 1995). Altered motor unit recruitment patterns have been characterized by changes in joint angles while running with DOMS (Goff, Hamill, & Clarkson, 1998). Strength and power decrements during DOMS have been widely reported (Donnelly, Maughan, & Whiting, 1990; R G Eston, Mickleborough, & Baltzopoulos, 1995; Kazunori Nosaka, Newton, & Sacco, 2002).

Much of the literature related to EIMD/DOMS and exercise performance has focused on the loss of muscle strength that occurs immediately following muscle damage and eventual recovery to baseline. Decrements in endurance exercise performance due to DOMS/EIMD has also been established. The first study to directly demonstrate a significant effect of EIMD on endurance performance in humans, Marcora and Bosio (2007), found that self-paced running time trial performance was reduced by 4% ($p < .01$) following eccentric exercise compared to baseline, while perceived exertion was not different between trials. Twist and Eston (2009) observed that 5 minute time trial performance on a cycle ergometer was significantly ($p < .05$) reduced at 48 hours after an eccentric exercise protocol consisting of 100 counter-movement vertical jumps. Davies et al. (2009) investigated cycling performance following squat exercise and found reduced time-to-exhaustion during severe exercise. Doncaster and Twist (2012) found that time-to-exhaustion in arm cranking exercise was reduced following bench press exercise.

While significant effort has been put into investigating therapies to reduce muscle damage due to exercise and to alleviate DOMS symptoms after EIMD has occurred, relatively little success in reducing EIMD and DOMS can be found in the literature. Previously investigated treatment methods include “cryotherapy, stretching, anti-inflammatory drugs, ultrasound, electrical current techniques, homeopathy, massage, compression, hyperbaric oxygen and exercise” (Cheung et al., 2003).

Among treatments that have been investigated, non-steroidal anti-inflammatory drugs (NSAIDs) have shown some beneficial effects in alleviating DOMS. Researchers have reported reduced soreness at 48 hours post-exercise for a treatment group taking ibuprofen, dexamethasone and aspirin, but no differences in muscle strength between treatments (Hasson et al., 1993; Hasson, Wible, Reich, Barnes, & Williams, 1992). Other data include less impairment of elbow extension in subjects taking aspirin before and following eccentric exercise (Francis & Hoobler, 1987). NSAIDs inhibit arachidonic acid metabolism through the cyclooxygenase pathway and prevent the production of pro-inflammatory prostaglandins, which are involved in the immune system response that can lead to secondary muscle damage (Warden, 2009). Arachidonic acid metabolism and the production of pro-inflammatory proteins is also important in conditions such as asthma (Mickleborough & Rundell, 2005) and arthritis (Simopoulos, 2002).

Nutritional supplementation of treatments containing omega-3 polyunsaturated fatty acids (ω 3FA) such as fish oil and PCSO-524TM have been found to alleviate symptoms of a number of inflammation-related disease states such as asthma (Broughton, Johnson, Pace, Liebman, & Kleppinger, 1997), exercise-induced bronchoconstriction (Mickleborough, Murray, Ionescu, & Lindley, 2003; Mickleborough, Vaughn, Shei, Davis, & Wilhite, 2013),

rheumatoid arthritis (Kremer et al., 1990) and osteoarthritis (Knott, Avery, Hollander, & Tarlton, 2011). Data from previous research has shown that ω 3FA supplementation can attenuate markers of delayed onset muscle soreness resulting from intensive exercise (Sen et al., 1997; Tartibian, Maleki, & Abbasi, 2009), can attenuate post-exercise rise in protein release from muscle cells (Ernst, Saradeth, & Achhammer, 1991) and attenuate post-exercise immunosuppression (Benquet et al., 1994).

A marine lipid extract that has shown results in terms of reducing inflammation is PCSO-524™ (Lyprinol®/Omega XL®), a nutritional supplement derived from the New Zealand green-lipped mussel (*Perna Canaliculus*) containing a mixture of triglycerides, sterol esters, sterols, polar lipids, free fatty acids and carotenoids (Doggrell, 2011). Supplementation with PCSO-524™ has been shown to reduce symptoms in several inflammation-related conditions including osteoarthritis (Gibson & Gibson, 1998), rheumatoid arthritis (Gibson & Gibson, 1998), asthma (Emelyanov et al., 2002) and exercise-induced bronchoconstriction (Mickleborough, Vaughn, Shei, Davis, & Wilhite, 2013) in human clinical trials. PCSO-524™ has shown to have effects in animal models of inflammation including reduced swelling associated with collagen type-II-induced arthritis in rats (Wakimoto et al., 2011; Whitehouse et al., 1997). Similarly to NSAIDs, the mechanism by which PCSO-524™ affects inflammatory processes includes inhibition of the cyclooxygenase pathway and the 5-lipoxygenase pathway (Whitehouse et al., 1997). PCSO-524™ may have additional anti-inflammatory and anti-oxidant effects through furan fatty acids, which have been identified in the extract and found to reduce swelling in a rat adjuvant arthritis model (Wakimoto et al., 2011).

Recently, PCSO-524™ supplementation has been investigated as treatment for EIMD/DOMS following intensive exercise with equivocal results. Pumpa et al. (2011) investigated the effects of PCSO-524™ supplementation on measures of muscle damage following eccentric exercise using 20 well-trained men as subjects. Muscle damage was induced with five bouts of eight minutes of downhill running at 80% of HR_{max} at -10% incline with two minutes of level walking between bouts. No significant differences ($p > .05$) were found between treatment and placebo groups for any of the measured outcome variables including visual analogue scale of DOMS pain, pressure sensitivity, thigh circumference, range of motion of the knee, isokinetic force, vertical jump, IL-1, IL-10, IL-6, tumor necrosis factor α , C - reactive protein, myoglobin and creatine kinase. The authors noted they observed little evidence of an inflammatory response following eccentric exercise in either treatment or placebo subjects. Without an inflammatory response, which is the proposed mechanism that PCSO-524™ may affect, it is unlikely that PCSO-524™ supplementation would result in differences between groups.

In contrast to the data of Pumpa et al. (2011), Baum et al. (2013) found PCSO-524™ supplementation resulted in less DOMS in recreational runners following a 30 km run compared to their soreness levels following a previous 30 km prior to treatment. The PCSO-524™ group experienced reduced soreness following their post-treatment run compared to the pre-treatment run (median pain score: 3.5 pre-treatment, 2.0 post-treatment) compared to no changes in the control group. Runners who trained fewer than three times per week experienced greater attenuation of muscle soreness compared to subjects who ran more frequently. In this study (Baum et al., 2013), PCSO-524™ had greater effects in subjects who were less well adapted to the exercise task.

In addition to potential attenuation of EIMD/DOMS, ω 3FA supplementation has been investigated for effects toward improving exercise performance (Mickleborough, 2013; Tiriyaki-Sönmez, Schoenfeld, & Vatansever-Ozen, 2011). A number of studies have shown a theoretical basis for the improvement of exercise performance through enhanced red blood cell deformability (Cartwright, Pockley, Galloway, Greaves, & Preston, 1985; Terano et al., 1983), reduction in fat mass (Hainault & Carlotti, 1993; A. M. Hill, Buckley, Murphy, & Howe, 2007; Malaguti, Baldini, Angeloni, Biagi, & Hrelia, 2008), enhanced lipid metabolism (Jump, Clarke, Thelen, & Liimatta, 1994; Raclot, Groscolas, Langin, & Ferré, 1997), reduced heart rate during submaximal exercise (Buckley, Burgess, Murphy, & Howe, 2009; Peoples, McLennan, Howe, & Groeller, 2008), enhanced mitochondria biogenesis (Flachs et al., 2005; Vaughan, Garcia-Smith, Bisoffi, Conn, & Trujillo, 2012) and improved fatigue resistance in isolated hindlimb muscles in rats (Peoples & McLennan, 2010, 2013).

Mitochondrial density and skeletal muscle oxidative capacity are related to endurance exercise performance (Hoppeler & Fluck, 2003). Untrained subjects can increase mitochondrial volume after a few weeks of endurance training and improve maximal aerobic exercise capacity (Hoppeler et al., 1985). There is evidence that ω 3FA supplementation may be able to induce similar adaptation in skeletal muscle. ω 3FA can increase mitochondrial biogenesis and increase metabolism in skeletal muscle cells *in vitro* (Vaughan et al., 2012). ω 3FA were found to upregulate glycolytic, oxidative and total metabolism and increase mitochondrial content in isolated cells. In a different study, ω 3FA were found to enhance expression of genes for mitochondrial proteins and enhance β -oxidation in white fat cells in mice (Flachs et al., 2005).

Reduced heart rate during exercise has been found in ω 3FA supplementation studies of elite Australian rules football players (Buckley et al., 2009) and well-trained male cyclists (Peoples et al., 2008). Buckley et al. (2009) observed reduced heart rate while running at 10 km/hr ($p = 0.05$) after five weeks of fish oil supplementation (1.56 g DHA, 0.36 g EPA per day). Peoples et al. (2008) supplemented nine well-trained male cyclists with eight capsules of fish oil (2.4 g DHA, 0.8 g EPA) per day for eight weeks. Heart rate during incremental workloads on a cycle ergometer to exhaustion (including peak heart rate) were reduced with no reduction in peak power output. These findings suggest that fish oil supplementation may enhance oxygen metabolism during exercise and reduce the work required of the cardiovascular system. In a rat model, oxygen consumption was lowered by ω 3FA supplementation during isolated hindlimb stimulation (Peoples & McLennan, 2013), which provides support for these findings.

Body composition is an important contributing factor in endurance exercise, especially in those events which require locomotion of one's body on an uphill or non-level terrain such as many cycling and running competitions. ω 3FA supplementation has been studied for potential effects on body composition in several different populations including athletes, sedentary normal weight people and overweight people (A. M. Hill et al., 2007; Malaguti et al., 2008; Warner Jr & Ullrich, 1989). While the results do not conclusively prove that ω 3FA supplementation alone reduces body weight, there is some evidence that it is effective in combination with exercise in improving body composition.

Hill et al. (2007) found fish oil supplementation combined with exercise resulted in lowered body fat compared with sunflower oil combined with exercise in hyperlipidemic subjects. The subjects supplemented with 6 g fish oil per day (1.56 g DHA, 0.36 g EPA) or

placebo (6 g sunflower oil) for 12 weeks. Half of the fish oil subjects (n=19) were assigned a three-times per week exercise regimen while half did not exercise (n=18). The placebo group was divided into exercise (n=18) and non-exercise (n=19) groups as well. In a similar study, Warner et al. (1989) found that fish oil and exercise resulted in lowered body fat composition compared to baseline and fish oil without exercise. However, this study did not include an exercise only trial so it is not known if fish oil supplementation provided an additional benefit over exercise only.

Human studies on the ergogenic effects of ω 3FA for endurance exercise performance are limited and findings equivocal. Only a few studies have directly investigated the effect of ω 3FA consumption on endurance exercise performance. Buckley et al. (2009) did not observe differences between the fish oil and placebo groups in time to exhaustion during running at the subject's 2200 m time-trial speed. Nieman et al. (2009) similarly did not find a significant effect from fish oil supplementation (2 g EPA, 0.4 g DHA per day for six weeks) on 10 km cycling time trial performance or measures of inflammation and muscle damage (plasma cytokines, serum C-reactive protein, creatine kinase). Whether PCSO-524™ supplementation affects endurance exercise performance has not been investigated.

In conclusion, there is evidence to suggest that treatments such as PCSO-524™ that inhibit COX and 5-LOX pathways may be effective in reducing symptoms of DOMS following intensive exercise (Baum et al., 2013; Tartibian, Maleki, & Abbasi, 2011). Additionally, ω 3FA are able to modify several factors related to exercise performance including body composition, lipid oxidation, mitochondrial biogenesis and oxidative metabolism (Mickleborough, 2013; Tiryaki-Sönmez et al., 2011). PCSO-524™ has been shown to inhibit the COX and 5-LOX pathways, contains ω 3FA in high concentration and

therefore may enhance mechanisms to improve endurance-exercise performance while EIMD/DOMS is being experienced. The present study investigated the relationship between PCSO-524™ supplementation, EIMD/DOMS, peripheral muscle fatigue and endurance exercise performance.

Statement of the Problem

ω 3FA consumption has been investigated as a potential ergogenic aid during exercise and for anti-inflammatory effects in various disease states and during DOMS. Endurance exercise performance can be reduced following eccentric exercise, as a result of EIMD and symptoms of DOMS. EIMD is associated with a number of changes in muscle, including increased inflammation. Though inflammation due to damage is expected and part of the healing process, unchecked inflammation may cause additional muscle damage and prolong the recovery process. PCSO-524™ is a nutritional supplement containing ω 3FA and furan fatty acids that has been shown to be effective in attenuating symptoms of inflammation-related diseases and may be an effective treatment for exercise-related inflammation. To date, no published research has investigated the effects of PCSO-524™ supplementation on endurance exercise performance and DOMS/EIMD in untrained subjects. This study investigated the effects of PCSO-524™ (Lyprinol®/Omega XL®) supplementation on endurance performance, DOMS, peripheral muscle fatigue and muscle damage in untrained men following eccentric exercise.

Purpose of the Study

The purpose of this study was to evaluate whether EIMD-related endurance exercise performance impairment, peripheral muscle fatigue and symptoms of DOMS in untrained

men following eccentric exercise is affected by PCSO-524™ marine lipid fraction supplementation.

Delimitations

Healthy, untrained men were recruited as volunteer subjects for this study. Subjects were recruited on the Indiana University campus via flyers, campus online classifieds and word-of-mouth. Data collection occurred in a laboratory setting where temperature, humidity and barometric pressure are relatively unchanging. Cycling performance tests were conducted on an electronically braked cycle ergometer with seat height and handlebar height set to the subject's preferences. A familiarization time trial was conducted to minimize learning effects and variability during experimental trials. Subjects were asked to refrain from caffeine and alcohol consumption 24 hours prior to study visits. Subjects were asked to maintain their normal diet and sleep routine for the duration of the study, to limit non-study exercise and be well-rested and hydrated prior to exercise tests. Subjects were asked to refrain from using anti-inflammatory medications or nutritional supplements during the study, and from participating in eccentric-type exercise training.

Limitations

The subjects were self-selected based on their exposure to study recruitment methods and their desire to participate in the study. The treadmill was custom modified to run in reverse to enable downhill running and a handrail was installed to provide safety for the subjects. Due to the size of the treadmill belt and the nature of this unusual activity, it was possible that subjects did not run with the same running mechanics that they would have used while running outside down a similar slope. Subjects were asked to perform to their fullest ability during the cycle ergometer time trial. However, it is not possible to know if subjects

performed to the best of their ability. Subjects were asked to limit their exercise habits during the study. Exercise activity was self-reported, but it is not possible to know to what degree the responses reflect the subjects' actual activity. Subjects were asked to record their supplementation intake and return any unused capsules. It is not possible to know if subjects consumed all of the capsules that they reported consuming. The cycle ergometer positioning was adjusted to the subject's preference based on starting adjustment suggested by an investigator. Many subjects had little or no previous cycling experience and may not have selected at optimal position, resulting in biomechanical impairments to their performance.

Assumptions

Subjects refrained from changing their exercise participation rate in order to improve performance in subsequent performance tests. Subjects remained on their normal diet and sleep patterns. Subjects refrained from caffeine and alcohol prior to performance tests and were well-rested and hydrated before performance tests. The downhill running protocol is valid for producing exercised-induced muscle damage in the subject population studied. The downhill running protocol would induce sufficient EIMD and DOMS to reduce subsequent time trial performance. Subjects were highly motivated to maintain the highest possible level of performance during cycle ergometer time trials. The cycle ergometer time trial protocol is a valid measure of endurance exercise performance in untrained men. The cycle ergometer time trial protocol would cause peripheral muscle fatigue in the quadriceps.

Hypotheses

The study was designed to test the following hypotheses:

1. Twenty minute cycling time trial mean power output will be greater in the PCSO-524™ supplementation group compared to the placebo group after eccentric exercise.

2. Potentiated quadriceps twitch force will decrease less following a 20 minute cycling time trial in the PCSO-524™ supplementation group compared to the placebo group.
3. The concentration of skeletal troponin I (sTnI) in blood will be lower in the PCSO-524™ supplementation group compared to the placebo group following eccentric exercise.
4. Perceived quadriceps soreness during squatting will be lower in the PCSO-524™ supplementation group compared to the placebo group following eccentric exercise.

Definition of Terms

Delayed Onset Muscle Soreness (DOMS) - “DOMS is the sensation of discomfort or pain in the skeletal muscles that occurs following unaccustomed muscular exertion.”

(Armstrong, 1984)

Exercise-induced Muscle Damage (EIMD) - Muscle damage following strenuous or unaccustomed exercise. Characterized by DOMS, strength loss, disruptions of myofibrillar structure and inflammation. (Paulsen, Mikkelsen, Raastad, & Peake, 2012)

Half maximal inhibitory concentration (IC₅₀) - Concentration of a compound required to inhibit a biological process by 50% *in vitro* (Griffiths & Sundaram, 2011)

Omega-3 polyunsaturated fatty acids (ω3FA) - Polyunsaturated fatty acids where the first double bond from the methyl (CH₃) end of the molecule is located between the third and fourth carbons. (Ruxton, Reed, Simpson, & Millington, 2004)

Oxygen consumption (VO₂) – rate of oxygen consumption, the volume of oxygen consumed in one minute

PCSO-524™ (Lyprinol®/Omega XL®) - A stabilized lipid extract of the New Zealand green-lipped mussel. Manufactured and distributed by Pharmed International. (Tenikoff, Murphy, Le, Howe, & Howarth, 2005)

Polyunsaturated fatty acids (PUFA) - Fatty acids containing two or more C=C double bonds. PUFA are named based on the position of C=C double bonds and their total length. (Ruxton et al., 2004)

Potentiated quadriceps twitch force ($Q_{tw,pot}$) - Quadriceps muscle twitch measured following a short (usually 5 second) maximal voluntary contraction and, in this study, induced via magnetic stimulation. (Amann, Pegelow, Jacques, & Dempsey, 2007)

Power – the rate of mechanical work done, measured in watts (expressed as work / time)

Rating of perceived exertion (RPE) – a category scale of rating for perception of exertion ranging from 6 (no exertion) to 20 (maximal exertion) (Borg, 1982)

Time trial – a self-paced cycling exercise in which the participant attempts to attain the highest possible average power

CHAPTER 2

REVIEW OF LITERATURE

PCSO-524™

PCSO-524™, marketed under the brand names Lyprinol® and Omega XL®, is a nutritional supplement extracted from the New Zealand green-lipped mussel (*Perna Canaliculus*) containing a mixture of triglycerides, sterol esters, sterols, polar lipids, free fatty acids and carotenoids (Doggrell, 2011). PCSO-524™ came into research interest in part due to folklore that coastal Maori, a group of people native to New Zealand, suffered from lower incidences of arthritis than the general New Zealand population and inland Maori. Coastal Maori consume the green-lipped mussel as part of their normal diet as a contributor to good health (Cobb & Ernst, 2006).

Each capsule of PCSO-524™ contains 50 mg lipid extract, 100 mg olive oil and 0.225 mg vitamin E (d- α -tocopherol). Lipids from five major classes including sterol esters (230 mg/mL), triglycerides (43 mg/mL), free fatty acids (260 mg/mL), sterols and polar lipids (23 mg/mL) are found in PCSO-524™. PCSO-524™ contains approximately 140 mg/mL eicosapentaenoic acid (EPA, 20:5) (21% by weight) and 87 mg/mL docosahexaenoic acid (DHA, 22:6) (13% by weight). Ninety-one fatty acid components have been identified including 5,9,12,15-octodecatetraenoic acid, 5,9,12,16-nondecatertraenoic acid, 7,11,14,17-eicosatetraenoic acid, 5,9,12,15,18-heneic SAPententaenoic acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid and oleic acid (Wolyniak, Brenna, Murphy, & Sinclair, 2005).

Researchers have investigated *P. Canaliculus* and products derived from it such as Seatone™ and PCSO-524™ for evidence of therapeutic effects on inflammatory-related conditions. *P. Canaliculus* was first observed to have effects on joint stiffness and pain in a

study designed to determine if the mussel had an effect on cancer outcomes (Croft, 1986). PCSO-524™ has been investigated extensively as a treatment for various inflammatory conditions including asthma (Emelyanov et al., 2002), exercise-induced bronchoconstriction (Mickleborough et al., 2013), a mouse model of allergic airways disease (Wood, Hazlewood, Foster, & Hansbro, 2010), experimentally-induced inflammatory bowel disease (Tenikoff et al., 2005) and a rat model of adjuvant-induced arthritis (Wakimoto et al., 2011).

Emelyanov et al (2002) investigated the effects of eight weeks of PCSO-524™ supplementation on symptoms of asthma including daytime wheeze, waking at night from asthma, daily use of short acting inhalers. Additionally, lung function tests were performed and exhaled hydrogen peroxide concentration, a marker of airway inflammation, was measured. Subjects received four capsules (200 mg active lipid extract) of PCSO-524™ or placebo per day for eight weeks. Subjects taking PCSO-524™ had lower daytime wheeze, higher morning peak expired flow and lower exhaled H₂O₂ concentration. The authors concluded that PCSO-524™ may have beneficial effects in patients with steroid-naïve atopic asthma.

The findings of Mickleborough et al. (2013) provide support for effectiveness of PCSO-524™ in the treatment of exercise-induced bronchoconstriction. Twenty subjects participated in randomized crossover trial, receiving three weeks of eight capsules (400 mg active lipid extract) per day of PCSO-524™ supplementation or placebo. After a two week washout period, subjects switched treatments and supplemented for three more weeks. PCSO-524™ supplementation improved a number of measures of airway inflammation and exercise-induced bronchoconstriction symptoms. PCSO-524™ subjects were found to have reduced bronchodilator use, attenuated reduction in forced expired volume in one second (FEV₁)

following a eucapnic voluntary hyperventilation trial, improved combined morning and evening peak flow, and reduced markers of inflammation in exhaled breath condensate.

Recent research has investigated the effects of PCSO-524™ in exercise states that may be affected by inflammation, particularly in relation to DOMS. Pumpa et al. (2011) investigated the effects of PCSO-524™ supplementation on measures of muscle damage following eccentric exercise in 20 well-trained men. Subjects were divided into two groups of ten and the subjects consumed 200 mg (4 capsules) of PCSO-524™ or an olive oil capsule placebo daily for a period of eight weeks. Following the supplementation period, each subject performed a downhill running protocol designed to induce muscle damage. No significant differences were found between groups on the measured outcomes, which included “performance measures (Kin-Com, counter movement and squat jump), pain assessments (visual analogue scale, algometer) and blood analyses (Interleukin-1, Interleukin-6, Interleukin-10, tumour necrosis factor- α , C-reactive protein, myoglobin, creatine kinase) ... assessed at 7 time points over 5 days (pre, post, 4, 24, 48, 72 and 96 h after the downhill run)” (Pumpa et al., 2011).

Baum et al. (2013) found that PCSO-524™ supplementation had an effect on DOMS symptoms following a 30km run by recreational runners. The PCSO-524™ group experienced reduced soreness following their post-treatment run compared to the pre-treatment run (median pain score: 3.5 pre-treatment, 2.0 post-treatment) compared to no changes in the control group (median pain score: 2.0 pre-treatment, 2.0 post-treatment). Median pre-treatment pain scores in the control group were not different from post-treatment pain scores in the PCSO-524™ group. The authors reported that the greatest differences in DOMS were seen in runners who trained no more than three times per week (PCSO-524™ group post-

supplementation run: 1.7 pain score, control group post-supplementation run: 4.0 pain score). This result suggests that PCSO-524™ supplementation may have the greatest benefit for the less well-trained athlete. Trained runners may experience less soreness from a long run due to being conditioned for the task.

The primary mechanism by which PCSO-524™ is likely to affect muscle damage and recovery processes resulting from muscle damage is during the inflammatory response that occurs following EIMD. Following a muscle damaging event, intracellular $[Ca^{2+}]$ may be elevated due to increased cell membrane permeability (Nielsen, Madsen, Jørgensen, & Sahlin, 2005). Phospholipase A₂, the regulatory enzyme for the production of eicosanoids, is activated by increased $[Ca^{2+}]$, catalyzing a reaction that releases arachidonic acid (AA) from cell membranes. AA is precursor of eicosanoids including two-series prostaglandins (PGE₂) and four-series leukotrienes (LT₄) through a series of reactions catalyzed by cyclooxygenase and 5-lipoxygenase (**Figure 1**) (Balsinde, Winstead, & Dennis, 2002; Gissel, 2005; Mickleborough & Rundell, 2005).

PCSO-524™ acts as a “dual inhibitor of arachidonate oxygenation by both cyclooxygenase (COX) and 5-lipoxygenase” with particular emphasis on the COX-2 pathway and has been shown to inhibit leukotriene B₄ (LTB₄) production in human neutrophils *in vitro* (Whitehouse et al., 1997). Whitehouse et al. (1997) determined that unfractionated PCSO-524™ inhibits LTB₄ production *in vitro* by isolating human polymorphonuclear leukocytes (PMN) and stimulating production of LTB₄ through the addition of AA. In the presence of PCSO-524™, LTB₄ production was not detectable compared to 19.8 ± 0.8 ng/10⁶ PMN in the control PMN. Prostaglandin production was similarly inhibited in isolated PMN by PCSO-524™ with IC₅₀ = 1.2 µg/ml.

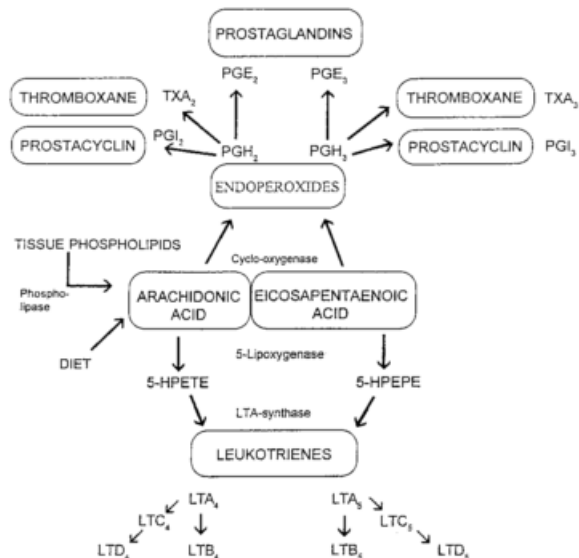


Fig. 1. Oxidative metabolism of arachidonic acid and eicosapentaenoic acid by the cyclooxygenase and 5-lipoxygenase pathways. 5-HPETE denotes 5-hydroperoxyeicosatetraenoic acid and 5-HPEPE denotes 5-hydroxyeicosapentaenoic acid.

Figure 1: Diagram of arachidonic acid and eicosapentaenoic acid via the cyclooxygenase and 5-lipoxygenase pathways (Simopoulos, 2002)

In addition to its effects on AA metabolism, PCSO-524™ contains furan fatty acids (Wakimoto et al., 2011), which can be incorporated into cell membranes to act as radical scavengers (Spiteller, 2005) and have been shown to have antioxidant effects *in vitro* (Okada et al., 1990). Furan fatty acids present in PCSO-524™ have been hypothesized as an overlooked component of the cardioprotective effects of diets containing high amounts of PUFAs including DHA and EPA (Spiteller, 2005). PCSO-524™ has been found to have similar anti-inflammatory properties to fish oil in a rat model of adjuvant arthritis at lower concentrations than fish oil required for anti-inflammatory effects (Whitehouse et al., 1997), suggesting that other components besides DHA and EPA contribute to its anti-inflammatory properties. In a study that compared PCSO-524™, EPA and isolated furan fatty acids in a similar rat arthritis model, furan fatty acids provided greater protective effects than EPA and similar to PCSO-524™ (Wakimoto et al., 2011).

PUFA Supplementation and Exercise Performance

Omega-3 fatty acids (ω 3FA) are polyunsaturated fatty acids containing a carbon-carbon double bond at the third carbon from the end of the molecule. Omega-3 fatty acids have been investigated for their impact on health and various disease states including protection from cardiac disease (Lavie, Milani, Mehra, & Ventura, 2009; Saravanan, Davidson, Schmidt, & Calder, 2010), improvement in muscle protein synthesis in older adults (Smith et al., 2011), reduction in symptoms of inflammatory disease (Simopoulos, 2002) and improvement in mood state disturbance (Su, 2009). Fish oil and flaxseed oil are common sources of ω 3FA that have been studied. EPA (a twenty carbon molecule with five carbon-carbon double bonds) and DHA (a twenty-two carbon molecule with six carbon-carbon double bonds) are among the most-researched ω 3FA. ω 3FA have also been investigated for potential ergogenic effects during exercise. While results from animal studies are promising, results from human exercise studies are less clear.

For long-distance migrant birds, fuel efficiency and highly effective oxidative metabolism are required to enable their strenuous seasonal journeys. One type of migrant bird, semipalmated sandpipers (*Calidris pusilla*, L.), prepares for a 3-day, ~4500km flight from eastern Canada to South America by feeding on amphipods containing high levels of ω 3FA (Maillet & Weber, 2006, 2007; Weber, 2009). During their refueling stop in the Bay of Fundy (New Brunswick, CA), the birds consume a diet comprised of approximately 45% ω 3FA (31% EPA, 14% DHA), doubling their body mass from ~20 g to 40 g in preparation for the flight. The consumption of ω 3FA during this stop is important not only because lipids are a concentrated fuel source for the long distance traveler, but because dietary fatty acid composition is related to capacity for endurance exercise in animals. For example, endurance

exercise capacity is affected the dietary fatty acid composition in rats (Ayre & Hulbert, 1997), Atlantic salmon (McKenzie & Higgs, 1998) and migrant songbirds (Pierce, McWilliams, O'Connor, Place, & Guglielmo, 2005).

In humans, the effect of fatty acid composition in the diet and ω 3FA consumption on endurance exercise performance is less clear. Only a few studies have directly investigated the effect of ω 3FA consumption on performance. Buckley et al. (2009) did not observe differences between the fish oil and placebo groups in time to exhaustion at the subject's 2200 m running time-trial speed. Nieman et al. (2009) similarly did not find a significant effect from fish oil supplementation (2 g EPA, 0.4 g DHA per day for six weeks) on 10 km cycling time trial performance or measures of inflammation and muscle damage (plasma cytokines, serum C-reactive protein, creatine kinase).

The predominant effect of ω 3FA supplementation that has been observed in humans during exercise is reduced heart rate during submaximal exercise (Buckley et al., 2009; Peoples et al., 2008). Fish oil supplementation has also been found to reduce heart rate at rest by 1.6 bpm in a meta-analysis (Mozaffarian et al., 2005). Buckley et al. (2009) observed reduced heart rate while running at 10km/hr ($p = 0.05$) after five weeks of fish oil supplementation (1.56 g DHA, 0.36 g EPA per day). Peoples et al. (2008) supplemented nine well-trained male cyclists with eight capsules of fish oil (2.4 g DHA, 0.8 g EPA) per day for eight weeks. Heart rate during incremental workloads on a cycle ergometer to exhaustion (including peak heart rate) were reduced with no reduction in peak power output. These findings suggest that fish oil supplementation may enhance cardiovascular efficiency during submaximal exercise.

Maintenance of red blood cell (RBC) deformability during exercise has been investigated in relation to ω 3FA consumption. Deformability refers to “the ability of a body to change shape in response to a deforming force” (Chien, 1987). RBCs deform from their normal shape as they pass through the microcirculation (Chien, 1987). During exercise, RBC deformability can decrease (Van der Brug, Peters, Hardeman, Schep, & Mosterd, 1995), which can lead to increases in platelet aggregation and increased transit time the microcirculatory system (Yalcin, Bor-Kucukatay, Senturk, & Baskurt, 2000). ω 3FA supplementation improves RBC deformability and decreases whole blood viscosity (Cartwright et al., 1985). It has been hypothesized that ω 3FA supplementation may be able to help maintain RBC deformability during exercise, potentially improving oxygen delivery to the skeletal muscles and increasing exercise performance (Guezennec, Nadaud, Satabin, Leger, & Lafargue, 1989).

The results of research on RBC deformability during exercise and ω 3FA supplementation are inconclusive. Guezennec et al. (1989) found less reduction in RBC deformability after hypobaric exercise in subjects supplemented with 6 g fish oil (1.08 g EPA, .72 g DHA) for six weeks compared to placebo. However, there was no significant difference between groups in RBC deformability at rest. In contrast to these results, Oostenbrug et al. (1997) did not observe a change in RBC deformability characteristics during exercise and no change in cycling time trial performance after three weeks of supplementation with 6 g fish oil per day (1.08 g EPA, .72 g DHA).

PUFA Supplementation and EIMD/DOMS

Several studies have investigated the effects of PUFA supplementation on EIMD and DOMS, with equivocal results. Two studies from Tartibian et al. (2009, 2011) reported

improved inflammatory, pain and mobility measures in fish oil-supplemented subjects. The 2009 study found attenuated range of motion decrements and less muscle soreness compared to placebo 48 hours after eccentric exercise in subjects who had taken 1 capsule (324 mg EPA, 216 mg DHA) per day for 30 days. The eccentric exercise protocol consisted of 40 minutes of bench stepping in five minute increments followed by one minute rest. The 2011 study found that fish oil supplementation moderated inflammatory and tissue injury markers following eccentric exercise. Experimental subjects demonstrated less increase in tumor necrosis factor α (TNF- α) and prostaglandin E₂ (PGE₂) following exercise, at 24 and 48 hours post-exercise. IL-6, CK and Mb levels were lower at 24 and 48 hours.

In contrast to data from Tartibian et al. (2009, 2011), Lenn et al. (2002) did not observe significant differences due to fish oil and isoflavone supplementation on muscle soreness following eccentric exercise of arm extensors. After 30 days of supplementation of 1.8 g/day fish oil (n=7), 120 mg soy isolate (n=8) or placebo (n=7), subjects performed 50 maximal effort eccentric contractions at a rate of 90°/sec. Physical measurements included muscle soreness, upper arm circumference, range of motion, and isokinetic strength. Blood measurements were taken immediately after, 3 hours, 24 hours, 48 hours and 72 hours following exercise and tested for cortisol, creatine kinase (CK), interleukin-6 (IL-6), TNF- α , lipid peroxidation and serum iron. The investigators reported changes in range of motion, increased soreness, increased arm circumference and decreased strength across groups but no significant differences between groups. No significant changes were found in blood markers of muscle damage and inflammation. The relatively short duration of exercise and small muscle mass (relative to other studies that used leg muscles, for example) exercised in this study may explain the lack of detectable increase in IL-6. Also noted was a high level of

variability in CK among subjects, which limited its usefulness in detecting differences between groups.

Additional data do support the hypothesis that the anti-inflammatory effects of PUFAs are relevant in exercise conditions. In a study using eccentric arm curl exercises to induce muscle damage, subjects supplementing with ω 3FA reported attenuated weighted and fully extended arm soreness. Arm circumference, arm volume and arm skin surface temperature were also measured without significant differences. The design of this study used each subject as his/her own control, where a mixture of dominant and non-dominant arm were used for the treatment and control conditions. This design was incorporated to avoid a repeated bout effect while still enabling repeated-measures within subjects. One limitation of this study is the lack of a placebo group (Jouris, McDaniel, & Weiss, 2011).

PUFA supplementation combined with other compounds has been found to reduce inflammation following arm curl exercises. Subjects supplemented with a dietary supplement containing DHA, tocopherols and flavonoids for 14 days had lower levels of IL-6 and C-reactive protein (CRP) at 10 days post-exercise (Phillips, Childs, Dreon, Phinney, & Leeuwenburgh, 2003). A plant extract containing high levels of omega-3 and various antioxidants also resulted in attenuated inflammatory markers including serum CK after 48 hours, LDH after 24 and 48 hours, cortisol at 24 and 48 hours, IgA at 0, 24 and 48 hours, potassium at 24 and 48 hours. Pain scores were also lower in the treatment group at 48 hours (Meamarbashi & Abedini, 2011).

Muscle Damage Processes and Inflammation

Muscle damage induced by exercise and recovery is a complex process that is not yet fully understood. The process of muscle damage following unaccustomed eccentric exercise

and recovery can be summarized as 1) initial mechanical injury, 2) muscle damage, 3) inflammatory response, 4) neutrophil release of oxygen free radicals and lysosomal proteases, which can lead to additional muscle damage and 5) eventual regeneration and repair (Toumi & Best, 2003). Muscle damage “involves a variety of histopathological changes, including swelling of muscle cells, loss of the intermediate filament proteins desmin and dystrophin, and inflammatory cell infiltration” (Toumi & Best, 2003). Data from previous studies has demonstrated that cytoskeletal disruption occurs early during exercise designed to cause EIMD in a rabbit model (Lieber, Thornell, & Fridén, 1996).

The first stage of EIMD is the initial or initiating event. The ‘popping sarcomere hypothesis’ describes the mechanical injury event as a lengthening of sarcomeres beyond their functional limit. The likelihood of cross-bridge formation decreases under conditions of increasing muscle length while generating tension. Sarcomeres can be stretched to the point at which passive tension is able to provide the required tension. Upon relaxation, some sarcomeres are able to reinterdigitate and resume normal force production while others are disrupted and unable to generate further active tension (Morgan & Allen, 1999).

Unaccustomed eccentric exercise has been used in human models as the initiating event in many studies. Initial signs of muscle damage from the initiating event include “a) disruption of the normal myofilament structures in some sarcomeres, observable with both light and electron microscope and b) loss of intramuscular proteins (e.g., creatine kinase enzymes) into the plasma, indicating damage to sarcolemma” (Armstrong, 1990).

While the presence of disrupted sarcomeres and damage to the excitation-contraction coupling system are both prominent early signs of muscle damage, there has been debate on which is the primary event. Proske and Morgan (2001) argue that the damage process is

initiated by overstretch of the sarcomeres. Warren et al. (2001), however argue that most of the decline in muscle force capability can be attributed to the excitation-contraction couple dysfunction, which is also known as excitation-contraction uncoupling. Lending support to the latter argument is evidence that caffeine injection into the muscle following fatigue can restore force production capability in mice (Warren et al., 1993).

The second stage of muscle damage is termed the autogenetic stage and is described as “the period immediately following the initiating event when proteolytic and lipolytic systems indigenous to the muscle fibers begin the process of degrading cellular structures” (Armstrong, 1990). Exercise may cause disruption of the sarcolemma, increasing cell membrane permeability and allowing intramuscular proteins such as creatine kinase and lactate dehydrogenase to be released to the extracellular space. Concomitantly, increased permeability of the cell membrane allows Ca^{2+} and other concentrated extracellular molecules to enter the cell at higher than normal concentrations. Increased $[\text{Ca}^{2+}]$ is a common factor in a number of potential autogenetic mechanisms in muscle cell injury (Figure 2), including being a precursor for phospholipase A₂ (PLA₂) activation. PLA₂ activation which leads to increased cell membrane permeability and release of arachidonic acid, initiating an inflammatory response to the muscle damage (Gissel, 2005).

POTENTIAL AUTOGENETIC MECHANISMS IN EXERCISE-INDUCED INJURY

- 1. Calcium-activated proteases**
- 2. Phospholipase A₂**
 - a. arachidonic acid**
 - i. leukotrienes (LTs)**
 - ii. prostaglandins (PGs)**
 - b. lysophospholipids**
- 3. Mitochondrial calcium accumulation**
- 4. Lysosomal proteases**
- 5. Free oxygen radicals**

Figure 4—Possible autogenetic processes involved in exercise-induced muscle injury. For further discussion of these processes, see the references in this paper and a previous review (1).

Figure 2: Autogenetic Mechanisms in Muscle Cell Following Exercise-Induced Injury (Armstrong, 1990)

The phagocytic stage, or inflammatory response, “is prevalent from 4-6 h after the initiating event through 2-4 d following exercise and is marked by a typical inflammatory response in the tissue” (Armstrong, 1990). The inflammatory response to eccentric exercise injury is characterized by neutrophilia, neutrophil activation, and the accumulation of neutrophils within the injured muscle as early as one to two hours after muscle damage (Fielding et al., 1993). Neutrophils are capable of releasing arachidonic acid, which can be metabolized through the cyclooxygenase pathway into pro-inflammatory prostaglandins (Walsh, Waite, Thomas, & DeChatelet, 1981) or by the 5-lipoxygenase pathway into leukotrienes (Mickleborough & Rundell, 2005). Connolly et al. (2003) provide a simple schematic (Figure 1) of the processes leading to inflammation influenced muscle damage following exercise. Following damage to the muscle cell membrane PLA₂ is activated, which leads to prostaglandin and leukotriene production.

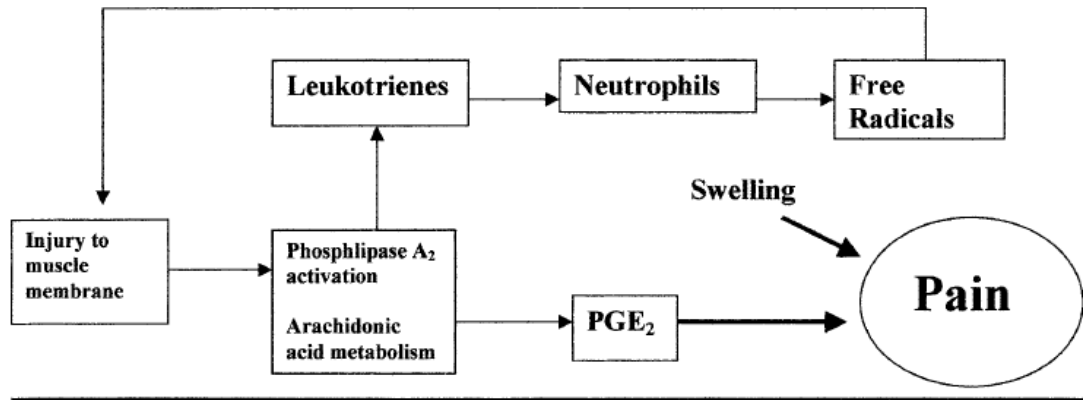


Figure 3: Schematic showing possible sequence of injury due to inflammatory processes following an exercise-induced muscle damage initiating event

Reactive oxygen species (ROS) are an emerging focus in EIMD and DOMS research (Close, Ashton, McArdle, & Maclaren, 2005). ROS are oxygen-centered molecules with an unpaired electron, which makes them highly reactive as the unpaired electron is unstable. ROS are generated by normal metabolic processes and the rate of generation is elevated during exercise. A ROS may pair with either another ROS, which is termed a termination reaction, and no further reactions occur for the two involved ROS. However, if a ROS pairs with a non-ROS, then a new ROS is formed which remains highly reactive. Continuing reactions may occur, which in the case of skeletal muscle membranes, can lead to damage to cell membrane phospholipids and compromise the integrity of the cell membrane itself (Close et al., 2005).

In the case of EIMD, the involvement of ROS in the damage and repair process is related to the inflammatory processes that occur following the initial damaging event. During and following eccentric muscle contractions, monocytes/macrophages, eosinophils and neutrophils are activated (Malm, Lenkei, & Sjödin, 1999) and produce ROS to attack damaged muscle cells (Close et al., 2005). In mouse models, the secondary damaging event has been shown to occur at three days post-exercise, is associated with an elevated ROS level

(A McArdle et al., 1999) and force loss attributed to ROS can be attenuated by treatment with polyethylene glycol-superoxide dismutase, a free radical scavenger (Zerba, Komorowski, & Faulkner, 1990).

There remains some debate as to whether inflammation resulting from EIMD is an overall positive or negative response in terms of muscle recovery and adaptation to the exercise stimulus. Toumi and Best (2003) describe the view as the thought that “the events following the initial injury, including inflammation, are necessary for optimal repair.” However, neutrophil invasion of the damage site and neutrophil activation has the potential to release a number of damaging molecules into the muscle, including “40 hydrolytic enzymes and toxic molecules in their granules and [neutrophils] can generate various oxidants such as superoxide anion, hydrogen peroxide, and hypochlorous acid.” Brickson et al. (2003) found that blocking the CD11b-dependent respiratory burst in stretch-injured rabbits with monoclonal antibody M1/70 resulted in less myofiber damage following the injury.

In addition to their potential contribution to secondary damage seen following eccentric exercise, production of ROS following muscle damage may be a necessary component of the recovery and adaptation, acting as a signaling component for regenerative processes (Anne McArdle, Vasilaki, & Jackson, 2002). Following cellular stress, heat shock protein (HSP) levels are elevated, which is hypothesized to provide protection against further stress and to facilitate recovery and remodeling (Hernando & Manso, 1997). Anti-oxidant supplementation attenuates the increase of HSP content in the muscle following stress (Khassaf et al., 2001). Thus, it appears that there may be a balance between the cell damaging effects of ROS production and the adaptive responses activated by ROS production.

Muscle Damage Exercise Models

A number of different protocols have been used in the literature to produce exercise-induced muscle damage. For endurance-related EIMD research, downhill running is a common method of inducing muscle damage. Studies have used varying running speeds, durations and treadmill inclines during downhill running to produce EIMD (**Table 1**) (Tiidus, 2008).

Other researchers have used downhill running intensities based on relative percentage of max HR (Shave, Dawson, & Whyte, 2002), a chosen treadmill speed (Koller et al., 1998), a chosen heart rate (Byrnes et al., 1985) and subject preference while running down stairs (Yu, Malm, & Thornell, 2002). Eston et al. (1996) used a protocol consisting of five bouts of 8 minutes running at 80% of predicted max HR on a treadmill set at -10° incline. The same protocol was used by Pumpa et al. (2011).

Table 1: Downhill Running Exercise Protocols

Study	Running Speed	Duration (min)	Incline
(Sorichter et al., 1997)	70% $\dot{V}O_{2max}$	20	-16%
(Shave et al., 2002)	70% max running velocity	30	-15%
(Koller et al., 1998)	9 km/hr	25	-25%
(Byrnes et al., 1985)	170 bpm HR	60	-10%
(Yu et al., 2002)	Subject preference	45	Unknown
(R G Eston et al., 1996)	80% predicted HR_{max}	5 x 8 min, 2 min rest between bouts	-10%
(Pumpa et al., 2011)	80% predicted HR_{max}	5 x 8 min, 2 min rest between bouts	-10%

Sorichter et al. (1997) used downhill running at 70% of VO_{2max} at a gradient of -16% for 20 min to induce muscle damage for the purposes of measuring the appearance of skeletal troponin I (sTnI) in plasma. This protocol was successful in producing the appearance of sTnI in blood plasma at elevated levels compared to those seen during a similar run on a level

treadmill (**Figure 4**). Subsequent research has supported the finding of elevated levels of sTnI in blood plasma following eccentric exercise (Chapman, Simpson, Iscoe, Robins, & Nosaka, 2013; Willoughby, 2003).

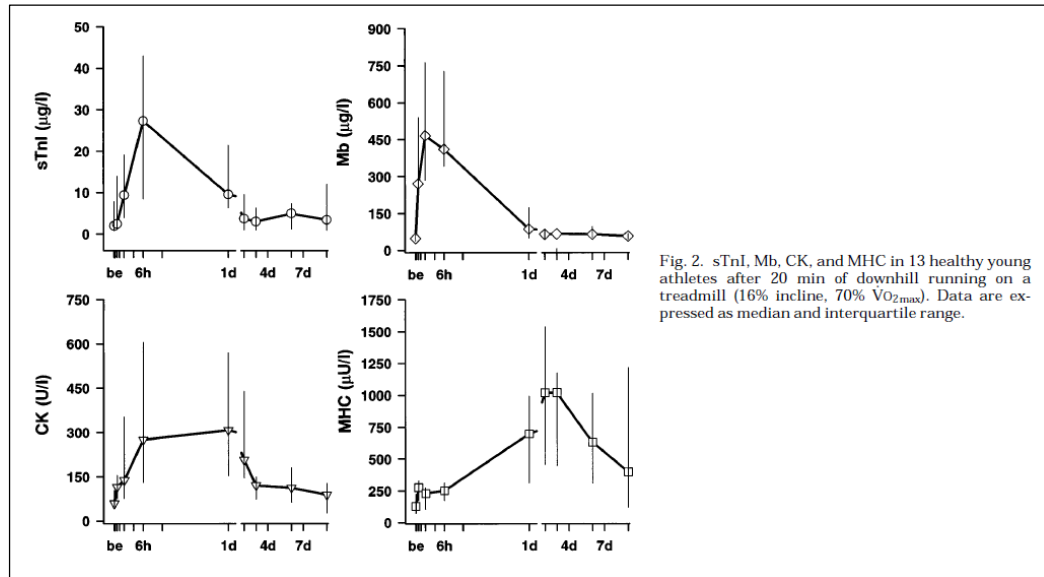


Fig. 2. sTnI, Mb, CK, and MHC in 13 healthy young athletes after 20 min of downhill running on a treadmill (16% incline, 70% $\dot{V}O_{2max}$). Data are expressed as median and interquartile range.

Figure 4: Time course of sTnI, Mb, CK and MHC following downhill running at -16% for 20 minutes at 70% of $\dot{V}O_{2max}$ (Sorichter et al., 1997)

Troponin is a three subunit protein complex involved in the regulation of muscle cell contraction. Troponin C (TnC) is the subunit that binds to calcium. Troponin I (TnI) is a central subunit, binding to actin, TnC and troponin T (TnT). TnT binds the troponin complex to tropomyosin. **Figure 5** depicts the troponin complex in conjunction with actin and tropomyosin. Contraction regulation occurs through the interaction of Ca^{2+} -dependent interaction of TnC and TnI. Ca^{2+} binding to TnC increases the affinity of TnC-TnI, switching TnI from binding at multiple sites on actin to sites on TnC. This configurational change pulls tropomyosin away from actin, exposing the active sites on actin which enables myosin to form cross bridges with actin (Gordon, Homsher, & Regnier, 2000; Plowman & Smith, 2011).

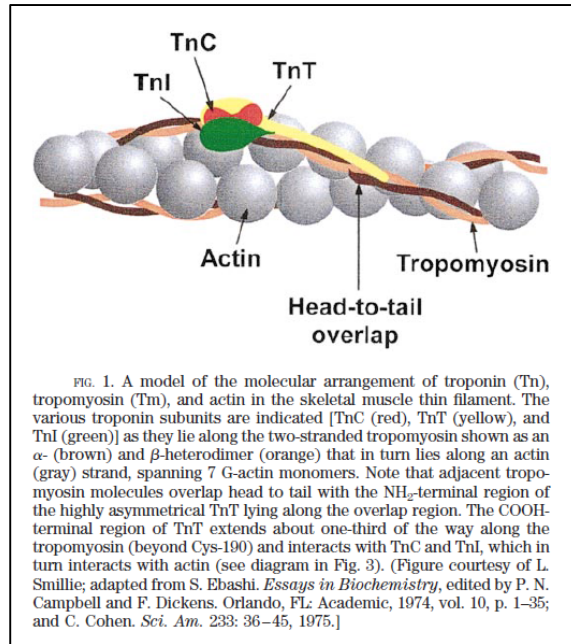


Figure 5: Troponin complex as part of muscle fiber thin filament (Gordon et al., 2000)

Only a few studies have investigated cycling performance following eccentric exercise or eccentric cycling exercise itself. Vertical jumping protocols, squatting, bench stepping and using modified cycle ergometers that require the subject to resist backward motor-driven movement of the pedals have been used (**Table 2**).

Table 2: Exercise Protocols Used in Cycling EIMD Studies

Study	Protocol
(Twist & Eston, 2009)	100 counter-movement vertical jumps
(Moysi et al., 2005)	25 reps of squats with 150% body mass load every 10 minutes during cycling
(Gleeson, Blannin, Zhu, Brooks, & Cave, 1995)	30 min of bench stepping (47-cm step, 15 steps min ⁻¹)
(Cannon & Fiatarone, 1994)	Resisting bicycle pedals driven backward by a motor for 45 min (3 x 15 min bouts) at an intensity of 78 ± 6% of HR _{max}
(Yu et al., 2002)	Resisting bicycle pedals driven backward by a motor for 30 min at or near concentric $\dot{V}O_{2max}$ (250 or 300W)
(O'Reilly et al., 1987)	3 x 15 min at 90, 80 and 70 % $\dot{V}O_{2max}$ on eccentric cycle ergometer

EIMD and Exercise Performance

EIMD has been shown to impair muscle strength and endurance exercise performance. One of the most tested and consistent findings in EIMD research is loss of strength immediately following exercise (Tiidus, 2008), with estimates of 50% of human EIMD studies reporting maximal voluntary contraction (Warren, Lowe, & Armstrong, 1999). Typically, eccentric resistance exercise produces an immediate loss in force production capability that recovers over the days and weeks following the exercise bout (**Figure 6**) (Balnave & Thompson, 1993; Clarkson, Nosaka, & Braun, 1992).

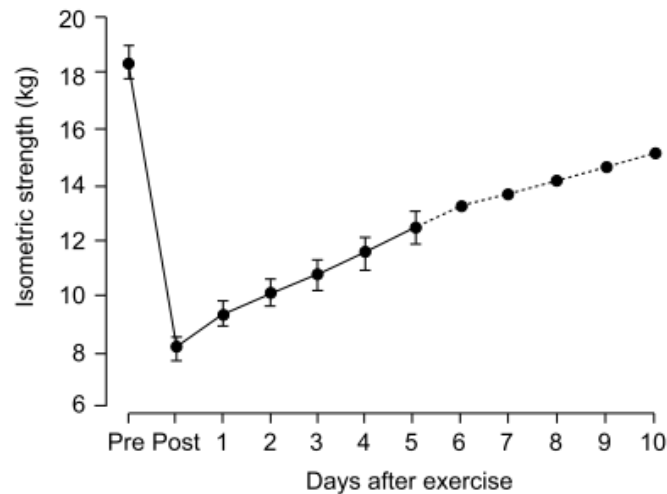


Fig. 1. Isometric strength (mean \pm standard error of the mean) of the forearm flexor muscles before (Pre), immediately after (Post) and 5 days *post* exercise for 109 individuals and means for 6 to 10 days *post* exercise for 15 individuals (reproduced from Clarkson et al.,^[64] with permission).

Figure 6: Typical Force Decrement in Forearm Flexor Muscles Following Eccentric Exercise (Clarkson et al., 1992)

There are numerous examples in the literature of decrements in strength and force production due to EIMD, a few of which are reviewed here. Davies and White (1981) found that muscle force production is reduced following eccentric exercise, which they demonstrated with direct electrical stimulation. EIMD brought about by 100 barbell squats

reduced muscle strength during isometric, eccentric and concentric contractions for 4 days and vertical jump performance. Also, plasma CK values were elevated for 3 days following the exercise bout (Byrne & Eston, 2002b). Balnave and Thompson (1993) reported a 17% decline in quadriceps MVC following a 40 minute downhill walk by nine healthy, sedentary subjects. Clarkson et al. (1992) found 50% decrements in MVC of the forearm flexor muscles following two sets of 35 maximal contractions, compiled from the pooled data from control subjects in several of their studies. Decreased strength was still evident at 10 days following the initial exercise bout (**Figure 6**).

Submaximal endurance exercise performance is affected by a previous bout of exercise that causes EIMD (Burt, Lamb, Nicholas, & Twist, 2012). While this study did not include a performance measure, the authors did report that ventilation was increased during both running and cycling and they postulated that additional recruitment of secondary muscles may have contributed to the difference. Running economy was found to decrease following EIMD in a group of well-trained runners and triathletes, a finding that may be due to altered motor unit recruitment patterns (Braun & Dutto, 2003). Marcora and Bosio (2007) found that self-paced running time trial performance was reduced by 4% ($p < .01$) following eccentric exercise compared to baseline without significant changes in perceived exertion. Davies et al. (2009) investigated cycling performance following squat exercise and found reduced time-to-exhaustion during severe exercise.

Doncaster and Twist (2012) found that time-to-exhaustion in arm cranking exercise was reduced following bench press exercise. Nine physically active men were asked to perform ten sets of six bench presses using a weight of 70% of their one repetition maximum (1-RM). Focus was given to the eccentric portion of the exercise, which was

completed in 3 seconds for each repetition, while the concentric portion of each repetition was completed in 1 second. Time to exhaustion for arm cranking occurred at an intensity equal to 80% between ventilatory threshold and $\dot{V}O_{2peak}$, an intensity within the severe exercise domain. Time to exhaustion in the treatment group (207.2 ± 91.9 s) was significantly ($p < .05$) lower than the control group (293.4 ± 75.6 s).

Eccentric exercise has previously been found to cause decrements in cycling performance. In seven recreationally trained men (Twist & Eston, 2009), EIMD was induced by 100 counter-movement jumps and the performance test consisted of a 5 minute time trial on a cycle ergometer. Mean power output and work performed during the time trial decreased from baseline (262.8 ± 17.7 W) at 48 hours (232.8 ± 15.3 W, $p < .05$) and had returned to baseline by 168 hours (266.1 ± 15.3 W, $p > .05$). RPE was increased at 60% (baseline: 13.7 ± 0.4 , 48 h: 15.4 ± 0.7 , $p < 0.05$) and 80% (baseline: 17.7 ± 0.5 , 48 h: 18.3 ± 0.7 , $p < 0.05$) of power at $\dot{V}O_{2max}$ during steady state cycling following EIMD. This study was the first to look at an “ecologically valid measure of cycling performance” in relation to EIMD and DOMS (Twist & Eston, 2009).

EIMD reduces $\dot{V}O_{2peak}$ and ventilatory threshold during cycling, two parameters that are associated with exercise performance (Black & Dobson, 2012). In a study of eleven recreationally active college students (5 men, 6 women), $\dot{V}O_{2peak}$ decreased from 46.2 ± 9.7 ml/kg/min to 41.8 ± 10.7 ml/kg/min ($p = .01$) and ventilatory threshold decreased from 34.2 ± 7.8 to 30.5 ± 8.5 ml/kg/min ($p = .031$). Muscle damage was induced by 3 sets of 8 single-leg knee contractions, completed on both legs, with the load initially set to 120% of 1-RM. Subjects lowered the weight from level to 90° over three seconds. When the load could not be lowered in a controlled manner, the weight was decreased by 5% and the set continued.

Additional studies have noted decrements in cycling performance following EIMD. Byrne and Eston (2002a) found significant ($p < .05$) decrements in Wingate cycling test results 1 hour, 1, 2, 3 and 7 days after an exercise bout of 10 sets of 10 repetitions of the eccentric portion of a barbell squat with a load of 80% of 1-RM. Sargeant and Dolan (1987) observed decreased anaerobic power output on a cycle ergometer following downhill walking at 6.44 km/hr at a -25% gradient. In this study, short term maximal power output (20 sec) on a cycle ergometer was reduced from baseline to $77 \pm 10\%$ ($p < 0.05$) at 24 h post exercise with performance decrements persisting through 96 h ($92 \pm 4\%$; $p < 0.05$).

However, some studies have not found changes in exercise performance following eccentric exercise. In a group of trained rugby and field hockey players, sprinting performance was not affected by a previous eccentric exercise bout of seven sets of 10 drop jumps. Sprinting performance was measured with a 30 meter sprint before exercise and at 12, 24, 48 and 72 hours. One limitation of this study is that there was no significant differences creatine kinase over time, which may indicate that the exercise protocol was not strenuous enough to produce significant damage (Semark, Noakes, St Clair Gibson, & Lambert, 1999). Gleeson et al. (1998) did not observe a significant change in time to exhaustion during cycling after 40 minutes of bench stepping exercise. The time to exhaustion measure was the time to completion of a $\dot{V}O_{2peak}$ test. $\dot{V}O_{2peak}$ was also not changed as a result of the exercise bout.

One phenomenon that affects the design of EIMD research protocols is known as the *repeated-bout effect*. The repeated-bout effect describes the tendency of EIMD and DOMS to be reduced in the weeks and months following an initial damaging exercise bout. Cleary et al. (2002) determined that the repeated bout effect lasts as long as nine weeks following the initial exercise bout, although further time points were not measured in this study. In a study

designed to test whether stride length affects symptoms of EIMD, no significant differences were found among stride lengths but the authors did note that symptoms of EIMD were lessened in the second trial where stride length was altered (R. G. Eston, Lemmey, McHugh, Byrne, & Walsh, 2000). Due to the repeated-bout effect, EIMD studies usually do not feature a cross-over design because subjects would not be expected to respond to an exercise bout in the same manner multiple times without lengthy delays between trials.

Delayed Onset Muscle Soreness

Delayed onset muscle soreness (DOMS), the feeling of pain and soreness that follows a bout of intensive or unaccustomed exercise, is a sensation familiar to most who have participated in sport and exercise. The symptoms and hypothesized causes of delayed onset muscle soreness were first described at the turn of the 20th century (Hough, 1902). Hough (1902) established in early EIMD/DOMS research that muscular performance can be reduced in the presence of DOMS. His findings suggest that performance is affected both by a reduction in voluntary effort and a reduction in the ability of the muscle to produce force.

The mechanisms underlying DOMS have been a topic of debate in the literature. Several hypotheses have been proposed to explain the cause of DOMS including lactic acid, muscle spasm, connection tissue damage, muscle damage, inflammation and enzyme efflux theory (Cheung et al., 2003). The current consensus is that these factors may each contribute to DOMS to some degree (Armstrong, 1984; Lewis et al., 2012).

DOMS may potentially affect any skeletal muscle that has been exercised to a sufficient extent, generally in exercises utilizing eccentric contractions. DOMS is generally localized in the distal portion of the muscle, typically near the muscle-tendon junction (Armstrong, 1984). The proposed causes for sensitivity localization are varied and include

increased concentration of pain receptors in this area (Kumazawa & Mizumura, 1977) or a higher incidence of muscle damage in specific portions of the muscle (Newham, Mills, Quigley, & Edwards, 1982). During severe DOMS results in pain that is not localized, but rather spread throughout the muscle (Newham, Mills, Quigley, & Edwards, 1983).

Eccentric contractions are usually used in research models to induce symptoms of DOMS in research subjects (Tiidus, 2008). Among the three types of contractions (eccentric, concentric and isometric), eccentric contractions produce the most tension on muscle and connective tissues (Lieber & Friden, 2002). Asmussen (1956) first established that DOMS is primarily associated with eccentric contractions.

Although many studies have measured DOMS and use it as an indirect indicator of EIMD, it is not clear that DOMS and EIMD are always correlated. Nosaka et al. (2002) found that DOMS did not increase in accordance with EIMD measured by other indirect markers, such as muscle strength. Additionally, the sensation of pain is individual, difficult to quantify and different subjects may interpret any given pain scale differently. The authors concluded that “it should be noted that preventative or treatment measures for DOMS are not necessarily the same as those for muscle degeneration and regeneration.”

A number of studies have investigated different treatments for DOMS that may reduce pain and performance decrements in the days following muscle damaging exercise including “cryotherapy, stretching, anti-inflammatory drugs, ultrasound, electrical current techniques, homeopathy, massage, compression, hyperbaric oxygen and exercise” (Cheung et al., 2003). In general, results have been equivocal and there is no consensus regarding the efficacy of treatments for EIMD and DOMS (Cheung et al., 2003).

Non-steroidal anti-inflammatory drugs (NSAIDs) which are commonly used by athletes to prevent and treat soreness, have been an area of interest in EIMD and DOMS research. (Paoloni, Milne, Orchard, & Hamilton, 2009). Some success in the treatment of DOMS has been seen with non-steroidal anti-inflammatory (NSAID) use, and NSAIDs are commonly recommended by doctors and used by athletes for the purpose of treating exercise-related muscle and joint pain (Paoloni et al., 2009).

Naproxen sodium (commonly sold as Aleve) improved strength recovery, lessened swelling measured by cross-sectional area and improve sleep and morning activities compared to placebo (Dudley et al., 1997). Like PCSO-524™, NSAIDs act to inhibit “the enzyme cyclo-oxygenase and thereby decreas[e] the production of prostaglandins which are capable of mediating the inflammatory response following injury” (Almekinders, 1999).

However, not all studies have found positive effects from NSAID treatment. Gulick et al. (1996) investigated a number of treatments including “a nonsteroidal anti-inflammatory drug, high velocity concentric muscle contractions on an upper extremity ergometer, ice massage, 10-minute static stretching, topical *Arnica montana* ointment, and sublingual *A. montana* pellets.” None of the treatments were effective in reducing DOMS and “the NSAID and *A. montana* treatments appeared to impede recovery of muscle function” (Gulick et al., 1996). Similarly, ibuprofen did not affect muscle soreness or reduce decrements in endurance performance following downhill running (Donnelly et al., 1990).

Some success has been found in other treatments for symptoms of EIMD. Phillips et al. (2003) saw reduced interleukin-6 (IL-6) and C-reactive protein, two proteins involved in the acute inflammatory response, in subjects taking a supplement containing tocopherols, flavonoids, and docosahexaenoate. Specialized clothing has also been hypothesized to reduce

DOMS symptoms. Zhang et al. (2000) found significant differences in pain, strength and several blood markers of muscle damage in response to a treatment involving an electromagnetic shielding fabric. A meta-analysis of 12 studies that evaluated the effectiveness of compression garments concluded that such clothing has a moderate effect on the severity of DOMS resulting from eccentric exercise (J. Hill, Howatson, van Someren, Leeder, & Pedlar, 2013).

Peripheral Muscle Fatigue

Fatigue is a “reversible decline in force production by muscles when contracting at or near their maximum capacity” (Bishop, 2012), where the ability of the muscle to perform work, maintain force or produce power is compromised (Asmussen, 1979; Bigland-Ritchie, Johansson, Lippold, & Woods, 1983). The decline in capacity may be identified by reduced power output during cycling, reduced speed during running or reduced ability to produce force during lifting exercises, for example.

The contributing factors to fatigue have been generally categorized as peripheral factors or central factors. The classic model of fatigue describes conditions in the periphery that inhibit the contractile function of muscle. Cellular changes that contribute to fatigue may include accumulation of inorganic phosphate, impaired calcium reuptake, acidosis, reduced glycogen availability and the production of reactive oxygen species in muscle tissue (Allen, Lamb, & Westerblad, 2008). Slowed cross bridge attachment may also be a molecular factor in fatigue (Jones, Turner, McIntyre, & Newham, 2009). Central nervous system factors contribute to fatigue during exercise. A reduction in central nervous system efferent output has been observed following strenuous exercise (Brasil-Neto, Cohen, & Hallett, 1994).

An integrated model of fatigue, the Central Governor Model, describes fatigue as a mechanism to protect whole-body homeostasis during exercise and the sensation of fatigue as an emotion reflecting sensory input from the periphery (Noakes, 2012). The reduction in power output during prolonged cycling exercise, for example, may be a result of a number of integrated factors including motivation, self-belief, pacing strategy, previous experience, environmental conditions and afferent feedback from the periphery (Noakes, 2011).

In order to isolate peripheral, central and integrated fatigue factors in muscle fatigue, techniques have been developed to study muscle function *in vivo* by way of stimulating efferent nerves, usually using surface electrodes, implanted electrodes or magnetic stimulation. Through bypassing the central nervous system, an “effort independent measure of muscle contractile performance” may be obtained (Kufel, Pineda, & Mador, 2002; Polkey et al., 1996).

Muscle contractile function is affected by the recent contraction history of the muscle fiber. Twitch force is either measured as potentiated or unpotentiated contractions. Potentiated twitches are those that are induced after recent maximal voluntary contraction or tetanic stimulation, while unpotentiated twitches are performed on a rested, not recently contracted muscle (Kufel et al., 2002). Fatigue can be detected in the quadriceps muscle effectively using a potentiated twitch model more effective than with unpotentiated twitches (Kufel et al., 2002; Polkey et al., 1996). Kufel et al. (2002) investigated both potentiated ($Q_{tw,pot}$) and unpotentiated ($Q_{tw,unpot}$) following 10, 15, 20 and 30 MVC maneuvers of 5 seconds each. $Q_{tw,pot}$ fell by as much as about 40% after 30 MVC maneuvers. Polkey et al. (1996) showed that quadriceps fatigue can be detected using a magnetic stimulator following a protocol in which subjects maintain contractions at 60% of MVC for as long as possible.

ω 3FA supplementation may be related to improved muscle fatigue resistance during exercise and metabolic stress. Pepe and McLennan (2002) investigated the effects of ω 3FA supplementation on oxygen consumption and recovery from ischemic stress in cardiac muscles. Male Wistar rats were supplemented with diets containing increased ω 3FA content (45% PUFA; 39% EPA and DHA), saturated fat (55% saturated fat) or a reference diet (46% PUFA; 34% n-6 PUFA) for 16 weeks. The researchers found that ω 3FA supplemented rats had a myocardial oxygen consumption and were able to recover from ischemic stress more readily than rats on the alternative diets.

Peoples et al. (2013) showed ω 3FA supplementation reduces skeletal muscle fatigue in a rat model during sustained electrically stimulated contractions. The diets of Wistar rats were manipulated to contain fat primarily from either saturated, n-3 or n-6 unsaturated fats. The n-3 group primarily consumed DHA. During 30 minutes of sustained isolated electrically stimulated hindlimb contractions, n-3 consuming rats developed greater maximum twitch tension, maintain higher peak twitch tension throughout compared to the saturated and n-6 groups. The n-3 group had attenuated changes in twitch development and faster twitch development, relaxation rates, shorter rise and fall time and twitch duration. Potential mechanisms for the differences seen in this study may include protection of the Ca^{2+} cycling process and an attenuated impairment of Ca^{2+} removal from the sarcoplasmic reticulum (Peoples & McLennan, 2013).

CHAPTER 3

METHODS

Subjects

Forty untrained men ages 18-26 were recruited from the surrounding Bloomington area to participate in the investigation. Thirty-four subjects completed the study. The reasons for subjects leaving the study were difficulty with venous blood draw (3 subjects), family emergency (1 subject), job conflict (1 subject), and not returning to the laboratory following supplementation (1 subject). Data from two subjects were discarded, initially based on inspection of DOMS data indicating implausible values or measurement error. Women were not recruited to this study as it is unclear whether the menstrual cycle and associated hormones affect markers of EIMD (Clarkson & Hubal, 2002).

Subjects were classified as ‘untrained’ if they exercised less than three times per week for less than 30 minutes during each session. Subjects were excluded if they had a history of significant pain in hips or knees, had participated in a strength training program with 60 days prior to their screening for the study, or regularly used anti-inflammatory medication or nutritional supplements. Subjects were screened for risks factors per American College of Sports Medicine guidelines for age, body mass index, blood pressure, cholesterol, and fasting blood glucose. Subjects were excluded from the study if they did not meet the conditions outlined in **Table 3**.

Table 3: Study screening criteria for exercise risk factors

	Criteria
Age	Subjects were accepted only if they were between 18 and 30 years of age.
Body Mass Index	Subjects were accepted only if they have a BMI < 30.
Blood Pressure	Subjects were accepted only if their blood pressure was less than 140/90 mmHg.
Cholesterol	Subjects were accepted only if their total cholesterol count was less than 200 mg/dL.
Fasting Blood Glucose	Subjects were accepted only if their fasting blood glucose was less than 100 mg/dL.

Untrained subjects were chosen for this study (as opposed to trained, well-trained or elite athletes) on the basis of data from previous research investigating eccentric exercise, EIMD/DOMS and PCSO-524™ supplementation that showed no effects among trained subjects (Pumpa et al., 2011) or greater effects among less-trained subjects (Baum et al., 2013). Pumpa et al. (2011) included well-trained men participating in sports including “Australian Football, cycling, middle and long distance running and rugby union”. Data from this study showed no differences in several measures of muscle damage and soreness between PCSO-524™ supplementation and placebo. Baum et al. (2013) noted greater differences in muscle soreness between PCSO-524™ supplementation and placebo groups among less-trained runners after a 30km run. Based on these results, the present study included untrained subjects as training status appears to play a role in the efficacy of PCSO-524™ supplementation to treat EIMD/DOMS.

Subjects were instructed to refrain from downhill running, stair running, resistance training, plyometric or other exercise that could cause muscle damage during the study. Subjects were instructed to refrain from using anti-inflammatory medication or nutritional supplements during the study. Subjects were instructed to refrain from increasing or modifying their exercise habits during the course of the study. Adherence to these instructions

was confirmed at each visit to the laboratory. The study was approved by the Indiana University Human Research Protection Program Committee (HRPP). All subjects gave their written informed consent to participate in the study as approved by the HRPP.

Study Design

A schematic of study procedures is presented below (**Figure 7**). Subjects were randomly assigned to either a PCSO-524™ supplementation group receiving 8 capsules of PCSO-524™ (800 mg olive oil, 400 mg lipid extract) (n=16) or a placebo group receiving 8 capsules containing olive oil (1200 mg olive oil) (n=16) for 30 days. A non-involved researcher prepared supplement bags to ensure both investigators and subjects were blinded.

A non-crossover design was chosen for this study to avoid the “repeated-bout effect” as a confounding variable. The repeated-bout effect describes protective effect of an initial damaging exercise bout on EIMD and DOMS during a subsequent exercise bout in the weeks and months following (Tiidus, 2008, pp. 69–70). Cleary et al. (2002) determined that the repeated bout effect lasts as long as nine weeks following the initial exercise bout. Other data has shown that the repeated bout effect can last as long as six months (Tiidus, 2008, p. 70). Due to the repeated-bout effect, EIMD studies usually do not use a crossover design because subjects would not be expected to respond to an exercise bout in the same manner multiple times without lengthy delays between trials.

Prior to the supplementation period, baseline measurements for peak aerobic exercise capacity (VO_{2max}), 20 minute cycle ergometer time trial performance, quadriceps muscle fatigue following the time trial, skeletal troponin I and quadriceps soreness were taken. Each subject completed a screening and familiarization session, a VO_{2max} testing session and exercise performance testing on different days.

After 26 days of supplementation, post-supplementation measures of skeletal troponin I and quadriceps soreness were taken. On the same day, subjects performed downhill running at -16% incline for 20 minutes at 70% of heart rate reserve (HRR), an eccentric exercise bout intended to produce muscle damage and peripheral muscle fatigue and modeled after the protocol used by Sorichter et al. (1997). Running speed was heart rate monitored. The target heart rate was 70% HRR, which was determined in the VO_{2max} test from resting and maximum heart rate. The downhill running protocol was performed on a treadmill (A. R. Young Power Transmission Engineers, Indianapolis, IN) that was adapted to allow the belt to run in reverse. Skeletal troponin I was evaluated at immediately following, two hours, 24 hours, 48 hours, 72 hours and 96 hours following the downhill running protocol. Perceived soreness/pain (DOMS) was evaluated at 24 hours, 48 hours, 72 hours and 96 hours following the downhill running protocol.

Twenty-four hours following the eccentric exercise bout, subjects performed a 20 minute cycle ergometer time trial. Quadriceps muscle fatigue was measured following the time trial. The timing for the time trial was chosen to correspond with expected peaks in strength loss, fatigue, range of motion and beginning of significant soreness (Tiidus, 2008), and thus have a potential effect on performance.

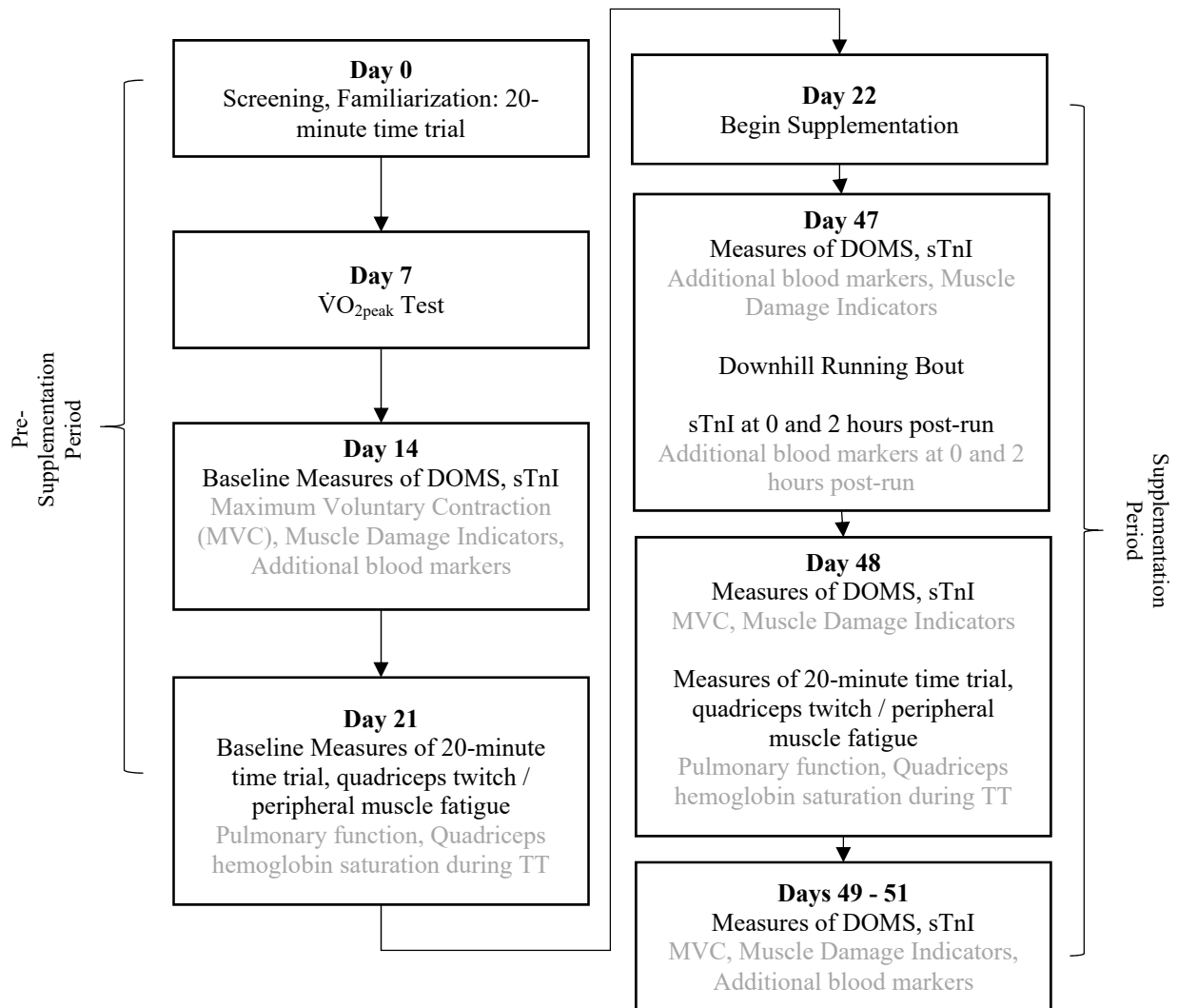


Figure 7: Schematic of Study Procedures. Procedures/Measurements in Light Grey were collected by another investigator during the same study. Muscle damage indicators include range of motion (ROM), thigh circumference and pressure sensitivity. Additional blood markers include heart-type fatty acid binding protein (h-FABP), myoglobin (MB), creatine kinase (CK), 8-hydroxydeoxyguanosine (8-OHdG), tumor necrosis factor (TNF- α) and interleukin-6 (IL-6).

Measurements

Maximal Aerobic Exercise Capacity ($\dot{V}O_{2max}$)

Subjects performed a maximal aerobic exercise capacity test on a motor driven treadmill (Model 18-60, Quinton, Seattle, WA). Subjects were briefed on the procedures of the test and safety precautions prior to beginning the test. A warm-up period of 5 minutes was

used to determine a running speed for each subject. After fitting the subject with a heart rate monitor (Polar Electro Inc., Lake Success, NY) and breathing mask (7450 Series V2, Hans Rudolph, Shawnee, KS USA), the test began with 5 minutes of seated rest while equipment function was verified and baseline metabolic measurements were collected.

The exercise portion of the test began with each subject running at 0% grade and a speed of 1.6 k/h less than the selected speed for 2 minutes. Following the initial stage, the speed was increased to the predetermined speed for 3 minutes. Then, the slope of the treadmill was increased to 4% for 3 minutes, and increased an additional 2% every 3 minutes until volitional exhaustion or valid test criteria were met (see below). Subjects were asked at the end of each minute if they could continue the test.

Tests were considered valid if they met two of the following criteria: 1) reaching a heart rate $\geq 90\%$ of the predicted maximum heart rate ($220 - \text{age}$), 2) a respiratory exchange ratio (RER) ≥ 1.10 , and 3) ≤ 150 mL increase in $\dot{V}O_2$ with an increase in treadmill slope.

Ventilatory and metabolic data were collected using open-circuit, indirect calorimetry. Dried expired gases were sampled at a rate of 300 mL/min for fractional concentrations of O_2 and CO_2 using an Applied Electrochemistry S-3A oxygen analyzer and a CD-3A carbon dioxide analyzer (Ametek, Thermox Instruments, Pittsburgh, PA). Inspired ventilation was measured with a pneumotachometer (Hans Rudolph 3813). \dot{V}_E was calculated using the Haldane Transformation.

Twenty Minute Cycle Ergometer Time Trial

All subjects completed three 20 minute time trials on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, Washington, USA). The first time trial was used for familiarization purposes. The second time trial occurred prior to supplementation. The third

time trial occurred after 26 days of supplementation and 24 hours following the downhill running protocol. Average power output was assessed at each 5 minute interval to assess performance. Heart rate (Polar, Polar Electro Oy, Kempele, Finland), power output, and rating of perceived exertion were recorded. The timing for the time trial was chosen to correspond with expected peaks in strength loss, fatigue, range of motion and beginning of significant soreness after eccentric exercise (Tiidus, 2008), and thus have a potential effect on performance.

Subjects were informed that the goal of the time trial was to achieve the highest possible average power output. Subjects were informed of their progress via a computer program (CompuTrainer 3D, RacerMate Inc., Seattle, Washington, USA) on a display that displayed elapsed time, current power output, average power output, heart rate and a simulated bicycle gear indicator. The cycle ergometer was adjusted for each subject's seat and handle height preferences prior to the initial time trial. Adjustments for cycling position were recorded for each subject and repeated for subsequent time trials. A continuously running fan was placed near the bicycle to provide a degree of air flow and cooling.

Potentiated Quadriceps Twitch Force

Quadriceps muscles are employed during cycling for a substantial portion of the pedal stroke (Jorge & Hull, 1986). During prolonged or high-intensity cycling, decrements in the force generating capability of the quadriceps muscles are observed (Lepers, Maffiuletti, Rochette, Brugniaux, & Millet, 2002). One of the methods that has been used to demonstrate non-volitional neuromuscular fatigue following cycling is measurement of quadriceps twitch force (Lepers et al., 2002).

In the present study, potentiated quadriceps twitch force was measured in the subject's

left leg to quantify an index of muscle fatigue following the cycle ergometer time trial. Muscle twitch force is typically measured in either a potentiated or unpotentiated state. Potentiated twitches are those that are induced after recent maximal voluntary contraction or tetanic stimulation, while unpotentiated twitches are performed while the subject has been at rest (Kufel et al., 2002). Fatigue of the quadriceps muscles is more reliably and easily detectable when a potentiated twitch is measured, as opposed to an unpotentiated twitch (Kufel et al., 2002).

The subject was supine on a table with a left knee joint angle of 90 degrees. The subject's ankle was wrapped in a non-compliant strap, placed just superior to the ankle malleoli. The strap was attached to a calibrated load cell (Model Z Tension Load Cell, Dillon, Fairmont, MN) for the measurement of force connected to a custom amplifier (Hector Engineering Co. Inc., Ellettsville, IN). A magnetic stimulator (Magstim 200, Magstim, Whitland, UK) connected to a double 70 mm coil was used to stimulate the femoral nerve, causing an involuntary contraction of the quadriceps muscle.

Nerve stimulation followed two protocols, following a protocol described previously (Amann et al., 2007; Kufel et al., 2002; Polkey et al., 1996):

Assessment of maximal nerve stimulation

Prior to the cycle ergometer time trial, a series of single twitches were obtained at varying levels of stimulator intensity (80%, 85%, 90%, 95%, and 100% of maximal stimulator power output) to determine when supramaximal stimulation had been reached. The position of the stimulator coil was placed over the femoral triangle and adjusted to determine an acceptable location for each subject. Stimulator placement was determined to be acceptable when repeatable and measurable quadriceps contractions were obtained. Stimulator placement

was marked on the subject's skin with an indelible marker to insure repeatability of the location and measurement. A typical twitch force curve for the MagStim 200 stimulator is pictured below. Typical stimulator output required to achieve supramaximal stimulation has been found to be a mean of 83% of stimulator output.

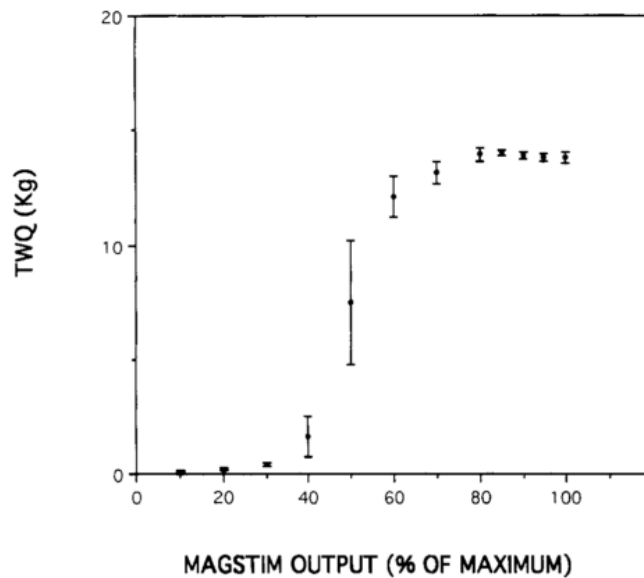


Figure 8: Twitch tension versus MagStim power output (*Polkey et al., 1996*). Twitch tension plateaus at a mean power output of 83% using a MagStim 200 stimulator (MagStim Co. Ltd., Whitland, Dyfed, Wales).

Assessment of fatigue

Prior to and immediately following the cycle ergometer time trial, an assessment of quadriceps twitch force ($Q_{tw,pot}$) was performed. Twitch force prior to the time trial was used as a baseline for twitch force obtained after the time trial. The assessment of fatigue protocol consisted of six repetitions of potentiation and magnetic stimulation with 30 seconds of rest between repetitions. For each repetition, subjects performed a maximal voluntary isometric contraction (MVC) of the quadriceps muscle for 5 seconds. At the end of the 5 second MVC, the subject received a supra-maximal magnetic stimulation of the femoral nerve, and a second stimulation after 5 seconds of rest. The force produced during the second twitch of each

repetition was recorded as $Q_{tw,pot}$. Force values from the first two repetitions were discarded based on previous findings that the degree of potentiation is smaller after the first two measurements (Amann et al., 2007). Force values from the final four repetitions were averaged to produce a $Q_{tw,pot}$ force value for each trial.

Delayed Onset Muscle Soreness

Quadriceps soreness was assessed using a numeric rating pain scale with “no soreness” indicated at one end (score 0) and “unbearably painful” at the other (score 10) (Downie et al., 1978). The subject stood with hands on hips and feet approximately shoulder width apart. The subject was asked to squat down to 90° (internal angle), rise to the start position and then indicate on the numeric scale the soreness felt in the lower limbs (Twist & Eston, 2005).

Skeletal Muscle Troponin I

Blood samples (10 mL) were taken from the antecubital vein prior to supplementation, following 26 days of supplementation, immediately following the downhill running protocol and at 2, 24, 48, 72, and 96 hours following the downhill running protocol. Each sample was put on ice for 30 minutes and centrifuged at 3000 RPM for 20 minutes. Plasma was pipetted into 4-6 storage tubes. Red-blood cells were stored in Vacutainer® into which the sample was collected. Plasma samples were stored at -80°C until being analyzed. RBC samples were also stored at -80°C but were not analyzed during this study.

Skeletal muscle troponin I, (Usen Life Science Inc., Wuhan, Hubei PRC) was analyzed from plasma using enzyme-linked immunoassay (ELISA) according to the manufacturer’s instructions. An ELx405™ Automated Plate Washer (Bio-Tek® Instruments, Inc., Winooski, VT) was used to wash the 96-well microplates during the pre-assay

preparations. The assay was performed using a Powerwave XSTM Spectrophotometer (Bio-Tek® Instruments, Inc., Winooski, VT).

Data Analysis

Data were analyzed using SPSS 20.0 (IBM Corporation, Chicago, IL, USA) statistical software. Dependent variables were analyzed for each condition for differences with a one-way, within-factors repeated measures analysis of variance (ANOVA) within groups and *a priori* independent t-test between groups to analyze simple main effects. Mauchly's test was conducted to determine whether sphericity was violated. If sphericity was violated, the repeated-measures ANOVA was corrected using the Greenhouse–Geiser correction factor. Statistical significance was set at $p < 0.05$. Data are presented as mean \pm standard deviation.

To determine an appropriate sample size for the study, post-hoc power analysis of existing literature was conducted using G*Power version 3.0.5 (Universität Kiel, Germany) (Faul, Erdfelder, Lang, & Buchner, 2007). Effect sizes are presented as mean differences between groups divided by standard deviation. Based on previous literature (Fontani et al., 2005; Guezennec et al., 1989; Santos et al., 2012; Tartibian et al., 2009, 2011), achieving an experiment-wise error rate of 0.05 required 15 subjects within each treatment group. In two studies, Tartibian et al. (2009, 2011) has shown that ingestion of ω 3FAs (n=9-15) for 30 days compared to placebo/control (n=9-15) significantly reduced inflammatory markers and perceived pain and symptoms following eccentric exercise, with effect sizes ranging from 0.64-0.75 for a study power of 0.82 and 0.84 respectively. Santos et al. (2012) showed that following 30 days ω 3FA supplementation (n=10), compared to placebo (n=10), a number of inflammatory markers were decreased directly after 5 days of military survival boot camp (increased physical stress/restricted caloric intake) for effect sizes ranging from 0.61-0.75 for

study power of 0.84. A sample size of 20 per group was chosen to provide an expected 96% power to detect differences. Study power was expected to be maintained at 80% if five subjects withdrew or were dropped from each group in the study.

CHAPTER 4

RESULTS

The purpose of this study was to evaluate whether PCSO-524TM supplementation attenuates EIMD-related endurance exercise performance impairment, peripheral muscle fatigue and symptoms of DOMS in untrained men following eccentric exercise.

Thirty-two subjects (treatment group: n=16, placebo: n=16) completed the study. Subject characteristics (**Table 4**) did not differ significantly ($p > .05$) for age, height, BMI or VO_{2max} and relative VO_{2max} . Mass was significantly different between groups ($p < .05$).

Table 4: Subject Characteristics

	PCSO-524 TM	Placebo	<i>p</i>
Age	21.7 ± 1.7	21.5 ± 2.4	0.80
Height (cm)	178.1 ± 5.8	174.2 ± 6.7	0.09
Mass (kg)	74.8 ± 8.8	66.6 ± 9.7	0.02
BMI	23.6 ± 2.9	21.9 ± 2.8	0.10
VO_{2max} (L)	3.38 ± 0.50	3.00 ± 0.63	0.07
VO_{2max} relative (mL/kg/min)	46.4 ± 6.2	45.63 ± 6.1	0.73

20 Minute Cycle Ergometer Time Trial Performance

Because body mass between the groups was significantly different, power output was compared both for mean power and mean power relative to body mass. There were no significant differences ($p > .05$) in power output between groups due to the supplement or within groups due to eccentric exercise on cycle ergometer time trial performance.

Mean power output during the time trial did not change significantly after eccentric exercise for the treatment ($F = .106, p = .75$) or placebo groups ($F = .122, p = .73$) (**Table 5**). Mean power output relative to body mass did not change significantly after eccentric exercise for treatment ($F = .077, p = .79$) or placebo subjects ($F = .288, p = .60$) (**Table 6**).

No significant differences were found in mean power output between groups before supplementation ($T = 1.079, p = .29$) or after eccentric exercise ($T = .1410, p = .17$). No significant differences were found in mean power output relative to body mass between groups before supplementation ($T = .258, p = .80$) or after eccentric exercise ($T = .026, p = .98$).

Table 5: Time Trial Power Output, Pre-Supplementation and 24 hours Post-Eccentric Exercise (Mean \pm SD)

Power Output (watts)	PCSO-524™	Placebo
Pre-Supplementation	153.3 \pm 36.5	139.1 \pm 38.2
24H Post-Downhill Run	154.9 \pm 30.9	137.8 \pm 37.3

Table 6: Time Trial Relative Power Output, Pre-Supplementation and 24 hours Post-Eccentric Exercise (Mean \pm SD)

Relative Power Output (watts/kg)	PCSO-524™	Placebo
Pre-Supplementation	2.07 \pm .46	2.11 \pm .57
24H Post-Downhill Run	2.08 \pm .39	2.08 \pm .55

RPE increased significantly for each group during each time trial ($p < .05$ for each group and time trial). The treatment group had significantly higher RPE at the end of the second time trial compared to the first ($T = 2.3, p = .04$; 95% CI = .05 – 1.32). The placebo group had significantly higher RPE at the beginning of the second time trial compared to the first ($T = 2.356, p = .03$; 95% CI = 0.13 – 2.61). RPE was not different between groups at 0, 5, 10, 15 or 20 minutes during the pre-supplementation time trial ($p > .05$ for all time points) and the post-supplementation time trial ($p > .05$ for all time points) (Table 4 and Table 5).

Table 7: Rate of Perceived Exertion during Pre-Supplementation Time Trial

Time (min)	PCSO-524™	Placebo	<i>p</i>
0	8.6 ± 1.5	8.7 ± 1.9	.92
5	12.6 ± 1.6	12.8 ± 2.0	.70
10	14.5 ± 1.6	14.6 ± 1.8	.92
15	15.9 ± 1.5	15.9 ± 1.8	1.00
20	17.3 ± 1.8	17.3 ± 1.9	.92

Table 8: Rate of Perceived Exertion during Post-Eccentric Exercise Time Trial

Time (min)	PCSO-524™	Placebo	<i>p</i>
0	9.0 ± 1.9	10.1 ± 2.6	.19
5	12.8 ± 1.3	12.9 ± 2.2	.84
10	14.6 ± 1.2	14.6 ± 2.0	.92
15	15.8 ± 1.4	15.5 ± 2.0	.69
20	17.9 ± 1.8	17.3 ± 1.8	.36

The treatment group had significantly lower heart rate during the post-supplementation time trial at 5 minutes ($T = 2.311$, $p = .04$; 95% CI = 0.6 – 15.4) and 10 minutes ($T = 2.239$, $p = .04$; 95% CI = 0.3 – 13.4) compared to the pre-supplementation time trial. The placebo group did not have any significant differences during any point of the two time trials ($p > .05$ for 0, 5, 10, 15, 20 minute time points). Heart rate was significantly lower in the treatment group only at the start of the second time trial (**Table 9**) compared to the placebo group (treatment: 106 ± 17 bpm, placebo: 127 ± 18 bpm, $p < .01$). No other significant differences ($p > .05$) were found for heart rate between groups during the two time trials.

Table 9: Heart Rate during Pre-Supplementation Time Trial

Time (min)	PCSO-524™	Placebo	<i>p</i>
0	113 ± 19	122 ± 15	.16
5	153 ± 16	160 ± 18	.25
10	159 ± 15	167 ± 17	.22
15	162 ± 14	172 ± 14	.08
20	171 ± 12	180 ± 14	.07

Table 10: Heart Rate during Post-Supplementation Time Trial

Time (min)	PCSO-524™	Placebo	<i>p</i>
0	106 ± 17	127 ± 18	< .01
5	145 ± 18	157 ± 21	.09
10	153 ± 19	165 ± 19	.08
15	158 ± 16	169 ± 21	.09
20	172 ± 14	181 ± 16	.13

Delayed Onset Muscle Soreness

Muscle soreness increased in both groups after the eccentric exercise protocol, peaking between 24 and 48 hours and declining toward baseline at 72 and 96 hours (**Error! Reference source not found.**). Significant effects for time were found in the treatment group ($F = 22.071, p < .001$) and placebo group ($F = 15.108, p < .001$). Pairwise comparisons between groups at each time point revealed significantly lower DOMS in the treatment group at 72 hours ($T = 2.475, p < .05; 95\% \text{ CI} = 0.252 - 2.623$) and 96 hours ($T = 2.100, p < .05; 95\% \text{ CI} = 0.038 - 2.712$). No significant differences ($p > .05$ for all time points) were found between groups at pre-supplementation, post-supplementation, 24 hours post-DHR and 48 hours post-DHR (**Table 11**).

Table 11: DOMS Pain Scores

Time point	PCSO-524™	Placebo	<i>p</i>
Pre-supplementation	2.1 ± 1.5	2.1 ± 1.5	.66
Post-supplementation	1.8 ± 1.8	1.4 ± 1.5	.53
24H Post-Run	4.5 ± 2.5	4.4 ± 2.1	.88
48H Post-Run	4.3 ± 2.0	4.8 ± 1.8	.46
72H Post-Run	2.5 ± 1.5	3.9 ± 1.8	.02
96H Post-Run	1.6 ± 1.4	2.9 ± 2.2	.04

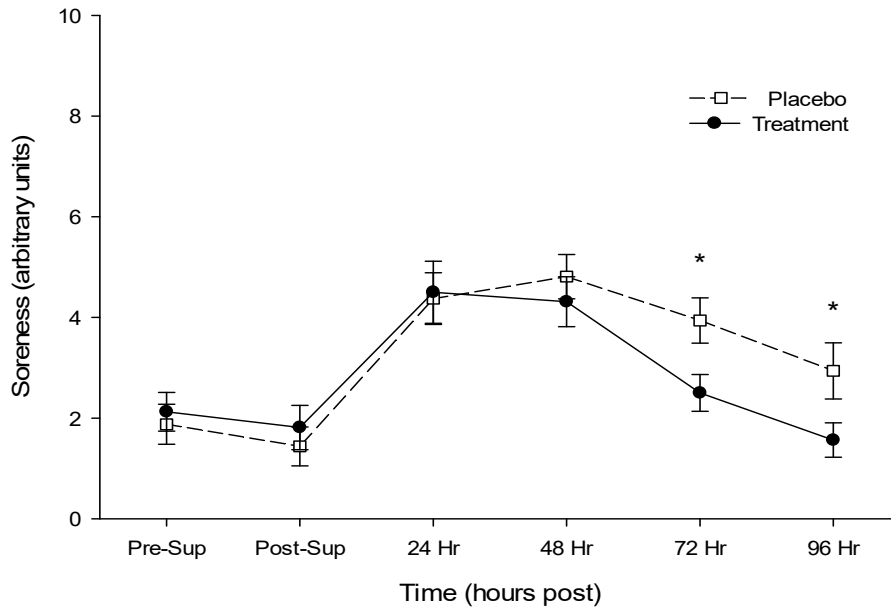


Figure 9: Quadriceps muscle soreness was lower at 72 and 96 hours post-eccentric exercise in the treatment (PCSO-524™ marine lipid fraction, n=16) group compared to placebo (n=16). Soreness is a numeric rating scale from 0 (no pain) to 10 (unbearably painful). Bars represent SE. * denotes significant difference between groups ($p < .05$)

Potentiated Quadriceps Twitch

Data is presented as a percent change in quadriceps twitch force ($Q_{tw,pot}$) from pre-time trial to post-time trial. *Post-hoc* paired, one-tailed t-tests revealed that treatment group $Q_{tw,pot}$ did not change significantly ($T = .38, p > .05$) between time trials, but the placebo group $Q_{tw,pot}$ change did significantly ($T = 2.095, p < .05$) between time trials. Between group differences in $Q_{tw,pot}$ were non-significant for the pre-supplementation time trial ($p = .21$) and the 24hrs post-eccentric exercise time trial ($p = .34$) (**Table 12**). It should be noted that post-supplementation quadriceps twitch data was not collected from one subject due to equipment error. Additionally, one outlier score was removed from the placebo group, leaving 16 PCSO-524™ subjects and 14 placebo subjects for this measure. The outlier score in the placebo group demonstrated a 211% increase in $Q_{tw,pot}$ from pre- to post-time trial at 24 hours post-

eccentric exercise, which is 3.4 SD from the mean with this score included and 7.1 SD from the mean with the score excluded.

Table 12: Percent Change in $Q_{tw,pot}$ following cycling time trial

$Q_{tw,pot}$ % Change (Pre to Post Time Trial)	PCSO-524™	Placebo	<i>p</i>
Pre-Supplementation Time Trial	-27.8 ± 26.2	-23.9 ± 24.0	.669
24hrs Post-Eccentric Exercise Time Trial	-30.4 ± 14.3	-39.5 ± 24.3	.243

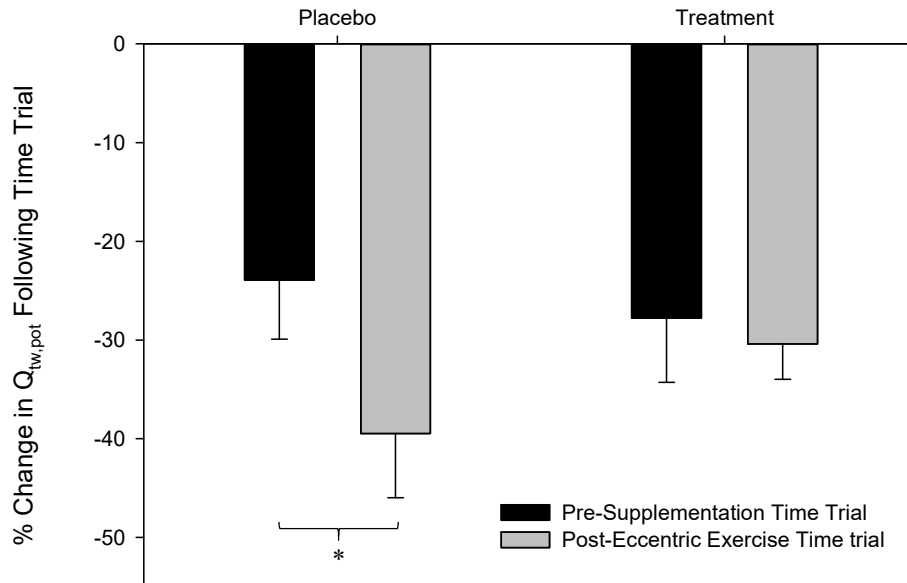


Figure 10: Potentiated quadriceps twitch force ($Q_{tw,pot}$) decreased significantly ($p < .05$) in the placebo group ($n=16$) after the post-eccentric exercise cycle ergometer time trial while no changes were observed in the treatment group ($n=16$). Bars are SE. * denotes significant difference between trials ($p < .05$)

Skeletal Muscle Troponin I

Significant effects for time (

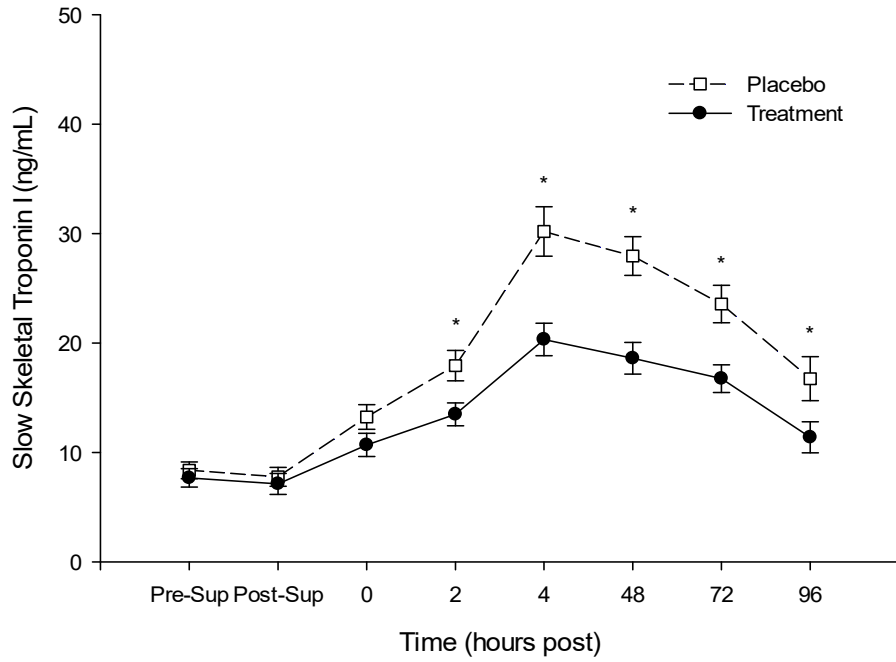


Figure 11: Concentration of slow skeletal troponin I in blood was higher at 2 – 96 hours in the placebo group (n=16) following eccentric exercise compared to the treatment group (n=16). Bars are SE. * denotes significant ($p < .05$) difference between groups.

) were found for skeletal muscle troponin I for the treatment group ($F = 30.964, p < .001$) and the placebo group ($F = 74.733, p < .001$). Pairwise comparisons (**Error! Reference source not found.**) between groups at each time point revealed significant differences at 2 hours ($T = 2.562, p < .05; 95\% \text{ CI} = 0.9 - 8.0$), 24 hours ($T = 3.561, p < .01; 95\% \text{ CI} = 4.4 - 15.4$), 48 hours ($T = 4.095, p < .001; 95\% \text{ CI} = 4.7 - 14.0$), 72 hours ($T = 3.222, p < .01; 95\% \text{ CI} = 2.5 - 11.2$) and 96 hours post-downhill run ($T = 2.177, p < .05; 95\% \text{ CI} = 0.3 - 10.4$). Differences between groups were not significant ($p > .05$) at pre-supplementation, post-supplementation and 0 hours post-eccentric exercise.

Table 13: Slow Skeletal Troponin I Appearance in Blood

sTnI (ng/ml)	PCSO-524 TM	Placebo	T	<i>p</i>
Pre-supplementation	7.7 ± 3.3	8.4 ± 3.1	.613	.54
Post-supplementation, pre-run	7.1 ± 3.8	7.8 ± 3.5	.504	.62
0 hours post-run	10.7 ± 4.2	13.2 ± 4.5	1.651	.11
2 hours post-run	13.5 ± 4.2	17.9 ± 5.6	2.562	.02
24 hours post-run	20.3 ± 6.0	30.2 ± 9.0	3.651	< .01
48 hours post-run	18.6 ± 5.8	28.0 ± 7.1	4.095	< .001
72 hours post-run	16.7 ± 5.0	23.6 ± 6.8	3.222	< .01
96 hours post-run	11.4 ± 5.7	16.7 ± 8.1	2.177	.04

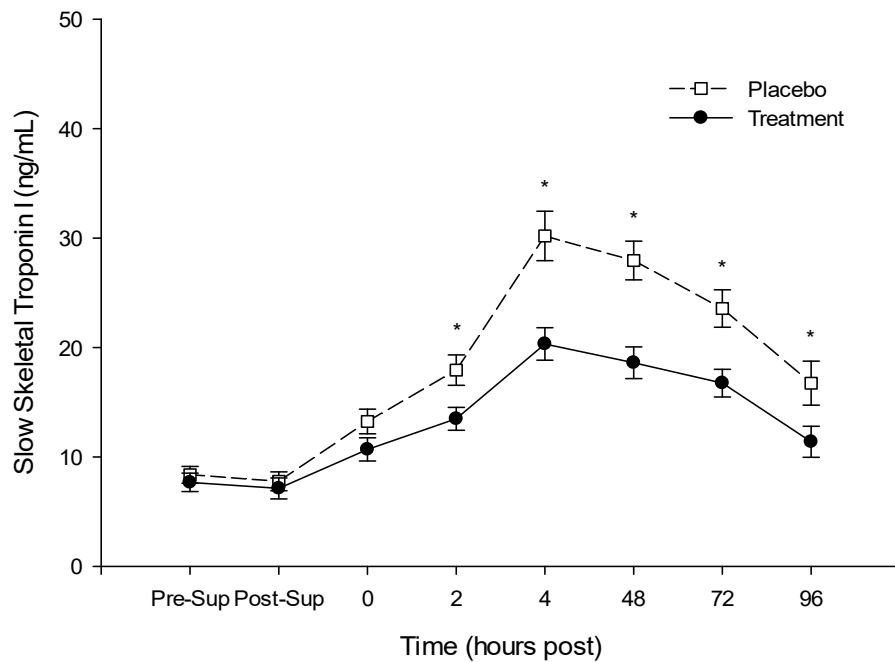


Figure 11: Concentration of slow skeletal troponin I in blood was higher at 2 – 96 hours in the placebo group (n=16) following eccentric exercise compared to the treatment group (n=16). Bars are SE. * denotes significant ($p < .05$) difference between groups.

Decision on the Hypotheses

1. Twenty minute cycling time trial mean power output was not greater in the PCSO-524™ supplementation group compared to the placebo group after eccentric exercise.
Hypothesis 1 was rejected.
2. Potentiated quadriceps twitch force decreased less following a 20 minute cycling time trial in the PCSO-524™ supplementation group compared to the placebo group.
Hypothesis 2 was accepted.
3. The concentration of skeletal troponin I (sTnI) in blood was lower in the PCSO-524™ supplementation group compared to the placebo group following eccentric exercise.
Hypothesis 3 was accepted.
4. Perceived quadriceps soreness during squatting was less in the PCSO-524™ supplementation group compared to the placebo group following eccentric exercise.
Hypothesis 4 was accepted.

CHAPTER 5

DISCUSSION

The purpose of this study was to evaluate whether or not endurance exercise performance, quadriceps muscle fatigue, muscle damage and muscle soreness following eccentric exercise by untrained men can be affected by PCSO-524™ marine lipid fraction supplementation. The data demonstrate that quadriceps muscle soreness, muscle fatigue and muscle damage can be lessened through chronic PCSO-524™ supplementation. However, endurance exercise performance following eccentric exercise was not affected by PCSO-524™ supplementation.

Delayed Onset Muscle Soreness

A novel finding of this study was that untrained men chronically supplemented with PCSO-524™ experienced less muscle soreness from 72 to 96 hours following eccentric exercise. DOMS-related pain was lower at 72 hours (treatment: 2.5 ± 1.5 , placebo: 3.9 ± 1.8 , $p < .05$) and 96 hours (treatment: 1.6 ± 1.4 , placebo: 2.9 ± 2.2 , $p < .05$) in the PCSO-524™ group compared to placebo. PCSO-524™ contains EPA, DHA and furan fatty acids, all molecules that have been found to inhibit the chain of events leading to DOMS (Wakimoto et al., 2011; Whitehouse et al., 1997).

Previously, PCSO-524™ supplementation had been found to attenuate DOMS in runners (Baum et al., 2013). Similar to Baum et al. (2013), the present study found PCSO-524™ was effective in attenuating symptoms of DOMS following strenuous exercise. Baum et al. (2013) noted a slight but significant reduction in DOMS in the 48 hours following a 30km run in runners who supplemented with 8 capsules PCSO-524™ per day for 11 weeks compared to no changes in DOMS for runners supplementing with a placebo. Median DOMS

scores in the PCSO-524™ group fell from 3.5 to 2.0 from the pre-supplementation to post-supplementation 30km run ($p < .05$), but no changes were found in the placebo group (median DOMS: pre = 2.0, post = 2.0, $p > .05$). In the present study, muscle soreness was lower in the treatment group at 72 and 96 hours following eccentric exercise but not at 24 – 48 hours as in Baum et al. (2013). The reason for the different time course for muscle soreness is unclear, although the cycling time trial at 24 hours post-eccentric exercise may have delayed recovery from muscle soreness in our study.

PCSO-524™ supplementation was most effective in alleviating symptoms of DOMS among runners who trained less than three times per week in the Baum et al. (2013) study, which is consistent with data from the present study. The present study confirms the finding that less well trained subjects respond to PCSO-524™ supplementation, as the subjects were all untrained men.

The inflammatory processes that are activated in response to exercise-induced muscle damage, and which PCSO-524™ supplementation can affect, involves a cascade of events which can lead to DOMS. Following a muscle cell damaging event, an influx of calcium into disrupted sarcomeres can activate PLA₂, which attacks membrane phospholipids and release fatty acids such as arachidonic acid (AA) (Gissel, 2005). When arachidonic acid is metabolized through the COX and 5-LOX pathways, pro-inflammatory prostaglandins and leukotrienes are produced. The production of these proteins and their diffusion into the intercellular space attracts monocytes to the area of injury. Monocytes then convert to macrophages that phagocytize the affected area and can produce reactive oxygen (ROS) species (Yaqoob & Calder, 1995). Edema, caused by an influx of fluid into the muscle, increases pressure in the muscle, which is thought to activate sensory neurons and lead to pain

felt as DOMS (Appell et al. 1992, Hasson et al. 1993). While an important step in the healing process, unchecked or overactive immune response to muscle damage may lead to additional damage and delayed recovery from muscle damage (Miller, Bailey, Barnes, Derr, & Hall, 2004).

The components PCSO-524™ have been shown to affect two aspects of the cascade of events leading to DOMS. First, EPA and DHA inhibit AA metabolism by replacing AA in the cell membrane (Heller, Koch, Schmeck, & van Ackern, 1998; Thies et al., 2001). When muscle damage occurs, activated PLA₂ may disrupt membrane phospholipids, but the metabolism of EPA and DHA leads to less inflammatory 3-series prostaglandins and 5-series leukotrienes. Secondly, furan fatty acids may be able to provide an antioxidant effect on ROS produced by macrophages in damaged muscle cell, limiting any further damage that could be caused by ROS (Wakimoto et al., 2011). Antioxidant supplementation such as Vitamin C has previously been shown to attenuate DOMS (Bryer & Goldfarb, 2006), and the furan fatty acids in PCSO-524™ may have a similar effect. In a mouse model, evidence of secondary muscle damage due to ROS was observed at three days post-exercise (A McArdle et al., 1999). In the present study, DOMS scores began to differ between treatment and placebo groups at three days post-eccentric exercise (treatment: 2.5 ± 1.5 , placebo: 3.9 ± 1.8 , $p < .05$). While speculative, the time course observed in the present study may suggest that a decrease of oxidative stress by furan fatty acids played a role in the observed results, which may be an avenue for future research.

In contrast to Baum et al. (2013) and the present study, Pumpa et al. (2011) did not find significant differences in DOMS after a downhill running protocol among subjects supplementing with PCSO-524™. Training status of the subjects in the Pumpa et al. (2011)

study may have contributed to the results observed with PCSO-524™ supplementation in relation to symptoms of EIMD. In the Pumpa et al. (2011) study, subjects were exclusively well-trained men participating in a variety of sports including Australian Football, cycling, middle and long-distance running and rugby. Given that less well trained individuals responded more to PCSO-524™ supplementation in the Baum et al. (2013) study and the present study, while well-trained individuals did not respond to supplementation in the Pumpa et al. (2011) study, it is possible that training status has some effect on the efficacy of PCSO-524™ supplementation in relation to DOMS.

Additionally, PCSO-524™ supplementation dosage may have contributed to the differences among these studies. Subjects in the Pumpa et al. study (2011) consumed four PCSO-524™ capsules per day (200 mg active lipid extract) for eight weeks. The present study used twice the dosage at eight capsules per day (400 mg lipid extract) for thirty days. Baum et al. (2013) reported supplementation of eight capsules per day (400 mg lipid extract) for 11 weeks. In studies investigating the effects of NSAIDs on DOMS symptoms, the supplement dosage has been found to be a factor in the effectiveness of treatment. Studies of ibuprofen as a treatment for DOMS have found contradictory results at difference dosages (Gulick et al., 1996).

The results for DOMS pain scores in the present study are consistent with the findings of Tartibian et al. (2009), where PUFA supplementation resulted in reduced indices of EIMD including muscle soreness, thigh circumference and range of motion. Tartibian et al. (2009) used fish oil (324 mg/day EPA, 216 mg/day DHA) supplemented over 30 days. Indices of DOMS were significantly lower at 48 hours in the treatment group (n=9) compared to the control (n=9) and placebo (n=9) groups.

Time Trials

The primary focus of the present study was to investigate the effects of eccentric exercise and PCSO-524™ supplementation on endurance exercise performance. While PCSO-524™ has not been investigated specifically as an ergogenic aid during exercise, ω 3FA have been investigated for ergogenic effects during exercise. PCSO-524™ contains a high proportion of ω 3FA (21% EPA, 13% DHA, 6% other ω 3FA by weight) (Wolyniak et al., 2005) and the results from ω 3FA supplementation research may apply to PCSO-524™ supplementation. The present study did not find a significant change in 20 min time trial performance (pre-supplementation: 153.3 ± 36.5 W, post-supplementation: 154.9 ± 30.9 W, $p > .05$) in subjects supplemented with PCSO-524™ for thirty days.

Previous research has found that vertical jumping impairs endurance cycling performance in a 5 minute cycle ergometer time trial (Twist & Eston, 2009). In contrast, our data showed that 20 minute cycling performance is not affected by a previous bout of eccentric exercise. Several differences in the design of the present study and that of Twist and Eston (2009) may account for these differences, including the eccentric exercise protocol, endurance performance test and subject characteristics.

The present study used untrained men as subjects, while Twist and Eston (2009) used physically active male subjects. The exercise performance testing protocol used in the present study consisted of a 20 minute time trial. In contrast, Twist and Eston used a 5 minute time trial. Additionally, Twist and Eston (2009) used a vertical jumping protocol to induce muscle damage while the present study used a downhill running protocol. Vertical jumping exercise would be expected to recruit primarily Type II muscle fibers, while in the present study,

exercise intensity was chosen at 70% of HRR to primarily target Type I fibers for fatigue and muscle damage.

Twist and Eston (2009) found significant decreases in mean power output during a 5 minute time trial following a vertical jumping protocol (baseline: 262.8 ± 17.7 W, 48 hours post-exercise: 232.8 ± 15.3 W; $p < .05$). In contrast, the present study did not find a change in 20 minute power output at 24 hours post-eccentric exercise with a mean power output during the pre-supplementation time trial of 146.2 ± 37.5 W among all subjects and during the post-downhill run time trial of 146.3 ± 34.8 W ($p > .05$).

Other studies have reported decrements in endurance exercise tasks following eccentric exercise, which contrast with the results of the present study. Doncaster and Twist (2012) observed that bench press exercises resulted in decreased strength and increased soreness in the elbow flexors and extensors at 24 and 48 hours following exercise. In the performance measure for this study, time-to-exhaustion during cycling arm cranking at 80% of the difference between ventilatory threshold and VO_{2peak} was lower in eccentric exercise subjects compared to control (treatment: 207.2 ± 91.9 sec, control: 293.4 ± 75.6 sec, $p = 0.036$). Burt and Twist (2011) found decreased performance among recreationally active subjects ($n = 8$, 7 men, 1 woman) in a 15 minute cycling time trial (preloaded with 5 minutes at ventilatory threshold) at 48 hours following plyometric exercises designed to cause EIMD (pre-exercise: 189.71 ± 38.19 W, post-exercise: 168.79 ± 37.68 W, $p < .05$). Marcora and Bosio (2007) also reported decreased endurance running performance during a 30 minute time trial at 48 hours following EIMD.

However, not all researchers have found decrements in endurance exercise performance following EIMD. Gleeson et al. (1998) did not observe a significant change in

time to exhaustion during cycling after bench stepping exercise. This result is similar to the lack of change in cycling performance seen in the present study after downhill running. Peak blood lactate was higher in the DOMS condition during the cycle ergometer exercise test, while $\dot{V}O_{2\text{peak}}$, HR_{peak} and endurance time were not significantly different. A possible explanation is additional recruitment of type II glycolytic muscles, which may explain increased blood lactate without a change in endurance performance (Gleeson et al., 1998). However, blood lactate was not measured in the present study, so it is not possible to conclude that increased recruitment of type II muscle fibers occurred.

Potential explanations for the lack of effect on cycling performance from eccentric exercise in the present study may be related to the timing of the exercise test relative to EIMD, the characteristics of the subjects and their relative unfamiliarity with high-intensity cycling. Previous studies (Burt & Twist, 2011; Marcora & Bosio, 2007; Twist & Eston, 2005, 2009) tested endurance performance at 48 hours following EIMD, while the present study tested endurance performance at 24 hours following EIMD. DOMS has been reported to peak 24 – 48 hours following EIMD (Tiidus, 2008). Cycling sprint performance was previously tested at 24 and 48 hours following EIMD and was reduced at both time points (Twist & Eston, 2005). It is possible, although unknown, that exercise performance in the present study may have been different if tested at 48 hours following EIMD.

Additionally, the training status of subjects in the present study and lack of familiarity with cycling and high-intensity exercise in general may have contributed to the lack of difference in performance following eccentric exercise. All subjects completed a familiarization time trial in the present study, which has been shown to improve reliability in subsequent time trials (Noreen, Yamamoto, & Clair, 2010). However, subjects in the Noreen

et al. (2010) study were trained cyclists who were familiar with time trials and high intensity cycling. Additionally, their study utilized an onscreen pacer during the second and third time trials, while the present study did not. In a different study, Zavorsky et al. (2007) found that time trial performance repeatability was higher in more highly trained cyclists (mean power: 285 ± 33 W) compared to recreationally trained cyclists (mean power: 204 ± 34 W) during a simulated 20 km time trial. In the present study, time trial performance following downhill running decreased in seventeen subjects (range: -26 to -1 W) and increased in fifteen subjects (range: 1 to 44 W). In light of the range of performance changes observed, the repeatability of the test among the subjects tested may have been an issue.

Peripheral Muscle Fatigue

Quadriceps muscle fatigue has previously been measured following an exercise task through magnetic stimulation of the femoral nerve and measurement of potentiated quadriceps twitch force (Kufel et al., 2002; Polkey et al., 1996). This measure provides a non-volitional “quantitative estimate of the degree of contractile fatigue elicited by the exercise” (Mador, Bozkanat, & Kufel, 2003). Quadriceps twitch force is measured before and after the exercise task and the percentage of force decrement from baseline is taken as the level of fatigue.

Quadriceps muscle fatigue following a cycling time trial, measured through potentiated quadriceps twitch force, was significantly different between groups in the present study. The placebo group experienced a significant decline in quadriceps twitch force after the post-eccentric exercise time trial compared to a baseline time trial (pre-supplementation: $-23.9 \pm 24.0\%$, post-eccentric exercise: $-39.5 \pm 24.3\%$, $p < .05$). The PCSO-524™ group, however, did not show a change in quadriceps fatigue from the pre-supplementation time trial to the post-eccentric exercise time trial (pre-supplementation: $-27.8 \pm 26.2\%$, post-DHR: $-30.4 \pm$

14.3%, $p > .05$). This suggests that PCSO-524™ supplementation has a protective effect on muscle damage during eccentric exercise and allows subjects to engage in intensive cycling exercise while inducing less quadriceps muscle fatigue.

The level of potentiated quadriceps twitch force fatigue observed in the present study is similar to levels of fatigue observed previously (Kufel et al., 2002). Kufel et al. investigated both potentiated ($Q_{tw,pot}$) and unpotentiated ($Q_{tw,unpot}$) following 10, 15, 20 and 30 MVC maneuvers of 5 seconds each. $Q_{tw,pot}$ fell by as much as about 40% after 30 MVC maneuvers, which compares to the $39.5 \pm 24.3\%$ fall in $Q_{tw,pot}$ observed in the present study in the placebo group after the downhill run and final cycling time trial exercise.

There is some evidence for a contractile fatigue protective effect from PUFA supplementation. PUFA supplementation can be effective in providing fatigue resistance in rat peripheral muscles during long-duration exercise (Peoples & McLennan, 2010, 2013). Rats were fed diets with fat content modified to either saturated fat, n-3 PUFA or n-6 PUFA. The n-3 PUFA diet contained a high proportion of DHA (28.9%) and EPA (9%), which are two primary components of PCSO-524™. Rats fed fish oil were more highly resistant to fatigue during continuous muscle twitch contractions and showed reduced skeletal muscle O_2 consumption during exercise and recovery (Peoples & McLennan, 2013).

Slow Skeletal Troponin I

The present study is the first to measure slow sTnI in plasma in response to an anti-inflammatory nutritional supplementation and eccentric exercise. The data indicate that eccentric exercise induced significant changes in slow sTnI plasma levels and PCSO-524™ supplementation attenuated the appearance of slow sTnI compared to placebo ($p < .05$). Significant differences in sTnI levels were observed at 2 hours following eccentric exercise

and persisted at 24, 48, 72 and 96 hours ($p < .05$). Peak sTnI levels observed in the present study (treatment: 20.3 ± 6.0 ng/ml, placebo: 30.2 ± 9.0 ng/ml) are comparable to those reported in bone injury (16 ± 2 ng/ml) and soft-tissue injury (10 ± 2 ng/ml) patients (Onuoha et al., 2001). The data from the present study indicate that the eccentric exercise bout caused muscle damage and disruption of structural protein troponin I.

Skeletal troponin I has been proposed as an alternative serum marker of skeletal muscle damage to commonly used markers such as creatine kinase (CK) and myoglobin (Mb) concentrations (Sorichter et al., 1997). These three markers have limitations as markers of muscle damage and a more specific protein marker is desirable. Proteins normally found in the cytoplasm of muscle cells such as CK and Mb are commonly used as markers of muscle damage following intensive exercise. However, CK has been observed to be highly variable following comparable exercise-induced muscle damage, which limits its usefulness when being used to examine differences in muscle damage between interventions (Clarkson & Ebbeling, 1988; K Nosaka & Clarkson, 1996). Mb is similarly found primarily in muscle cell cytoplasm and its appearance in blood following muscle damage indicates damage to the muscle cell membrane (Dahlqvist, Voss, Lauridsen, Krag, & Vissing, 2014).

Skeletal muscle troponin I (sTnI) is a structural protein in the sarcolemma involved in the regulation of muscle cell contraction (Plowman & Smith, 2011). Due to its location in the cell and specificity to muscle cells, sTnI has been investigated as a marker of damage specific to the contractile structure, providing information about permeability of the muscle cell membrane and disruption of structural elements (Dahlqvist et al., 2014).

sTnI has previously been found to increase in plasma following exercise-induced muscle damage as a result of downhill running (Sorichter et al., 1997). Sorichter et al. (1997)

found sTnI levels peaked about 6 hours following downhill running at 27.3 $\mu\text{g/ml}$ (27300 ng/ml). In the present study, baseline slow sTnI levels were 8.4 ± 3.1 ng/ml in the placebo group and increased to 30.2 ± 9.0 ng/ml at 24 hours following the downhill running bout. There are differences among reported slow sTnI levels in the literature. sTnI increased in response to eccentric knee extensor exercise from 71.34 ± 26.58 ng/ml to 128.55 ± 40.49 ng/ml (Willoughby, 2003), which is a higher rate of appearance than the current study but lower than that seen by Sorichter et al (1997). It is not clear why there is a large variation in the levels of sTnI observed in these studies. Sorichter et al. (1997) did not report whether fast or slow sTnI was measured. It is possible that the assay used was not specific to either isoform.

It is possible that the different manufacturers of assays used in these studies contributed to some of the differences observed. Another explanation may be the mode of exercise and the training status of the subjects in each study. Sorichter et al. (1997) used physical education teacher trainees, but did not describe the training status. It is likely that the subjects were somewhat active since they were involved in physical education. Willoughby et al. (2003) used recreationally active but non-trained men. The present study used untrained subjects who were specifically screened to minimize any recent exercise experience.

In addition to the levels of sTnI observed in the present study compared to previous studies, the general time course of sTnI appearance and return to baseline is of interest. In the present study, sTnI had increased from baseline by two hours, peaked at 24 hours and returned to baseline about 96 hours following downhill running. The time course observed by Sorichter et al. (1997) was somewhat different, with a peak at 6 hours post-run and the decline toward baseline having already begun at 24 hours and returning to baseline by 48 hours. One

difference to note is that the present study included the cycling time trial bout at 24 hours following downhill running, which may have introduced additional muscle damage. Willoughby et al. (2003) measured sTnI through 48 hours where levels for eccentric exercise were still elevated (98.30 ± 34.32 ng/ml). Additionally, the time course of fast sTnI appearance and return to baseline following elbow flexor exercises was observed to peak at 96 hours (Chapman et al., 2013). Since the exercise used by Chapman et al. (2013) was maximal contractions, Type II muscle fibers were damaged (as demonstrated by increased fast sTnI levels) but Type I muscle fibers were not (slow sTnI levels did not increase). The time course observed by Willoughby et al. (2003) is more similar to the present study than those observed by Sorichter et al. (1997). Clearly, there is variability in the time course of sTnI that likely depends on the mode of exercise, the degree of muscle damage introduced and the subject population.

Future Directions

The present study has demonstrated that PCSO-524™ supplementation attenuates symptoms of DOMS and peripheral muscle fatigue following eccentric exercise in untrained men. Muscle damage was induced by the downhill running protocol and less muscle damage was observed with PCSO-524™ supplementation compared to placebo, as evidenced by slow sTnI levels in the hours and days following exercise. Further research is needed to determine whether a dose-dependent treatment effect can be observed and whether different modes of exercise are affected differently by PCSO-524™ supplementation.

The present study did not find a difference in cycling endurance performance following an eccentric exercise bout. Future performance-based research is needed to determine whether PCSO-524™ attenuates performance decrements that follow eccentric

exercise, using a protocol that reliably results in performance changes following eccentric exercise. If untrained subjects are studied, then a previously validated eccentric exercise protocol such as vertical jumping and a different cycling performance trial such as that used by Twist and Eston (2009) may be considered.

Evidence exists that use of NSAIDs may retard adaptation to training stimulus and prolong recovery in exercise-damaged muscles (Machida & Takemasa, 2010; Warden, 2009). Thus, the benefits of the pain relieving effects of these drugs may be outweighed by the long-term potential negative effects from their use. It has not been determined if PUFA supplementation during exercise has the same drawbacks. In contrast, PUFA supplementation has been found to improve protein synthesis in healthy adults, older adults and patients recovering from esophageal cancer surgery (Noreen, Sass, et al., 2010; Ryan et al., 2009; Smith et al., 2011). Future research is needed to determine if the anti-inflammatory effects of PCSO-524™ are accompanied by the detrimental effects of reduced adaptation to training stimulus as seen with other anti-inflammatory treatments.

Further research in this area may wish to look more closely at the relationship between PCSO-524™ and DOMS. Data from the present study and Baum et al. (2013) suggest that PCSO-524™ may be an effective pain reliever following intensive exercise that causes muscles soreness. PCSO-524™ has been shown to be an effective anti-inflammatory and has effects on the cyclooxygenase and 5-lipoxygenase pathways, which is similar to popular pharmaceutical treatments such as NSAIDs.

Conclusions

In summary, this study is the first to demonstrate that muscle soreness in untrained men following eccentric exercise can be decreased with a prior period of PCSO-524™

supplementation. The reduction in DOMS symptoms was seen in the 72 to 96 hours following exercise. Based on data from the present study and previously demonstrated effectiveness of PCSO-524™ as a treatment for inflammation-related conditions such as arthritis, it is possible that reduced inflammation is one mechanism by which DOMS can be decreased. Endurance exercise performance in untrained males is not affected by a bout of eccentric exercise taking place 24 hours prior. Cycling time trial performance among untrained men following eccentric exercise is not affected by thirty days of PCSO-524™ supplementation. Quadriceps muscle twitch force decreased less among PCSO-524™ subjects following a cycling time trial, indicating that PCSO-524™ supplementation can lessen muscle fatigue during endurance exercise that follows a prior bout of eccentric exercise. This has implications for PCSO-524™ in endurance athletic activities where strength endurance impairment may be a competitive disadvantage. These data further demonstrate the complexity of exercise-induced muscle damage, delayed onset muscle soreness and endurance exercise performance. PCSO-524™ supplementation appears to be a viable treatment for the purpose of attenuating DOMS, peripheral muscle fatigue following endurance exercise, and muscle damage resulting from eccentric exercise, but has no effect on exercise performance in untrained men.

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APPENDICES

APPENDIX A

INFORMED CONSENT STATEMENT

INDIANA UNIVERSITY INFORMED CONSENT STATEMENT FOR

Effect of Marine Lipid Fraction PCSO-524™ on Markers of Exercise-Induced Muscle Damage and Cycling Endurance Performance in Untrained Males

You are invited to participate in muscle damage research. We are studying the effect of a lipid extract on exercise performance. The extract is from New Zealand green-lipped mussel (Omega XL®). You were selected as a possible subject because: 1) you're male, 2) you're 18-30 years old, and 3) you exercise not more than three times per week and not more than 30 minutes each time that you exercise.

Please read this form. You should get any questions answered before starting the study.

The study is being conducted by Dr. Timothy D. Mickleborough, David Platt and Jacob Sinex, Department of Kinesiology, Indiana University. It is funded by Pharmed International Ltd.

STUDY PURPOSE

The purpose of this study is to determine if taking lipid extract for thirty days reduces muscle damage from intense exercise.

NUMBER OF PEOPLE TAKING PART IN THE STUDY:

If you want to be in the study, you'll be one of 40 people participating.

PROCEDURES FOR THE STUDY:

If you want to be in the study, you'll visit the lab nine times over about seven weeks. During the visits, you will run and ride a bike. Below is the study schedule. Items listed as "lab days" mean that you must come to the lab to be tested. "At home" means that you do the task at home on your own. Details about each procedure are included below the Schedule of Study Procedures.

You are responsible for transportation to and from the lab. The lab is located at the HPER Building at 1025 E. 7th Street, Room 80, Bloomington, IN 47405. The total amount of time estimated to finish the study is under 13 hours. Parking is free in designated parking spots behind the building.

Schedule of Study Procedures

Week 1	<p>Visit 1 (Day 0): Lab day: Estimated time 2 hours</p> <p><u>Familiarization Session and 20 Minute Bicycle Time Trial</u></p> <p>On the first day, we'll check your cholesterol, glucose, and heart rate, and take height and weight measurements. After that you'll be asked to fill out a questionnaire and we'll answer any questions you might have. If all your values are normal we'll ask you to ride an exercise bike for 20 minutes as fast as you can to become familiar with what it's like to do a time trial for the real test.</p> <p><u>Questionnaires and Diary (Days 1-7): At home</u></p> <p>We ask you to fill out two forms every day. One enables us to monitor your well-being and mood every day. The other helps us monitor mood and emotional demands for athletes. Once a week we will ask you to fill out a longer form on how you feel. Typically these forms will take about 10 minutes to finish for the entire week.</p>
Week 2	<p>Visit 2 (Day 7): Lab day: Estimated time 45 minutes</p> <p><u>Maximal Aerobic Exercise Capacity Test</u></p> <p>We'll ask you to run on a treadmill at increasing incline levels until you decide that you cannot continue to run at the current incline level. This test is called</p>

	<p>a VO2 max test.</p> <p><u>Questionnaires and Diary (Days 7-14): At home</u> Like the previous week, we will ask you to fill out forms monitoring your activity, well-being, and mood every day.</p>
Week 3	<p><u>Visit 3 (Day 14): Lab day:</u> Estimated time 20-35 minutes.</p> <p><u>Measures of Muscle Damage and Soreness</u></p> <p>We will do various tests to measure your muscle strength and soreness. These tests are expected to take 15-30 minutes.</p> <p><u>Blood Collection</u></p> <p>We'll also collect 2 teaspoons (10 mL) of blood. This should only take 5 minutes to finish.</p> <p><u>Questionnaires and Diary (Days 14-21): At home</u> Like the previous weeks, we'll ask you to fill out forms monitoring your activity, well-being, and mood every day.</p> <p><u>Food Frequency Questionnaire (Days 14-21): At home</u> We will ask you to fill this out on any day you choose between Days 14-21. It is just one form. You only fill this out once during this period. It should take around 10 minutes to complete.</p>
Week 4	<p><u>Visit 4 (Day 21): Lab Day:</u> Estimated time 1 hour, 30 minutes</p> <p><u>Measurements of Peripheral Muscle Fatigue</u> We will measure your muscle fatigue before and after the 20 minute time trial.</p> <p><u>Pulmonary Function Test</u> We'll test your lung function before and after the 20 minute time trial.</p> <p><u>20 Minute Cycling Time Trial</u> You will ride an exercise bike for 20 minutes as fast as you can.</p> <p><u>Supplementation</u> You begin taking the supplement or placebo (a pill that looks like the supplement but does not have the active ingredients) after this lab visit. You will take eight capsules per day (4 in the morning and 4 in the evening) until the end of the study.</p> <p><u>Questionnaires and Diary (Days 21-28): At home</u> Like the previous weeks, we'll ask you to fill out forms monitoring your activity, well-being, and mood every day.</p>
Weeks 5-7	<p><u>Supplementation (Days 28-47): At home</u></p> <p>You will not visit the lab during this period. You will continue taking eight capsules each day (4 in the morning and 4 in the evening) during weeks five</p>

	<p>through seven.</p> <p><u>Questionnaires and Diary (Days 28-47): At home</u> Like the previous weeks, we'll ask you to fill out forms monitoring your activity, well-being, and mood every day.</p>
<p>Weeks 7-8</p>	<p><u>Visit 5 (Day 47): Lab Day:</u> Estimated time 3 hours, 20 minutes</p> <p><u>Measures of Muscle Damage and Soreness</u></p> <p>We will do various tests to measure your muscle strength and soreness at the beginning of this visit. These tests are expected to take 15-30 minutes.</p> <p><u>Blood Collection</u></p> <p>We'll also collect 2 teaspoons (10 ml) of blood at the beginning of this visit. This should take about 5 minutes.</p> <p><u>Downhill-Running Regimen</u></p> <p>We'll have you run downhill on a modified treadmill for 20 minutes. Including setup, this test should take no longer than 35 minutes.</p> <p><u>Blood Collection</u></p> <p>We will ask you to wait in the lab for 2 hours watching TV, reading or otherwise sitting quietly after you complete the downhill running. After 2 hours, we'll collect 2 teaspoons (10 ml) of blood. This should take about 5 minutes.</p> <p><u>Food Frequency Questionnaire (Days 47-50): At home</u> We will ask you to fill this out on any day you choose between days 47-50. It is just one form. You only fill this out once during this period. It should take about 10 minutes to complete.</p> <p><u>Visit 6 (Day 48): Lab day:</u> Estimated time 1 hour, 30 minutes</p> <p>We'll ask you to come back to the lab 24 hours after your last visit at the same time of day for the following tests and measures.</p> <p><u>Measures of Muscle Damage and Soreness</u></p> <p>We will do various tests to measure your muscle strength and soreness at the beginning of this visit. These tests are expected to take 15-30 minutes.</p> <p><u>Blood Collection</u></p> <p>We'll also collect 2 teaspoons (10 ml) of blood at the beginning of this visit. This should take about 5 minutes.</p> <p><u>Measurements of Peripheral Muscle Fatigue</u> We will measure your muscle fatigue before and after the 20 minute time trial.</p> <p><u>20 Minute Cycling Time Trial</u> You will ride an exercise bike for 20 minutes as fast as you can.</p>

Visit 7 (Day 49): Lab day: Estimated time 1 hour

We'll ask you to come back to the lab 24 hours after your last visit at the same time for the following tests and measures.

Measures of Muscle Damage and Soreness

We will do various tests to measure your muscle strength and soreness at the beginning of this visit. These tests are expected to take 15-30 minutes.

Blood Collection

We'll also collect 2 teaspoons (10 ml) of blood at the beginning of this visit. This should take about 5 minutes.

Pulmonary Function Test

We'll test your lung function.

Visit 8 (Day 50): Lab day: Estimated time 30 minutes

We'll ask you to come back to the lab 24 hours after your last visit at the same time for the following tests and measures.

Measures of Muscle Damage and Soreness

We will do various tests to measure your muscle strength and soreness at the beginning of this visit. These tests are expected to take 15-30 minutes.

Blood Collection

We'll also collect 2 teaspoons (10 ml) of blood at the beginning of this visit. This should take about 5 minutes.

Visit 9 (Day 51): Lab day: Estimated time 30 minutes

We'll ask you to come back to the lab 24 hours after your last visit at the same time for the following tests and measures.

Measures of Muscle Damage and Soreness

We will do various tests to measure your muscle strength and soreness at the beginning of this visit. These tests are expected to take 15-30 minutes.

Blood Collection

We'll also collect 2 teaspoons (10 ml) of blood at the beginning of this visit. This should take about 5 minutes.

Questionnaires and Diary (Days 47-51): At home

Like the previous weeks, we'll ask you to fill out forms monitoring your activity, well-being, and mood every day.

Prescreening

To ensure that you're healthy enough for this study, we'll check your cholesterol. This involves a finger prick to collect a few drops of blood. We'll run your blood through a machine specially built for this purpose. We'll check your blood sugar in a similar manner. We will check your height with a ruler, weight by standing still on a scale. We'll also measure blood pressure with a cuff, and heart rate using an infrared device that we'll place on your finger. We'll also ask you to fill out a 16 question Yes/No survey. Based on this information, this will tell us if you can safely participate in the study.

Activity Diary

We'll ask you to keep an exercise log. We'll ask you to write down how many times you exercise and for how long. We'll also want you to write down how you exercise and how hard it was.

Daily Analysis of Life Demands of Athletes (DALDA) Questionnaire

This survey monitors your well-being. We want to make sure that you're healthy throughout the study. You'll fill out a survey about your general outlook on life. This form has two parts. One part asks you about the sources of stress. The other part asks you about how moody or stressed out you feel. We also want to know any health changes that occur during the study. You should fill this form out the same time every day. The DALDA is 34 questions long.

Profile of Mood States (POMS) Questionnaire

We'll ask you to complete another mood questionnaire each day in the morning. There is a short version of the form that we'll ask you to fill out six out of seven days each week. The number of questions for the short form is 37 questions. There is a long version of the form with 65 questions that we'll ask you to fill out once per week. The questions ask you how you feel right now. Question types include how happy you feel or how much energy you have, for example.

Food Frequency Questionnaire

We'll ask you to complete a questionnaire about what you eat before starting the supplement and again at the end of the 30-day supplementation period. This form helps us see if changes in your diet will affect the results. This questionnaire has 27 questions. If you have questions about changing your diet during the study, ask us. We're here to help!

Supplementation

After the second time trial, we'll ask you to begin taking some capsules. The capsules could contain the active supplement or olive oil. The olive oil capsule (placebo) has been shown to not have the health benefits of the supplement. We'll assign you to either take the olive oil capsule or the actual supplement completely by chance. You have an equal chance of being assigned the olive oil capsule or the supplement. Neither you nor the study investigator will know which capsule you are taking during the study. We'll ask you to take eight capsules a day, on the schedule of four capsules in the morning and four in the evening. You must return any unused capsules and the containers (even if empty) back to us at the next lab day visit. We'll ask you to record the times that you take the capsules each day on the Activity Diary.

20-minute Bicycle Time-Trial

We'll set up the exercise bike for you and show you how to use it. After that you can warm up at whatever speed you want. Next, we'll ask you to ride the bike as fast as you can for 20 minutes. You can change gears whenever you want. We'll ask you to do the 20-minute time trial on your first, fourth, and sixth visit to the lab. While doing the time trials, we'll measure how much oxygen your body is providing the muscles, how much of that oxygen your muscles are using, and how hard your heart is working. The monitors we use to measure these things are non-invasive. We'll wrap an infrared sensor on your leg with an elastic bandage to estimate the amount of oxygen reaching the tissues in your leg. Measuring heart rate requires you to wear a strap around your chest. We will ask you to wear a mouthpiece connected to hoses during the time trial so we can collect exhaled gas to measure your oxygen consumption and breathing rate. We will ask you to wear a nose clip so all of your breathing will be through your mouth.

Rating of Perceived Exertion and Shortness of Breath: We'll ask how hard it was to exercise during your bicycle time trial tests in the lab. We use two scales: a 15 point rating of perceived exertion (how hard you feel like you are working) and a 10 point scale to rate your difficulty of breathing. We'll do this every 4 minutes while you're riding the bike.

Maximal Aerobic Capacity Exercise Test

This test measures your highest exercise capacity. Top athletes take this test to see how fit they are. We'll give you an opportunity to become familiar with the equipment and warm up before the test. We'll make sure that you're ready before beginning the test. You will wear a mouthpiece connected to hoses during the test and your nose will be plugged with a nose clip. We'll also take your heart rate with a monitor. For the start of the test, you will sit for 5 minutes while we get resting measurements. Then you will get up and begin running on the treadmill at an easy pace. We'll ask you to continue running while we increase the grade (incline) 2% every three minutes until you decide that you can't run anymore. At that point you'll have completed the test. If at any point you feel dizzy or lightheaded, let us know. Safety is our number one priority. After the test, you will cool down by running or walking at an easy pace.

Pulmonary function:

We will measure how well your lungs are functioning with two devices. One measures lung airflow. Another device measures lung air pressure. Both tests require you to inhale and exhale as fast as you can while sitting down. Each device requires you to blow through a tube. Your nose will be plugged with nose clips. We'll have you do three trials to measure airflow. If the results aren't within 10% of each other we'll ask you to do more trials until we get three that have results that are close enough to each other. The test for air pressure measures how strong your breathing muscles are. This test will be done 5 times separated by 30 seconds. If the results are not close enough to each other, we will ask you to do more tests.

Downhill-Running Regimen:

We'll ask you to run for 20 minutes downhill (-16° grade) on a treadmill. This test will be performed on your fifth visit to the lab. You will warm up by running slowly while the treadmill is flat. We'll then decrease the grade to -16° and ask you to run at a moderate pace (a percentage of the pace that you ran during the maximal aerobic exercise capacity test). After 20 minutes, you will cool down by running or walking at an easy pace. We'll ask you to wear a mouthpiece to measure how much oxygen your muscles are using and a heart rate monitor. You'll probably feel sore for about one week after you are finished running. You'll feel this most likely in your legs.

Measurements of Peripheral Muscle Fatigue

Before and after the 20 minute bicycle time trials, we'll ask you to do some leg extensions in your right leg. We'll ask you to be seated with your leg bent. Your leg will be wrapped above your ankle. We will stimulate the muscles in your thighs with a wire and a small voltage which you should feel like a small pull in your leg. Three single pulls will be obtained with breaks of 30 seconds between each one. We'll increase the voltage intensity from 50%, 60%, 70%, 80%, 85%, 90%, 95%, and 100% of safe allowed voltage by the machine. We'll ask you to contract your quads as hard as you can for 5 seconds. At the end of the 5 seconds, we will stimulate your nerve twice with 5 seconds of rest in between. After 30 seconds of rest, we will repeat this procedure 5 times. We'll do these tests on your third, fifth, sixth, seventh, eighth, and ninth visits to the lab.

Blood Collection:

We will be drawing blood from a vein in your arm. We will collect about 2 teaspoons (10 mL) 8 times during the study. Drawing blood should only take 5 minutes. In total, we will take about 16 teaspoons

of blood from you. We want to see how much damage your muscles have and how quickly they recover from the damage.

One of the tests that we will be using measures how your body modifies muscle cells in response to muscle damage. We will not be profiling your DNA for any specific markers or genes.

Measures of Muscle Damage and Soreness

We will check for muscle pain a few ways. We'll ask you to lie on your stomach on a table with your legs outstretched and measure how much you can flex your leg. We will also check for swelling by measuring around your thigh. We'll also measure how much power your muscles can produce. We have a special leg extension machine that measures force. We'll ask you to sit in a chair and push a bar away from you with your leg. We'll give you a chance to familiarize yourself with it before actually doing measurements. Additionally, we'll ask you about how much pain you're having in your legs. We'll measure it by asking you to squat and tell us how painful it is. Finally, we'll measure pain with a device that measures a certain amount of pressure. After pushing on your thighs, we'll ask you how much it hurts.

RISKS OF TAKING PART IN THE STUDY:

While on the study, the risks and discomforts are:

1. **Supplementation:** Common side effects of the supplement include vomiting, nausea, bloating, and burping. Rare side effects include easy bleeding/bruising and serious allergic reactions. In people allergic to shellfish, the supplements can cause a serious allergic reaction. Signs of serious allergic reactions include feelings of a "lump" in the throat, hoarseness, or feeling tightness in the chest and/or audible wheezing, nausea, vomiting, stomach pain, diarrhea. You might also have an allergic reaction if your skin turns red or becomes swollen with raised, itchy bumps.
2. **Maximal exercise testing on a treadmill:** Risks for this test include lightheadedness, chest discomfort, leg cramps, significant leg fatigue, nausea, occasional irregular heartbeats, and abnormal blood pressure responses. The risk of heart attack during this test, although minor (approximately 1 to 2 in 10,000), does exist. There is also a small risk of contamination or infection from the mouthpieces.
3. **Downhill treadmill running:** The risk of death while performing this type of exercise is about the same as drowning in a bathtub. The likelihood that you will have leg pain for along time after you finish the study is also minimal. You may also have shortness of breath, muscle soreness or cramping, and lightheadedness. A few days after the test, we expect you to have moderate to severe muscle soreness in your legs. Pain should disappear by about one week after the test.
4. **Time Trial on Exercise Bicycle:** You may feel discomfort due to being unfamiliar with being seated on a bicycle while exercising for a sustained period of time. You may also have wrist pain, shoulder pain, neck pain, back pain, buttocks pain, leg pain and numbness in your hands and/or feet.
5. **Peripheral Muscle Fatigue Testing:** You may have temporary dizziness, fainting, or nausea during this test. Additionally, the electrodes might cause your skin to be irritated. You could develop a rash if you have sensitive skin. During stimulation you may be surprised by the involuntary muscle contraction or experience discomfort in your legs.
6. **Blood Draw:** You may have fainting, soreness, bruising, infection from drawing blood, and/or swelling at the site where we draw your blood.
7. **Pulmonary Function Testing:** You may experience headache, light-headedness, or possibly fainting.

8. Measures of Muscle Damage and Soreness: You could have some pain and discomfort from the pressure sensitivity testing. You may also have pain or be injured while lifting weights with your legs. Additionally, you will be performing this test after completing the downhill running test which is intended to produce muscle damage. We expect that you will experience some pain or discomfort while completing this test.
9. Questionnaires/Diary: You might have anxiety or discomfort when filling out these forms.
10. There is also a risk of loss of confidentiality: such as computers could be hacked or have a virus and data taken which would identify you with your collected data. Examples of data could be name, addresses, and the results of tests.

To minimize the risks listed above, the following measures will be employed:

1. During any of the tests or trials, you can ask to stop the test or trial and we will stop right away.
2. Supplementation: If you are allergic to shellfish or fish oil then you will not be able to participate in the study. You should report any adverse effects or unusual symptoms to the principal investigator.
3. Maximal exercise testing on a treadmill: We will use a disposable mouthpiece whenever possible. When rubber mouthpieces are used, they will be cleaned in a detergent solution and disinfected following each use. High risk subjects, such as subjects that are overweight, have allergies to fish products, or have a history of muscle soreness, asthma or cardiac problems, will be unable to participate in the study. Trained researchers will monitor you during the test.
4. Downhill treadmill running: We'll monitor your heart rate and breathing and stop the test if you have any adverse effects.
5. Time Trial on Cycle Ergometer: We'll ensure that you're comfortable on the bike before starting. We'll make any modifications or adjustments to the bike to ensure your comfort. We'll monitor your heart rate and stop the test if you have any adverse effects.
6. Peripheral Muscle Fatigue Testing: You will be sitting during this test to minimize injury if you faint. If it is too painful you can request us to stop the test.
7. Blood Draws: You will be sitting to minimize injury if you faint. Sites will be sterilized, and trained individuals will draw blood to minimize the risk of infection.
8. Pulmonary Function Testing: You will be sitting during these tests to minimize injury if you faint. If severe wheezing begins, the test will be immediately stopped and you will be given oxygen if necessary.
9. Measures of Muscle Damage and Soreness: You will be able to tell us if you are experiencing too much pain and the tests will be stopped at that time.
10. Questionnaires/Diary: If you are uncomfortable with any questions, you can choose not to answer them. Also, we'll monitor your responses during the supplementation period and compare them to responses before you began the supplement. If there are large changes, we'll refer you to IU Health for consultation.
11. Confidentiality: We'll keep all data in a locked room on password-protected computers with virus protection in rooms with limited access.

BENEFITS:

You are not expected to benefit from participating in this study. However, you will have access to all of your own data regarding your personal lung function and markers of inflammation.

ALTERNATIVES TO TAKING PART IN THE STUDY:

You can choose not to participate in this study.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. Data will be stored on password-protected computers in locked rooms with limited public access. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Your identity will be held in confidence in reports in which the study may be published and databases in which results may be stored.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the study investigator and his/her research associates, the IU Institutional Review Board or its designees, and (as allowed by law) state or federal agencies, specifically the Office for Human Research Protections (OHRP) who may need to access the collected research data.

PAYMENT

You will be paid \$1,000 for taking part in this study. We'll pay you as follows:

First lab visit	\$0
Second lab visit:	\$30
Third lab visit:	\$30
Fourth lab visit	\$30
Fifth lab visit	\$230
Sixth lab visit	\$200
Seventh lab visit	\$160
Eighth lab visit	\$160
Ninth lab visit	\$160

You will be compensated for completing each session. If you withdraw from the study, you will be compensated for the completed visits. If you discontinue a test in the middle of a lab visit, you will be paid on a rate of \$10 per hour for that visit in addition to payment for the completed visits. Payments will be made after each session and mailed to an address that you specify. If you would prefer, we can wait and mail the entire amount after you are done participating in the study. Payment takes about 3 weeks for the university to process and send out the check.

COMPENSATION FOR INJURY

In the event of physical injury resulting from your participation in this research, necessary medical treatment will be provided to you and billed as part of your medical expenses. Costs not covered by your health care insurer will be your responsibility. Also, it is your responsibility to determine the extent of your health care coverage. There is no program in place for other monetary compensation for such injuries. However, you are not giving up any legal rights or benefits to which you are otherwise entitled. Because you are participating in research which is not conducted at a medical facility, you will be responsible for seeking medical care and for the expenses associated with any care received.

CONTACTS FOR QUESTIONS OR PROBLEMS:

If you have any questions about the study or the procedures, or you experience adverse effects as a result of participating in this study, you may contact Jacob Sinex or David Platt (812-856-9126) or Dr. Timothy D. Mickleborough (812-855-0753). If you cannot reach the researchers during regular business hours (i.e., 8:00AM-5:00PM), please call the IU Human Subjects Office at (812) 856-4242 or (800) 696-2949.

For questions about your rights as a research participant or to discuss problems, complaints or concerns about a research study, or to get information, or give input, contact the IU Human Subjects office at (812) 856-4242 or (800) 696-2949.

VOLUNTARY NATURE OF STUDY

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your decision whether or not to participate in this study will not affect your current or future relations with the investigators or Indiana University. The Principal Investigator may remove subjects from the study if it felt that subjects are not abiding by the restrictions placed on subjects entering the study.

SUBJECT'S CONSENT

In consideration of all of the above, I give my consent to participate in this research study.

I will be given a copy of this informed consent document to keep for my records. I agree to take part in this study.

Subject's Printed Name: _____

Subject's Signature: _____ **Date:** _____
(must

be dated by the subject)

Printed Name of Person Obtaining Consent: _____

Signature of Person Obtaining Consent: _____ **Date:** _____

APPENDIX B

MEDICAL QUESTIONNAIRE

Modified Physical Activity Readiness Questionnaire (PAR-Q)

Name		Email
DOB		Age
		Phone
Yes	No	Have you ever been told by a physician that you have cardiac, peripheral vascular, or cerebrovascular disease?
Yes	No	Have you ever been told by a physician that you have COPD, asthma, interstitial lung disease, or cystic fibrosis?
Yes	No	Have you ever been told by a physician that you have Diabetes mellitus (types 1 and 2), thyroid disorders, renal or liver disease?
Yes	No	Have you ever felt or do you currently experience pain, discomfort in the chest, neck, jaw, arms, or other areas?
Yes	No	Have you ever felt or do you currently experience shortness of breath at rest or with mild exertion?
Yes	No	Have you ever felt or do you currently experience dizziness or a loss of consciousness at rest or during mild exercise?
Yes	No	Have you ever felt or do you currently experience shortness of breath when lying flat or severe shortness of breath and coughing that generally occurs at night?
Yes	No	Have you ever had or currently have swelling of the feet or ankles?
Yes	No	Have you ever experienced or currently experience rapid heart beat under resting conditions or does your heart skip beats under any conditions?
Yes	No	Have you ever experienced or currently experience intermittent muscle pain (ache, cramp, numbness or sense of fatigue) in your calf muscle, which occurs during exercise and is relieved by rest.
Yes	No	Have you ever been told by a physician that you have that you have a known heart murmur?
Yes	No	Have you ever experienced or currently experience unusual fatigue or shortness of breath with usual activities?
Yes	No	Are you a current cigarette smoker or quit within the previous 6 months or are routinely exposed to environmental tobacco smoke?
Yes	No	Do you have a father, brother or son with heart disease before the age of 55 years old or a mother, sister or daughter with heart disease before the age of 65 years old?
Yes	No	Do you currently participate in more than 30 min of moderate intensity physical activity on at least three days of the week for at least three months?
Yes	No	Are you currently taking any medication (prescription or over the counter)? If yes, please list on

		the reverse side.
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Signature	Date
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Regular exercise is associated with many health benefits, yet any change of activity may increase the risk of injury. By signing you affirm that you have read each question carefully and answered every question honestly.

Medications you're currently taking

Name	Reason for taking

Height _____ (m)	Calculated BMI _____
Weight _____ (kg)	
Blood Pressure	_____ / _____ mm Hg
Cholesterol (HDL only)	_____ mg/dL
Blood glucose	_____ mg/dL
Resting Heart Rate	_____ bpm

For Office use only:

Risk Stratification:

Low

Moderate

High

APPENDIX C

PERSONAL HISTORY QUESTIONNAIRE

Subject#:

Study #: 1211009997

General Personal History Questionnaire

How many days per week do you typically exercise?

1 2 3 4 5 6 7

How long is your typical exercise session?

Less than 30 – 60 60 – 120 over 120
30 minutes minutes minutes minutes

Indicate your status for each of the following:

I have been diagnosed with asthma or exercise-induced asthma. Yes No

I have participated in a strength training program during the past 60 days. Yes No

I consume fish oil or other omega-3 nutrition supplements on a regular basis. Yes No

I frequently run up and downhill or up and down stairs for exercise. Yes No

I have a history of experiencing pain in my hips or knees. Yes No

I have an allergy to fish, seafood, or shellfish. Yes No

Do you take any medications, supplements or vitamins? Yes No

If yes, write name, amount, frequency, and how long you have taken them:

Name of medication or supplement	How much	How often	How long taking it (in weeks, months, years)

APPENDIX D

SUPPLEMENTATION AND EXERCISE DIARY

APPENDIX E

DATA COLLECTION SHEETS

Subject #:

Visit 1 Data Collection Sheet

Study #: 12110009997

Date:

Time from Start	HR	RPE	Dyspnea	Average Power	Average RPM
Rest		---	---	---	---
0 min				----	----
5 min					
10 min					
15 min					
20 min					

Seat Height (cm):

Aerobic Capacity Data Collection Sheet

Subject: _____ Weight: (kg) _____

Date: _____

Pbar _____

Rel _____

Hum: _____

Mode: Run

Temp: _____

VO2max Filename: _____

Target RPM: _____

Target Pace (mph): _____

Time (min)	Speed (mph)	Grade (%)	RPM	HR
0	Rest	-	-	
1	Rest	-	-	
2	Rest	-	-	
3	Rest	-	-	
4	Rest	-	-	
5	run - 1 mph	0		
6	run - 1 mph	0		
7	run	0		
8		0		
9		0		
10	run	4		
11		4		
12		4		
13	run	6		
14		6		
15		6		
16	run	8		
17		8		
18		8		
19	run	10		
20		10		
21		10		

	FEO2	FECO2	VE stpd	VE btps	VO2	VCO2	VO2ml	RER	fb
Max									

Subject:

Visit 3 Data Sheet

Date:

Algometer Site	15 cm distal to the ASIS	4 cm proximal to the SPP	midpoint of the ASIS and SPP along the axis	2 cm lateral of midpoint
Trial 1				
Trial 2				
Trial 3				
Average				

	Squatting Soreness Score	Thigh Circumference (cm)	Knee Flexion ROM (degrees)	Knee Flexion Iso Torque (v)
Trial 1				
Trial 2				
Trial 3				
Average				

Subject:

Visit 4 Data Sheet

Date:

Pulmonary Function (Pre)	FVC	FEV1	FEF25-75
Trial 1			
Trial 2			
Trial 3			

Height (cm):

Weight (kg):

Time Trial	Seat Height (cm):	Average Power (watts)	Average RPM	HR	RPE	Dyspnea
Rest	---	---	---		---	---
0 Min		---	---			
5 Min	---					
10 Min	---					
15 Min	---					
20 Min	---					

Pulmonary Function (Post)	FVC	FEV1	FEF25-75
Trial 1			
Trial 2			
Trial 3			

Supplement Bag #

Subject:

Visit 5 Data Sheet

Date:

Algometer Site	15 cm distal to the ASIS	4 cm proximal to the SPP	midpoint of the ASIS and SPP along the axis	2 cm lateral of midpoint	2 cm medial of midpoint
Trial 1					
Trial 2					
Trial 3					
Average					

	Squatting Soreness Score	Thigh Circumference (cm)	Knee Flexion ROM (degrees)	Knee Flexion Iso Torque (v)
Trial 1				
Trial 2				
Trial 3				
Average				

Minute	HR	RPE	Pace	Rev Count
0				
5				
10				
15				
20				

Blood Collection		Max HR:	
Time 1:		Rest HR:	
Time 2:		HRR:	
Time 3:		70% HRR:	
		Target HR:	

Subject:

Visit 6,7,8,9 Data Sheet

Date:

Algometer Site	15 cm distal to the ASIS	4 cm proximal to the SPP	midpoint of the ASIS and SPP along the axis	2 cm lateral of midpoint	2 cm medial of midpoint
Trial 1					
Trial 2					
Trial 3					
Average					

	Squatting Soreness Score	Thigh Circumference (cm)	Knee Flexion ROM (degrees)	Knee Flexion Iso Torque (v)
Trial 1				
Trial 2				
Trial 3				
Average				

APPENDIX F

RAW DATA – CYCLING TIME TRIAL PERFORMANCE

Subject	Group	Pre-Sup Mean_Power	Post-Run Mean_Power
80	Placebo	138	122
67	Placebo	141	143
56	Placebo	119	116
52	Placebo	203	202
46	Placebo	118	119
25	Placebo	104	101
60	Placebo	132	122
54	Placebo	148	122
31	Placebo	178	185
4	Placebo	170	189
23	Placebo	117	120
20	Placebo	155	146
3	Placebo	94	89
103	Placebo	219	202
105	Placebo	90	93
108	Placebo	99	134
	Mean:	139.1	137.8
	SD:	38.2	37.3
	CV:	0.275	0.271
45	Treatment	182	180
53	Treatment	145	148
96	Treatment	164	145
8	Treatment	140	170
85	Treatment	140	154
98	Treatment	134	141
10	Treatment	185	182
70	Treatment	256	230
6	Treatment	124	113
113	Treatment	143	119
110	Treatment	151	136
117	Treatment	133	134
40	Treatment	87	131
116	Treatment	143	141
104	Treatment	180	201
118	Treatment	146	153
	Mean:	153.3	154.9
	SD:	36.5	30.9
	CV:	0.238	0.199

Subject	Group	HR_0_Pre	HR_5_Pre	HR_10_Pre	HR_15_Pre	HR_20_Pre
80	Placebo	125	160	165	169	172
67	Placebo	115	162	171	174	174
56	Placebo	130	156	176	182	188
52	Placebo	114	171	181	187	193
46	Placebo	118	169	176	176	188
25	Placebo	104	128	143	164	179
60	Placebo	102	136	146	160	169
54	Placebo	145	180	185	181	187
31	Placebo	103	174	174	179	195
4	Placebo	138	162	171	174	179
23	Placebo	130	165	172	176	190
20	Placebo	138	169	168	164	185
3	Placebo	130	164	167	174	184
103	Placebo	141	182	182	182	187
105	Placebo	108	163	174	179	179
108	Placebo	108	119	117	125	136
	Mean:	121.8	160	166.8	171.6	180.3
	SD:	14.6	17.7	17.5	14.5	14
	CV:	0.12	0.111	0.105	0.084	0.078
45	Treatment	62	176	171	176	182
53	Treatment	114	171	184	182	180
96	Treatment	120	163	165	172	186
8	Treatment	121	158	174	169	167
85	Treatment	105	128	134	138	144
98	Treatment	126	160	169	174	185
10	Treatment	143	181	181	178	185
70	Treatment	129	145	156	160	171
6	Treatment	114	135	138	136	176
113	Treatment	129	148	156	160	176
110	Treatment	82	141	154	162	167
117	Treatment	116	175	174	176	178
40	Treatment	122	140	142	153	154
116	Treatment	111	145	151	150	164
104	Treatment	107	141	148	150	171
118	Treatment	110	140	154	162	160
	Mean:	113.2	152.9	159.4	162.4	171.6
	SD:	19.1	16.4	15.2	14.1	11.9
	CV:	0.169	0.107	0.095	0.087	0.069

Subject	Group	HR_0 Post	HR_5 Post	HR_10 Post	HR_15 Post	HR_20 Post
80	Placebo	111	152	158	162	171
67	Placebo	135	156	160	167	174
56	Placebo	110	140	158	171	188
52	Placebo	126	176	185	190	197
46	Placebo	138	178	178	181	194
25	Placebo	96	117	130	132	176
60	Placebo	110	128	133	128	133
54	Placebo	144	160	162	166	180
31	Placebo	139	170	188	190	194
4	Placebo	148	171	177	185	185
23	Placebo	141	164	171	176	190
20	Placebo	140	182	188	182	190
3	Placebo	114	143	141	145	171
103	Placebo	154	182	182	200	197
105	Placebo	97	164	176	179	185
108	Placebo	121	128	152	152	167
	Mean:	126.5	156.9	164.9	169.1	180.8
	SD:	18.3	20.5	18.9	20.9	16.1
	CV:	0.145	0.131	0.115	0.124	0.089
45	Treatment	82	156	162	160	174
53	Treatment	76	160	171	169	188
96	Treatment	99	145	154	156	179
8	Treatment	109	176	182	188	194
85	Treatment	121	140	146	152	154
98	Treatment	129	160	167	171	190
10	Treatment	134	174	176	179	181
70	Treatment	125	150	145	150	171
6	Treatment	102	136	128	142	155
113	Treatment	110	124	120	129	169
110	Treatment	97	132	152	143	156
117	Treatment	104	150	176	177	190
40	Treatment	117	126	133	147	154
116	Treatment	90	118	126	150	171
104	Treatment	113	148	158	162	179
118	Treatment	90	124	145	148	154
	Mean:	106.1	144.9	152.6	157.7	172.4
	SD:	16.8	17.7	19.2	15.8	14.4
	CV:	0.158	0.122	0.126	0.1	0.084

Subject	Group	RPE_0 Pre	RPE_5 Pre	RPE_10 Pre	RPE_15 Pre	RPE_20 Pre
80	Placebo	9	13	15	17	18
67	Placebo	7	13	15	15	16
56	Placebo	7	10	12	13	15
52	Placebo	11	14	15	17	19
46	Placebo	7	12	13	15	17
25	Placebo	6	12	14	15	15
60	Placebo	8	12	14	17	18
54	Placebo	10	17	18	17	18
31	Placebo	7	14	16	17	19
4	Placebo	7	10	13	15	17
23	Placebo	7	10	13	15	17
20	Placebo	12	15	17	18	20
3	Placebo	11	13	14	15	17
103	Placebo	11	15	16	17	19
105	Placebo	9	14	16	19	19
108	Placebo	10	11	12	12	13
	Mean:	8.7	12.8	14.6	15.9	17.3
	SD:	1.9	2	1.8	1.8	1.9
	CV:	0.218	0.156	0.123	0.113	0.11
45	Treatment	12	16	17	16	17
53	Treatment	7	13	17	19	19
96	Treatment	9	13	15	18	20
8	Treatment	9	13	14	15	15
85	Treatment	7	13	15	17	17
98	Treatment	7	12	14	16	17
10	Treatment	7	9	12	15	18
70	Treatment	10	14	15	16	17
6	Treatment	6	13	14	15	19
113	Treatment	9	13	15	15	18
110	Treatment	8	12	14	16	17
117	Treatment	9	13	17	18	20
40	Treatment	9	11	12	13	13
116	Treatment	9	10	12	14	15
104	Treatment	10	13	15	16	17
118	Treatment	10	13	14	15	17
	Mean:	8.6	12.6	14.5	15.9	17.3
	SD:	1.5	1.6	1.6	1.5	1.8
	CV:	0.174	0.127	0.11	0.094	0.104

Subject	Group	RPE_0	RPE_5	RPE_10	RPE_15	RPE_20
---------	-------	-------	-------	--------	--------	--------

		Post	Post	Post	Post	Post
80	Placebo	7	12	14	14	16
67	Placebo	11	14	15	16	17.5
56	Placebo	10	12	13	14	15
52	Placebo	12	15	18	19	20
46	Placebo	10	13	14	15	19
25	Placebo	6	9	13	13	15
60	Placebo	15	15	16	17	17
54	Placebo	11	14	15	15	17
31	Placebo	10	12	15	17	19
4	Placebo	9	12	13	15	17
23	Placebo	6	9	11	14	17
20	Placebo	12	17	19	19	20
3	Placebo	12	13	14	13	15
103	Placebo	13	15	16	17	19
105	Placebo	7	13	15	17	19
108	Placebo	10	11	13	13	15
	Mean:	10.1	12.9	14.6	15.5	17.3
	SD:	2.6	2.2	2	2	1.8
	CV:	0.257	0.171	0.137	0.129	0.104
45	Treatment	9	15	17	16	17
53	Treatment	9	15	17	18	20
96	Treatment	7	12	14	14	20
8	Treatment	12	13	14	15	17
85	Treatment	10	12	15	16	18
98	Treatment	7	11	13	15	19
10	Treatment	7	11	15	18	20
70	Treatment	7	13	14	15	17
6	Treatment	6	14	15	15	17
113	Treatment	11	14	15	17	20
110	Treatment	10	13	14	16	16
117	Treatment	8	12	15	17	20
40	Treatment	12	12	13	13	15
116	Treatment	9	11	13	14	15
104	Treatment	9	13	14	16	18
118	Treatment	11	13	15	17	18
	Mean:	9	12.8	14.6	15.8	17.9
	SD:	1.9	1.3	1.2	1.4	1.8
	CV:	0.211	0.102	0.082	0.089	0.101

APPENDIX G

RAW DATA – DELAYED ONSET MUSCLE SORENESS

Group	Pre Sup	Post Sup	24 hr Post-Run	48 hr Post-Run	72 hr Post-Run	96 hr Post-Run
Placebo	2	0	2	4	3	1
Placebo	5	3	3	2	3	3
Placebo	2	3	7	8	7	6
Placebo	2	2	4	3	3	2
Placebo	0	0	1	3	1	1
Placebo	1	0	4	5	4	2
Placebo	5	4	7	6	5	4
Placebo	1	1	8	7	5	2
Placebo	2	1	4	4	3	3
Placebo	2	0	3	3	2	1
Placebo	0	0	2	5	3	1
Placebo	2	3	6	7	7	5
Placebo	1	2	6	4	4	1
Placebo	4	4	3	4	3	2
Placebo	0	0	6	7	7	7
Placebo	1	0	4	5	3	7
Mean:	1.9	1.4	4.4	4.8	3.9	3
SD:	1.6	1.5	2.1	1.8	1.8	2.2
CV:	0.842	1.071	0.477	0.375	0.462	0.733
Treatment	5	3	8	7	4	3
Treatment	3	4	6	4	3	2
Treatment	1	1	1	1	1	1
Treatment	2	1	6	3	2	2
Treatment	3	2	4	5	3	2
Treatment	1	0	1	5	2	3
Treatment	2	1	1	1	0	0
Treatment	0	0	5	6	2	0
Treatment	2	0	4	4	1	1
Treatment	1	0	4	4	2	0
Treatment	0	2	7	5	3	1
Treatment	2	3	5	6	4	1
Treatment	1	0	2	2	1	0
Treatment	4	3	6	5	3	2
Treatment	5	6	9	8	6	5
Treatment	5	6	9	8	6	5
Mean:	2.3	2	4.9	4.6	2.7	1.8
SD:	1.7	2	2.7	2.2	1.7	1.6
CV:	0.739	1	0.551	0.478	0.63	0.889

APPENDIX H

RAW DATA – POTENTIATED QUADRICEPS TWITCH

Group	Pre Sup	Post Sup
Placebo	-30.62	-16.85
Placebo	-17.73	-40.22
Placebo	-12.62	
Placebo	0.91	-61.69
Placebo	-29.11	-8.33
Placebo	-31.41	-31.55
Placebo	-56.19	-75.05
Placebo	-23.11	-54.17
Placebo	-37.34	-34.01
Placebo	-9.05	-10.61
Placebo	-40.66	-32.91
Placebo	0.95	
Placebo	34.3	0.23
Placebo	-31.65	-55.56
Placebo	-67.12	-65.54
Placebo	-32.25	-66.49
Mean:	-23.9	-39.5
SD:	24	24.3
CV:	-1.004	-0.615
Treatment	2.28	-18.87
Treatment	-54.18	-30.01
Treatment	-26.14	-42.41
Treatment	-23.7	-9.73
Treatment	-51.79	-34.05
Treatment	-37.17	-47.32
Treatment	-41.6	-33.61
Treatment	-38.81	-15.29
Treatment	-1.78	-50.73
Treatment	-52.29	-41.42
Treatment	-7.09	0.77
Treatment	-16.8	-17.86
Treatment	-54.17	-37.29
Treatment	15.22	-40.42
Treatment	-68.8	-35.82
Treatment	12.75	-32.2
Mean:	-27.8	-30.4
SD:	26.2	14.3
CV:	-0.944	-0.472

APPENDIX I

RAW DATA – SKELETAL MUSCLE TROPONIN I

Group	Pre Sup	Post Sup	0 hr Post-Run	2 hr Post-Run	24 hr Post-Run
Placebo	5.5	8.3	15.4	23.2	37.4
Placebo	9.2	7.2	12.3	19.4	39.3
Placebo	6.8	8.3	17.4	26.3	40.2
Placebo	14.4	12.4	19.3	24.2	39.3
Placebo	3.3	2.8	7.3	13.3	21.4
Placebo	4.7	6.3	13.7	15.3	24.3
Placebo	11.6	13.4	17.4	20.2	31.4
Placebo	8.2	5.3	9.6	13.2	24.3
Placebo	11.8	7.3	12.1	14.8	18.4
Placebo	6.3	4.2	7.4	9.5	31.4
Placebo	6.7	4.8	7.1	14.3	35.3
Placebo	5.9	4.2	10.4	12.6	14.3
Placebo	9.8	10.3	18.3	23.2	39.3
Placebo	11.1	12.4	19.4	26.4	41.3
Placebo	7.4	4.7	8.3	11.3	19.4
Placebo	11.1	12.4	16.4	19.7	26.4
Mean:	8.4	7.8	13.2	17.9	30.2
SD:	3.1	3.5	4.5	5.6	9
CV:	0.369	0.449	0.341	0.313	0.298
Treatment	6.3	8.3	11.3	17.5	10.85
Treatment	13.2	12.4	15.3	20.3	26.5
Treatment	5.2	2.8	6.4	12.6	19.3
Treatment	8.9	5.6	8.4	9.5	25.3
Treatment	7.4	5.6	9.3	11.2	19.6
Treatment	9.6	11.2	14.4	17.5	23.4
Treatment	12.4	13.2	19.4	18.4	24.3
Treatment	13.7	14.2	17.3	16.5	26.4
Treatment	3.8	2.9	6.4	9.5	19.4
Treatment	4.2	3.2	5.3	8.4	17.5
Treatment	5.7	4.2	7.3	7.9	10.3
Treatment	5.8	5.2	9.8	10.3	12.6
Treatment	6.9	6	12.6	18.3	31.5
Treatment	10.2	9.2	12.3	14.3	17.5
Treatment	3.1	3.8	5.9	14.3	23.4
Treatment	6.3	6.1	9.5	9.2	17.4
Mean:	7.7	7.1	10.7	13.5	20.3
SD:	3.3	3.8	4.2	4.2	6
CV:	0.429	0.535	0.393	0.311	0.296

Group	48 hr Post-Run	72 hr Post-Run	96 hr Post-Run
Placebo	34.2	31.3	28.4
Placebo	26.3	23.5	12.4
Placebo	36.3	35.3	30.3
Placebo	31.3	29.2	22.5
Placebo	25.3	20.3	6.5
Placebo	27.4	23.8	24.8
Placebo	33.2	28.4	19.4
Placebo	24.3	19.4	8.6
Placebo	19.4	14.5	6.7
Placebo	26.5	19.3	12.5
Placebo	31.3	24.4	18.3
Placebo	13.4	9.4	7.4
Placebo	34.5	25.6	20.4
Placebo	39.4	32.4	25.6
Placebo	17.4	17.9	14.5
Placebo	27.3	22.5	9.5
Mean:	28	23.6	16.7
SD:	7.1	6.8	8.1
CV:	0.254	0.288	0.485
Treatment	8.5	12.5	7.4
Treatment	22.4	20.4	10.5
Treatment	15.4	13.5	12.3
Treatment	22.1	15.6	13.5
Treatment	15.9	17.6	8.9
Treatment	19.3	12.4	5.3
Treatment	19.4	18.5	6.9
Treatment	25.4	24.5	19.5
Treatment	24.3	22.9	17.5
Treatment	16.4	15.4	12.5
Treatment	12.4	13.5	5.6
Treatment	13.2	11.4	7.6
Treatment	29.4	28.5	26.4
Treatment	14.7	12.2	10.3
Treatment	25.3	16.4	7.6
Treatment	13.5	12.6	10.3
Mean:	18.6	16.7	11.4
SD:	5.8	5	5.7
CV:	0.312	0.299	0.5

CURRICULUM VITAE

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EDUCATION

- M.S. 2014 Indiana University, Department of Kinesiology
Major: Exercise Physiology
Thesis: Cycling Endurance Performance and Peripheral Muscle Fatigue Following Marine Lipid Fraction PCSO-524TM Supplementation and Eccentric Exercise in Untrained Males
Advisor: Timothy Mickleborough, Ph.D.
- B.S. 2006 Indiana University, School of Informatics
Major: Informatics, Minor: Business

HONORS AND AWARDS

- Indiana University – Student Academic Appointment 2011
Indiana University – Graduated with Highest Distinction 2006

PROFESSIONAL EXPERIENCE

- January 2013 – July 2013 Research Assistant, Department of Kinesiology, Indiana University, Bloomington, Indiana, Advisor: Timothy Mickleborough, Ph.D.
- August 2011 – September 2012 Graduate Assistant, The President’s Challenge Physical Fitness Program, Department of Kinesiology, Indiana University, Bloomington, Indiana, Supervisor: Jeff McClaine

ABSTRACTS

Timothy D. Mickleborough, FACSM, Eric Ress, **Jacob Sinex**, David Platt, Molly B. Hirt, Chapman F. Robert, FACSM. Marine Lipid Fraction PCSO-524TM Attenuates Muscles Damage and DOMS Following Eccentric Exercise in Untrained Males. American College of Sports Medicine Annual Meeting 2014.

Mickleborough, T.D., Vaughn, C.L., Davis, E.M., Shei, R-J., **Sinex, J.A.**, Platt, D., and Wilhite, D.P. Marine lipid fraction PCSO-524TM (Lyprinol®/Omega XL®) attenuates

airway inflammation and hyperpnea-induced bronchoconstriction in asthmatic individuals. *Medicine and Science in Sports and Exercise*, 2013. 45(5): S246.

PROFESSIONAL ORGANIZATIONS

American College of Sports Medicine 2014 - Present