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Adding omega-3 fatty acids to a protein-based supplement during pre-season training results in reduced muscle soreness and the better maintenance of explosive power in professional Rugby Union players

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Abstract

Evidence suggests that omega-3 fatty acid supplementation could reduce muscle soreness and maintain muscle function following eccentric exercise-induced muscle damage. The aim of this applied field study was to investigate the effectiveness of consuming a protein-based supplement containing 1546 mg of omega-3 PUFA (551 mg eicosapentaenoic acid (EPA) and 551 mg docosahexaenoic acid (DHA)) twice daily (FO) compared to a protein-based placebo (P) on muscle soreness, countermovement jump (CMJ) performance and psychological well-being in 20 professional Rugby Union players during 5 weeks of pre-season training. Players completed a 5-point likert soreness scale with 5 indicating “no soreness” and a questionnaire assessing fatigue, sleep, stress and mood each morning of training, plus they performed countermovement jump (CMJ) tests once or twice per week. Data were analysed using magnitude-based inferential statistics and are presented as percent beneficial/trivial/harmful. On day 35, there was a likely (% beneficial/trivial/harmful: 94/5/1) moderate (0.75, standardized mean difference (SMD)) beneficial effect of FO vs. P on the change in lower body muscle soreness compared with day 0 (FO: -3.8±21.7%; P: -19.4±11.2%). There was a likely (92/7/0) moderate (SMD: 0.60) beneficial effect of FO vs. P on CMJ performance (change from baseline to day 35, FO: +4.6±5.9%; P: -3.4±8.6%). From day 20, a moderate beneficial effect of FO on fatigue was observed. In terms of practical relevance, the moderate beneficial effect of adding fish oil to a protein-based supplement on muscle

soreness translated into the better maintenance of explosive power in elite Rugby Union players during pre-season training.

Keywords: muscle recovery, fatigue, rugby, fish oil

Introduction

Athletes involved in contact sports such as Rugby Union are regularly exposed to muscle damage during both training and match play (Naughton, Miller, & Slater, 2017). The physiological demands of Rugby Union result in an acute inflammatory response and elevations in blood creatine kinase and myoglobin concentrations during acute recovery. Such perturbations are indicative of structural damage to the muscle and/or extracellular matrix (Takarada, 2003; Cunniffe et al., 2010). The level of structural damage to the muscle fibres during Rugby depends, in part, on the number of tackles experienced, which causes muscle trauma (Takarada, 2003; Cunniffe et al., 2010). Further, repetitive sprints with rapid decelerations result in eccentric muscle contractions which also contribute to muscle damage (Naughton, Miller, & Slater, 2017). This damage is initiated by overstretching of the sarcomere, and leads to membrane disruption of the sarcolemma and t-tubules, excitation-contraction coupling dysfunction, entry of calcium ions into the sarcolemma and ultimately loss of muscle strength (Takarada, 2003; Peake, Neubauer, Della Gatta, & Nosaka, 2017). While the exact aetiology of muscle damage and its associations with soreness remain unresolved (Close, Ashton, Cable, Doran, & MacLaren, 2004), there is evidence that muscle soreness is linked to inflammation, connective tissue damage, muscle damage, or is caused by structural damage to the extracellular matrix during exercise (Lewis, Ruby, & Bush-Joseph, 2012; Peake et al., 2017).

The rugby pre-season period is characterised by intensive daily training sessions with an emphasis on speed, strength, endurance and contact. Therefore, coaches progressively overload training to transition players from the off-season to the competitive season. Despite the focus of pre-season being on training adaptation, and the notion that interfering with the recovery process may blunt the adaptive response to exercise (Markworth, Maddipati, & Cameron-Smith, 2016), optimising recovery during this period is pertinent for players to maintain the intensities required for each training session. Pre-season also is a period during which nutritional strategies for the competitive season can be practiced and evaluated. In theory, attenuating muscle damage with nutritional interventions during this period could facilitate players to better maintain performance, potentially via psychological and physiological mechanisms (McLean, Coutts, Kelly, McGuigan, & Cormack, 2010; Tavares, Smith, & Driller, 2017b).

The most common nutritional strategy investigated for recovery from muscle damaging exercise is protein. Amino acid ingestion facilitates muscle protein turnover during recovery (Tipton, Ferrando, Phillips, Doyle and Wolfe, 1999), synthesizing new muscle proteins and repairing old damaged proteins. In theory, promoting the repair of damaged proteins reduces the severity of exercise-induced muscle damage and accelerates recovery. However, since mixed results have been reported for protein ingestion and muscle recovery from damaging exercise (Pasiakos, Lieberman, & McLellan, 2014), alterative nutritional interventions warrant investigation.

Omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation also has received attention for its potential to aid recovery (Gray, Chappell, Jenkinson, Thies, & Gray, 2014; Lenn et al., 2002; Tartibian, Maleki, & Abbasi, 2010). Fish oils contain the n-3 PUFA's, 20:5n3 (eicosapentaenoic acid, EPA) and 22:6n3 (docosahexaenoic acid, DHA). The incorporation of EPA and DHA into cellular membranes exerts anti-inflammatory properties by initiating a reduction in the pro-inflammatory hormones (Calder, 2015; Erikson, 1996). In addition, n-3 PUFA ingestion reduces oxidative stress, evidenced by a reduction in the expression of plasma thiobarbituric acid and H_2O_2 – induced lymphocyte DNA damage following exercise (Gray et al., 2014). Hence, it has been suggested that omega-3 PUFA ingestion can act as a free radical scavenger (Barbosa et al., 2003), which can modulate the production of prostaglandin E2, whereby there is a decrease in PGE-2 production and an increase in PGE-3 (Calder, 2006). Dietary n-3 PUFA also are essential components of nerve endings and the myelin sheath of neurons of the nervous system (Pu et al., 2013; Laye, Nadjar, Joffre, & Bazinet, 2018). Specifically, DHA is readily incorporated into the neuronal membrane where it appears to exert its biological action. For example, 21 days of n-3 PUFA supplementation during training was shown to enhance neuromuscular development in athletes (Lewis, Radonic, Wolever, & Wells, 2015). Hence, there is scientific rationale to link n-3 PUFA ingestion with a reduced inflammatory response to exercise and improved neuromuscular function (Lewis et al., 2015).

The majority of previous studies that investigated the impact of n-3 PUFA supplementation on recovery from exercise-induced muscle damage were conducted within a controlled laboratory setting, whereby muscle damage was initiated by protocols (i.e., knee extension/flexion on isokinetic dynamometer, downhill running)

that do not necessarily reflect the typical movement patterns of elite athletes (Corder, Newsham, McDaniel, Ezekiel, & Weiss, 2016; Gray et al., 2014; Jouris, McDaniel, & Weiss, 2011; Lembke, Capodice, Hebert, & Swenson, 2014; Lenn et al., 2002; McGlory et al., 2016). For example, Jouris et al (2011) reported an attenuation of muscle soreness with the ingestion of 3 g/day of n-3 PUFA over a 7 day supplementation period following an eccentric bicep curl exercise protocol (Jouris et al., 2011). The beneficial role of n-3 PUFA ingestion was primarily attributed to an anti-inflammatory response. However, the lack of a blinded placebo condition means that a "placebo effect" cannot be eliminated as a possible reason for the findings. In contrast, two experimental studies (Gray et al. 2014; Lenn et al. 2002) reported no changes in blood CK concentrations, performance or muscle soreness with fish oil supplementation following eccentric based exercise. However, given that the change in muscle function following exercise was negligible, Gray et al. (2014) acknowledge the exercise protocol utilized exerted only mild muscle damage and may explain why no differences were detected. Given these equivocal findings, there is scope to investigate the influence of n-3 PUFA ingestion on exercise recovery using a training model of muscle damage that simulates the functional movements of elite athletes within an applied field setting (Cockburn, Bell, & Stevenson, 2013).

Therefore, the primary aim of the present study was to assess the impact of a twice daily supplement of n-3 PUFA combined with protein on muscle recovery in professional Rugby Union players during a 5 week training camp. We hypothesised that twice daily supplementation with 1546mg omega-3 PUFA (551 mg EPA and 551 mg DHA) would reduce the perception of muscle soreness, attenuate the decline in

countermovement (CMJ) jump performance and improve general well-being in elite Rugby Union players during pre-season training.

Methods

This double-blind, parallel designed, intervention study received ethics approval from the University of Otago, Human Health Ethics Committee. A verbal and written explanation of study procedures was provided to all participants, before providing written informed consent to participate. Thirty-three professional Rugby Union players volunteered to participate in the study. Participants were assigned to either a fish oil (FO) or placebo (P) supplement group, stratified by playing position and body composition goals (*e.g.* as part of pre-season players are placed into one of three groups based on their current skinfold measures and their individual optimal skinfolds. These groups are 1) gain muscle mass, 2) maintain current body composition, 3) lose body fat). While 16 players were assigned to FO and 17 to P, only 9 in FO and 11 in P completed the study. The mean age of participants was 22 years and 7 months (SD: 2 years 11 months; range: 18 years 11 months - 27 years 11 months). Two participants dropped out of the study due to gastrointestinal illness and 7 to training related injury. Six players did not complete all data collection.

Pre-season training schedule. All players performed 5 weeks of pre-season training as scheduled by team coaching staff. Training took place 5 days per week from 8am to 5pm, with sessions including strength and conditioning, match skills/simulated match play and flexibility on Monday, Tuesday, Thursday and Friday. Wednesday sessions were a recovery day, consisting of light training and meetings. Weekly training distances covered were 19.3±3.8 km (FO 17.6±7.0 km P 21.0±6.5 km).

During weeks 4-5, players travelled to Australia and completed a pre-season rugby match and a rugby 10s tournament.

Resistance training comprised of a strength intensification phase where all players were required to perform the same exercises, including sets and repetitions. The load for each repetition was based on individual 1RMs. The on-field training load was monitored via GPS (Statsports viper system, Statsports Group Ltd, N. Ireland). On-field training load was measured as fast running distance (FAST = velocity 4-7 m/s), sprint distance (SPRINT = velocity > 7 m/s) and High Metabolic Load Distance (HMLD = W/kg > 25.5). Equations developed by di Prampero et al., (2005) and Osgnach, Poser, Bernardini, Rinaldo, & di Prampero, (2010) enabled the calculation of metabolic power output (HMLD), which our research (Smith, Tarrant and McIntosh, In press) suggests more accurately accounts for the energetic costs of acceleration and deceleration that occur during the intermittent and intensive running that is common in rugby (Gaudino et al., 2013; Kempton, Sirotic, Rampinini, & Coutts, 2015).

Dietary supplementation. Participants consumed two, 200 mL protein-based drinks daily for 5 weeks. The intervention group (FO) consumed drinks that contained multiple nutrients including 1546 mg of omega-3 PUFA (551 mg EPA and 551 mg DHA), whereas the placebo (P) group consumed drinks matched for protein (15.0g/200mL), carbohydrate (14.5g/200mL) and fat content (8.4g/200mL), but without omega 3 PUFA (Smartfish, Oslo, Norway). All drinks were served in identical white packages. While training in New Zealand, participants consumed one test drink after morning training and the other following afternoon training. These

drinks were consumed alongside a protein shake containing an additional 15g of whey protein (BSc, Whey Protein Powder, Body Science, Auckland, New Zealand). As players left training on Friday they were provided with four additional drinks and asked to consume one in the morning and one in the afternoon with meals on Saturday and Sunday. When in Australia (days 26-33) coaching staff provided players with drinks twice per day. All players were aware of best nutrition practices during flights, particularly regarding the importance of hydration. Accordingly, there was no difference in hydration status assessed via Urine Specific Gravity (Atago Ltd, Tokyo, Japan) from the first void of day 1 (FO: 1.024±0.005; P: 1.025±0.004) to the first void of day 35 (FO: 1.021± 0.010, P: 1.022±0.004).

Breakfast, lunch and all snacks were consumed at training, i.e. Monday to Friday each week and all meals whilst in Australia were provided by the team nutritionist.

Data collection

Ear lobe blood samples were collected at baseline, day 19 and day 35, following at least 48 hours without strenuous exercise and were later analysed for percent fatty acid composition. All blood samples were collected upon arrival at training prior to breakfast (7:30-8:00 am). The ear lobe was cleaned with an alcohol swab then punctured by a lancet (Becton, Dickinson contact activated lancet, Auckland, New Zealand). Blood was dispensed into eppendorf tubes containing EDTA and stored at -80^{o}C. At these timepoints, players were asked to record their weekly dietary fish consumption. Additional measurements were collected during the pre-season training period, including CMJ peak force, perceived feelings of muscle soreness, a McLean questionnaire on fatigue, sleep, stress and mood (McLean et al., 2010), skinfold

assessments and strength (table 1). On day 35, participants were asked to predict whether they had been assigned to FO or P conditions.

Skinfold measurements. A level 1 trained and accredited International Society for the Advancement of Kinanthropometry (ISAK) anthropometrist performed skinfold measurements from eight sites (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, thigh, calf) on the right hand side of the body using body fat calipers (Holtain Ltd., Crosswell, United Kingdom) and tape measures (Lufkin Executive Thinline, W606PM). Skinfold measurements were collected at baseline and on the final day of the study and the average technical error of measurement (TEM) was 0.9%.

Countermovement jump performance. To measure neuromuscular fatigue, peak force (N) was measured using a CMJ test. All vertical jumps were performed between 08:00 and 10:00 AM following breakfast, at baseline, days 5, 12, 16, 19, 22 and on day 35. Participants completed a standardized warm-up of dynamic stretches and bodyweight movements. Participants performed three CMJ with a brief rest between each jump on two force plates one under each foot (PASCO PS 2142, Roseville, CA, USA) to enable the measurement of peak force at a sample rate of 500Hz. The total ground reaction force was calculated as the sum of the left and right force plate measures. Participants began each CMJ standing on the force plates with their knees fully extended and hands on their hips. Participants descended to a self-selected depth and jumped as high and quickly as possible. The best attempt, determined by peak force, was used for data analysis. CMJ is regularly performed, therefore all participants were familiar with the protocols. Force platform data were analysed using

Pasco Capstone v1.4.0 software (PASCO, Roseville, California, USA). Our unpublished data from 18 elite Rugby Union players demonstrated an acceptable level of test-retest reliability (ICC: 0.89; CV: 4.6%).

Subjective muscle soreness and wellness questionnaire. This questionnaire has been previously utilised to reflect changes in training load amongst Rugby League players (McLean et al., 2010; Tavares, Healey, Smith, & Driller, 2017). In this study, the questionnaires were used to determine subjective scores for muscle soreness, fatigue, mood, sleep and stress (McLean et al., 2010). Participants rated responses on a 5 point likert scale (1-5). A higher score represented a positive response. All players were familiar with this questionnaire as it formed part of the team's habitual monitoring procedure. Upper and lower body muscle soreness scores were rated using a diagram of a person with muscle groups divided into six compartments for both sides of the body (quadriceps, groin, calf, hamstrings, gluteus and upper body). Each muscle group was rated from 1-5 with 1 being "very sore" and 5 being "feeling great". Therefore the maximum score for the lower body was 50 and upper body was 10. All questionnaires were completed prior to training.

Blood treatment and analysis. Plasma, erythrocyte, and buccal cell lipids were extracted using the method of Bligh and Dyer (Bligh & Dyer, 1959). Samples were then analysed by Gas Chromatograph with flame ionization detection (HP-5890 Series, Hewitt-Packard, Wilmington, USA), as previously described (Dodds, McCoy, Rea, & Kennish, 2005). In brief, samples were thawed, dried and then extracted with chloroform and methanol at 200^{o}C and 13.8 MPa, before further drying, rinsing and evaporation with nitrogen. The recovered lipids were reconstituted and hydrolyzed.

One mL of distilled water and 2 mL of hexane were mixed with the solution. The organic portion was removed and an internal standard added, which allows for the calculation of absolute values for % omega 3 PUFA concentrations.

Data handling and statistical analysis.

All data were analysed using magnitude-based inferential statistics to determine inferences about the true effects of the intervention on CMJ, strength and the various subjective measures (Hopkins, 2007; Hopkins, 2018). This statistical approach allows for quantitative (trivial, small, moderate, large or very large) descriptions of the magnitude of difference between trials and establishes the likelihood of the experimental condition (i.e. FO) having a beneficial, trivial or harmful effect on the outcome of interest. Standardised Mean Differences (SMD) were calculated as the difference between the means divided by the pooled standard deviation. The following quantitative criteria for the SMD was used to explain the practical significance of the findings: trivial <0.20; small 0.20-0.59; moderate 0.60-1.19; large 1.20-1.99; very large 2.0-3.90; and almost perfect >4.00. Quantitative chances of real differences in variables between groups were assessed qualitatively as <1%, almost certainly not; 1% to 5%, very unlikely; 5% to 25%, probably not; 25% to 75%, possibly; 75% to 97.5%, likely; 97.5% to 99%, very likely; >99%, most likely. If the chances of a variable having beneficial and harmful differences which were >5%, the true effect was deemed to be unclear (Hopkins, 2010). Quantitative data are presented as percent beneficial/trivial/harmful. Data are provided as mean ± standard deviation (SD) and standardized mean differences (SMD), unless otherwise stated.

Results

Training

The average weekly distance covered in the intensive metrics was FAST: FO 2.5±0.9 km, P 2.7±0.7 km; HMLD: FO 2.3±0.8 km P 2.5±0.7 km; SPRINT: FO 177±156 m P 166±170 m. There were small but unclear differences between FO and P for both FAST (SMD = 0.36, 81/0/19) and HMLD (SMD = 0.28, 76/1/22), while trivial differences between FO and P for SPRINT (SMD = 0.07, 43/1/56).

Compliance and blood fatty acid concentrations

Compliance to consuming two drinks per day ranged from 79-100% while in New Zealand, with twelve players consuming 100% of their training drinks. Coaching staff reported all drinks were consumed in Australia. At baseline % omega-3 PUFA concentrations were 1.12±0.89 % and 1.41± 1.01 % for FO and P respectively, changing to 3.81±5.66 % and 1.30±1.13 % after 5 weeks for FO and P respectively (figure 1). Accounting for baseline values, there was a very large likely beneficial effect of FO on omega-3 PUFA concentrations compared to PLA (SMD 2.84, 87/6/8).

Dietary intakes and randomization

Dietary fish consumption ranged from 0 to 3 servings per week at both the mid- (FO: 1.3 ± 0.8 servings per week and P: 0.9 ± 1.3 servings per week) and final (FO: 1.83±1.00 servings per week and P: 1.80±1.03 servings per week) time point. Twenty-seven percent of participants in P correctly stated that they were assigned to the placebo, whereas a further 27% incorrectly believed they were assigned to the FO condition and remaining participants were unsure.

Anthropometrics

In FO, body mass increased from 106.9±8.3 kg to 107.1±8.1 kg whereas in P, body mass increased from 106.7±16.5 kg to 107.3±16.6kg (SMD -0.03, trivial; 100% most likely trivial effect). In FO, the sum of eight skinfolds was 70.8±14.4 mm at baseline and 67.5±15.5 mm post intervention, whereas P was 74.4±17.2 mm at baseline decreasing to 71.5±17.6 mm post intervention. There was an unclear trivial effect on the change in skinfolds from pre to post between FO and P (SMD: 0.02 trivial; 1/95/3 unclear).

Countermovement Jump

Peak force during the CMJ at baseline was 2705.2±271.4 N (range 2399.8–3031.8 N) and 2858.5±339.7 N (range 2269.4–3183.7 N) for FO and P groups, respectively. By day 35 of the training camp, 7 participants in P recorded lower CMJ peak force scores compared with baseline resulting in a mean decrease of 3.4±8.6% (2772.4±481.1 N). In contrast, peak force during the CMJ increased by 4.6±5.9% by day 35 in FO (2816.0±309.0 N), with only two participants producing less force on day 35 compared to baseline. Expressed as change in CMJ peak force from baseline, there was a likely beneficial effect of FO on CMJ performance at day 16 and day 35 (figure 2).

Muscle soreness

Expressed as a change from baseline, with the exception of day 12, a likely or very likely beneficial effect of FO on lower body muscle soreness was observed at all timepoints (figure 3b). At baseline, median (IQR) upper body muscle soreness was 8(1.5) and 8(2) (maximum value 10 which equates to “no soreness”) for FO and P

respectively. On Day 22 (two days after the in -house match), expressed as change from baseline, there was a likely harmful effect of FO on upper body muscle soreness whereby the muscle soreness was 4.57 out of a potential score of 10 (no muscle soreness) for FO and 6.82 for P (Figure 3a). Interestingly, the two players in FO that recorded the maximum soreness at this time point were forwards and one player played for the developmental squad against the senior squad for the match, so may have been subjected to heavier impacts throughout the match. Repeating statistical analysis of the upper body muscle soreness data set with this player's data at the 22 day timepoint removed reduced the SMD to -0.84 moderate (7/11/82 unclear).

Subjective measures of fatigue, sleep, stress and mood

Expressed as a change from baseline, a likely moderate beneficial effect (less fatigue) of FO was observed for subjective fatigue from Day 20-35 (figure 4), with the mean decrease for P being greater than 1 (on a 5 point likert scale) on all days compared to a mean score decrease of less than 1 for FO between days 20-35. However, there was only trivial or small unclear effects on stress or mood responses (data not shown). On Day 22, there was a moderate (0.83) likely beneficial (88/9/3) effect of FO compared to P on sleep quality, however at all other timepoints there were trivial or small unclear effects (data not shown).

Discussion

This is the first study, to our knowledge, to investigate the impact of fish oil derived omega-3 PUFA ingestion on muscle recovery within the real world, applied, field setting of elite Rugby Union. We report a very large likely beneficial effect of FO on increasing blood omega-3 PUFA concentrations compared to P over the course of the

pre-season training period. Consistent with our working hypothesis, we observed a moderate beneficial effect of adding 1546 mg of omega-3 PUFA (551 mg of EPA and 551 mg DHA) to a protein-based drink over 5 weeks of pre-season training on lower body muscle soreness, fatigue and CMJ performance in professional Rugby Union players competing in the Super Rugby competition. In contrast, there was a trivial effect of adding omega-3 PUFA to a protein-based drink on sleep, stress and mood. These data suggest that twice per day supplementation with a protein-based drink containing omega-3 PUFA has the potential to attenuate lower body muscle soreness and fatigue and better maintain neuromuscular performance in elite Rugby Union players during pre-season training. The present study findings could have important implications for Rugby Union players in terms of minimizing muscle soreness and fatigue and maintaining performance when the recovery period between training sessions or match-play is short. In the current study the time between the match and the physiological measurements was similar to the time period between matches and training typically scheduled during the competitive season. In theory, reducing the severity of muscle soreness between matches and training could have beneficial effects on training performance by allowing players to train at higher intensities during the season. Furthermore, the reduced muscle soreness with fish oil ingestion could have a psychological impact by reducing feelings of fatigue, thus improving a player's approach to training and matches.

Several previous studies, conducted in controlled laboratory settings, report a beneficial effect of fish oil supplementation on the perception of muscle soreness following intense eccentric exercise (Corder et al., 2016; Tsuchiya et al., 2016; Jouris et al., 2011; Lembke et al., 2014; Tinsley et al., 2016). Consistent with these

experimental study findings, the present field-based study, which included a training protocol reflective of professional rugby with progressive resistance training and high volumes of anaerobic training, revealed a moderately beneficial effect of fish oil ingestion on muscle soreness during preseason Rugby training. Specifically, a moderate beneficial effect of fish oil ingestion was observed on lower limb muscle soreness, whereas the effect on upper body soreness was unclear. We speculate that the beneficial effect of fish oil ingestion was mediated, at least in part, by the modification of the 3-series eicosanoids, 3-series prostaglandins and 5-series leukotriene. The 3-series eicosanoids exhibit lower inflammatory properties than the 2-series eicosanoids (PGE_2, 4-series leukotriene) and are proposed to decrease the inflammatory response to exercise and attenuate muscle soreness (Lenn et al., 2002). In support of this theory, muscle soreness is proposed to be associated with biochemical muscle damage to the sarcomere and free radical damage (Lewis et al., 2012). This damage leads to an inflammatory response which may contribute to the sensation of soreness and potentially a decrement in performance (Jakeman, Lambrick, Wooley, Babraj, & Faulkner, 2017). Alternatively, adding fish oil to a protein-based drink may attenuate muscle soreness by protecting the structural integrity of the muscle cell from damage. We previously demonstrated that the increase in plasma creatine kinase concentrations following eccentric exercise was reduced with the addition of fish oil to a protein-based supplement (Philpott, 2018). These data indicate a reduced leakage of creatine kinase from the muscle cell into circulation following fish oil ingestion. Hence, the exact mechanism for fish oil ingestion reducing muscle soreness remains unclear.

The better CMJ performance scores with fish oil ingestion may also be due to a reduced perception of muscle soreness. Alternatively, the benefit of fish oil ingestion on CMJ performance may have been mediated at the neuromuscular level since explosive power is determined by both the stretch-shortening cycle as well as the contractile properties of the muscle (add ref). Accordingly, previous studies demonstrate that decrements in CMJ performance in professional Rugby League players during the initial 48 hours following matchplay are due, in part, to changes in neural fatigue (McLean et al., 2010). Interestingly, a recent study reported that 21 days of fish oil supplementation exhibited positive changes in neuromuscular function (Lewis et al., 2015) which could explain the findings in the present study. Due to the nature of conducting research in an applied field setting with elite athletes, it was not possible to collect venous blood samples for analysis of blood markers of muscle damage, inflammation or oxidative stress. Furthermore, it was not possible to conduct more direct measurements of neuromuscular function such as EMG. Therefore, it was not possible to definitively explain the mechanisms mediating the link between fish oil ingestion and better CMJ performance. Future applied research designs are warranted in the context of fish oil ingestion and recovery to measure performance outcomes alongside more mechanistic biochemical and neuromuscular measurements.

The beneficial effects of fish oil ingestion on muscle soreness were observed 36 hours following international travel. Jet-lag occurs when greater than three time zones have been crossed (O'Connor & Morgan, 1990). In the present study, players crossed three time zones between Australia and New Zealand. Symptoms of jet-lag include fatigue, inability to sleep and potentially decrements in CMJ (Chapman, Bullock, Ross, Rosemond, & Martin, 2012). On day 35, there were beneficial effects of FO on CMJ,

and moderately lower fatigue scores. Hence, we may speculate that FO ingestion attenuated the detrimental impact of jet-lag on muscle recovery in our cohort of elite Rugby Union players and thus a follow-up study is warranted.

Despite the present study revealing a beneficial effect of adding omega-3 fatty acids to a protein-based supplement on muscle soreness and CMJ performance, we did detect a detrimental effect on upper body muscle soreness on day 22. This observation may be due to the preseason training schedule including a match on day 20 where we were unable to control workload between test drink conditions as previously achieved with training sessions. Also noteworthy was the observation that the highest upper body muscle soreness scores were reported by two forwards who are typically involved in the majority of collision impacts. One player was a flanker and this position is characterized by frequent involvement in breakdown play and subsequent impact on the upper body. These physical demands may have impacted the soreness results at this time point, especially since only trivial or moderately beneficial effects of FO on upper body soreness were detected at all other timepoints. In the present study, the primary endpoint measurement of performance was focused on CMJ performance. Therefore, we cannot discern the impact of fish oil ingestion on upper body performance in this study. It is possible that the study duration was not sufficient for maximal incorporation of EPA and DHA into the muscle phospholipid membrane. This notion is based on the findings of McGlory et al., (2014) that reported an increase in n-3 polyunsaturated fatty acid muscle lipid composition after 2 weeks of fish oil supplementation at a dose of 5g/day, with these rates of incorporation continuing to rise after 4 weeks of supplementation (McGlory et al., 2014). Given the lower relative dose of fish oil administered in the present study, it may be argued that

our supplementation regimen was not optimal. However, based on the work of McGlory et al (2014), 5 weeks of supplementation was likely sufficient to detect a significant increase in the level of n-3 PUFA incorporation into muscle in our cohort of elite Rugby Union players.

Despite the beneficial effects of adding fish oil to a protein-based supplement on fatigue, other markers of wellness, stress, mood and sleep quality were only trivially affected. However, no player reported a score lower than "normal" for stress or mood and only one player rated sleep as lower than "normal" throughout the study. These data suggest that although the training camp was intensive, it had little impact on player mood, stress or sleep quality. Therefore, any potential impact of fish oil ingestion on these measures could not be detected in this study.

Conclusion

The addition of fish oil (1546 mg of omega-3 PUFA; 551 mg EPA and 551 mg DHA) to a protein-based drink for 5 weeks provided an effective strategy to reduce muscle soreness and fatigue and better maintain CMJ performance during preseason training in elite Rugby Union players. These beneficial effects may confer benefits to muscle recovery between training sessions and also improve subsequent matchplay performance, especially when the period between matches is short.

References

Barbosa, D. S., Cecchini, R., El Kadri, M. Z., Rodriguez, M. A., Burini, R. C., & Dichi, I. (2003). Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty acids. *Nutrition, 19*(10), 837-842

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol, 37*(8), 911-917. doi: 10.1139/o59-099

Calder, P. C. (2006). Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids, 75*(3), 197-202. doi: 10.1016/j.plefa.2006.05.012

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta, 1851*(4), 469-484. doi: 10.1016/j.bbalip.2014.08.010

Chapman, D. W., Bullock, N., Ross, A., Rosemond, D., & Martin, D. T. (2012). Detrimental effects of west to east transmeridian flight on jump performance. *Eur J Appl Physiol, 112*(5), 1663-1669. doi: 10.1007/s00421-011-2134-6

Close, G. L., Ashton, T., Cable, T., Doran, D., & MacLaren, D. P. (2004). Eccentric exercise, isokinetic muscle torque and delayed onset muscle soreness: the role of reactive oxygen species. *Eur J Appl Physiol, 91*(5-6), 615-621. doi: 10.1007/s00421-003-1012-2

Cockburn, E., Bell, P. G., & Stevenson, E. (2013). Effect of milk on team sport performance after exercise-induced muscle damage. *Med Sci Sports Exerc, 45*(8), 1585-1592. doi: 10.1249/MSS.0b013e31828b7dd0

Cockburn, E., Hayes, P. R., French, D. N., Stevenson, E., & St Clair Gibson, A. (2008). Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. *Appl Physiol Nutr Metab, 33*(4), 775-783. doi: 10.1139/H08-057

Corder, K. E., Newsham, K. R., McDaniel, J. L., Ezekiel, U. R., & Weiss, E. P. (2016). Effects of Short-Term Docosahexaenoic Acid Supplementation on Markers of Inflammation after Eccentric Strength Exercise in Women. *J Sports Sci Med, 15*(1), 176-183

Cunniffe, B., Hore, A. J., Whitcombe, D. M., Jones, K. P., Baker, J. S., & Davies, B. (2010). Time course of changes in immuneoendocrine markers following an international rugby game. *Eur J Appl Physiol, 108*(1), 113-122. doi: 10.1007/s00421-009-1200-9

di Prampero, P. E., Fusi, S., Sepulcri, L., Morin, J. B., Belli, A., & Antonutto, G. (2005). Sprint running: a new energetic approach. *J Exp Biol, 208*(Pt 14), 2809-2816. doi: 10.1242/jeb.01700

Dodds, E. D., McCoy, M. R., Rea, L. D., & Kennish, J. M. (2005). Gas chromatographic quantification of fatty acid methyl esters: flame ionization detection vs. electron impact mass spectrometry. *Lipids, 40*(4), 419-428

Erikson, L. (1996). Does dietary supplementation of cod liver oil mitigate musculoskeletal pain? . *Eur J Clin Nutr, 50*, 689-693

Gaudino, P., Iaia, F. M., Alberti, G., Strudwick, A. J., Atkinson, G., & Gregson, W. (2013). Monitoring training in elite soccer players: systematic bias between running speed and metabolic power data. *Int J Sports Med, 34*(11), 963-968. doi: 10.1055/s-0033-1337943

Gray, P., Chappell, A., Jenkinson, A. M., Thies, F., & Gray, S. R. (2014). Fish oil supplementation reduces markers of oxidative stress but not muscle soreness after eccentric exercise. *Int J Sport Nutr Exerc Metab, 24*(2), 206-214. doi: 10.1123/ijsnem.2013-0081

Hopkins, W. G. (2007). A spreadsheet for deriving a confidence interval, mechanistic inference and clinical inference from a p value. *Sportscience, 11, 16–20.*

Hopkins, W. G.(2010). Spreadsheets for Analysis of Controlled Trials, With Adjustment for a Subject Characteristic. *Sportscience*, 10, 46–50.

Hopkins, W. G.(2018). Spreadsheets for Analysis of Controlled Trials, Crossovers and Time Series. *Sportscience*, 21, 1–4.

Jackman, S. R., Witard, O. C., Jeukendrup, A. E., & Tipton, K. D. (2010). Branched-chain amino acid ingestion can ameliorate soreness from eccentric exercise. *Med Sci Sports Exerc, 42*(5), 962-970. doi: 10.1249/MSS.0b013e3181c1b798

Jakeman, J. R., Lambrick, D. M., Wooley, B., Babraj, J. A., & Faulkner, J. A. (2017). Effect of an acute dose of omega-3 fish oil following exercise-induced muscle damage. *Eur J Appl Physiol, 117*(3), 575-582. doi: 10.1007/s00421-017-3543-y

Jouris, K. B., McDaniel, J. L., & Weiss, E. P. (2011). The Effect of Omega-3 Fatty Acid Supplementation on the Inflammatory Response to eccentric strength exercise. *J Sports Sci Med, 10*(3), 432-438

Kempton, T., Sirotic, A. C., Rampinini, E., & Coutts, A. J. (2015). Metabolic power demands of rugby league match play. *Int J Sports Physiol Perform, 10*(1), 23-28. doi: 10.1123/ijspp.2013-0540

Laye, S., Nadjar, A., Joffre, C., & Bazinet, R. P. (2018). Anti-Inflammatory Effects of Omega-3 Fatty Acids in the Brain: Physiological Mechanisms and Relevance to Pharmacology. *Pharmacol Rev, 70*(1), 12-38. doi: 10.1124/pr.117.014092

Lembke, P., Capodice, J., Hebert, K., & Swenson, T. (2014). Influence of omega-3 (n3) index on performance and wellbeing in young adults after heavy eccentric exercise. *J Sports Sci Med, 13*(1), 151-156

Lenn, J., Uhl, T., Mattacola, C., Boissonneault, G., Yates, J., Ibrahim, W., & Bruckner, G. (2002). The effects of fish oil and isoflavones on delayed onset muscle soreness. *Med Sci Sports Exerc, 34*(10), 1605-1613. doi: 10.1249/01.MSS.0000031099.08661.90

Lewis, E. J., Radonic, P. W., Wolever, T. M., & Wells, G. D. (2015). 21 days of mammalian omega-3 fatty acid supplementation improves aspects of neuromuscular function and performance in male athletes compared to olive oil placebo. *J Int Soc Sports Nutr, 12*, 28. doi: 10.1186/s12970-015-0089-4

Lewis, P. B., Ruby, D., & Bush-Joseph, C. A. (2012). Muscle soreness and delayed-onset muscle soreness. *Clin Sports Med, 31*(2), 255-262. doi: 10.1016/j.csm.2011.09.009

Markworth, J. F., Maddipati, K. R., & Cameron-Smith, D. (2016). Emerging roles of pro-resolving lipid mediators in immunological and adaptive responses to exercise-induced muscle injury. *Exerc Immunol Rev, 22*, 110-134

McGlory, C., Galloway, S. D., Hamilton, D. L., McClintock, C., Breen, L., Dick, J. R., . . . Tipton, K. D. (2014). Temporal changes in human skeletal muscle and blood lipid composition with fish oil supplementation. *Prostaglandins Leukot Essent Fatty Acids, 90*(6), 199-206. doi: 10.1016/j.plefa.2014.03.001

McGlory, C., Wardle, S. L., Macnaughton, L. S., Witard, O. C., Scott, F., Dick, J., . . . Tipton, K. D. (2016). Fish oil supplementation suppresses resistance exercise and feeding-induced increases in anabolic signaling without affecting myofibrillar protein synthesis in young men. *Physiol Rep, 4*(6). doi: 10.14814/phy2.12715

McLean, B. D., Coutts, A. J., Kelly, V., McGuigan, M. R., & Cormack, S. J. (2010). Neuromuscular, endocrine, and perceptual fatigue responses during different length between-match microcycles in professional rugby league players. *Int J Sports Physiol Perform, 5*(3), 367-383

Naughton, M., Miller, J., & Slater, G. J. (2017). Impact-Induced Muscle Damage and Contact-Sport: Aetiology, Effects on Neuromuscular Function and Recovery, and the Modulating Effects of Adaptation and Recovery Strategies. *Int J Sports Physiol Perform*, 1-24. doi: 10.1123/ijspp.2017-0268

Nosaka, K., Sacco, P., & Mawatari, K. (2006). Effects of amino acid supplementation on muscle soreness and damage. *Int J Sport Nutr Exerc Metab, 16*(6), 620-635

O'Connor, P. J., & Morgan, W. P. (1990). Athletic performance following rapid traversal of multiple time zones. A review. *Sports Med, 10*(1), 20-30

Osgnach, C., Poser, S., Bernardini, R., Rinaldo, R., & di Prampero, P. E. (2010). Energy cost and metabolic power in elite soccer: a new match analysis approach. *Med Sci Sports Exerc, 42*(1), 170-178. doi: 10.1249/MSS.0b013e3181ae5cfd

Pasiakos, S. M., Lieberman, H. R., & McLellan, T. M. (2014). Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: a systematic review. *Sports Med, 44*(5), 655-670. doi: 10.1007/s40279-013-0137-7

Peake, J. M., Neubauer, O., Della Gatta, P. A., & Nosaka, K. (2017). Muscle damage and inflammation during recovery from exercise. *J Appl Physiol (1985), 122*(3), 559-570. doi: 10.1152/japplphysiol.00971.2016

Philpott, J. D., Donnelly, C., Walshe, IH., Dick, J., Galloway, SDR., Tipton, KD., and Witard, OC. (2018). Adding fish oil to whey protein, leucine and carbohydrate over a 6 week supplementation period attenuates muscle soreness following eccentric exercise in soccer players. *Int J Sport Nutr Exerc Metab.* doi: 10.1123/ijsnem.2017-0161

Pu, H., Guo, Y., Zhang, W., Huang, L., Wang, G., Liou, A. K., . . . Gao, Y. (2013). Omega-3 polyunsaturated fatty acid supplementation improves neurologic recovery and attenuates white matter injury after experimental traumatic brain injury. *J Cereb Blood Flow Metab, 33*(9), 1474-1484. doi: 10.1038/jcbfm.2013.108

Smith, T.K., Tarrant, N., McIntosh. N. (In press). *Examination of the efficacy of GPS generated metabolic load measures for monitoring intensive intermittent running load in rugby union* . European College of Sports Science, Dublin, Ireland.

Stiefel, P., Ruiz-Gutierrez, V., Gajon, E., Acosta, D., Garcia-Donas, M. A., Madrazo, J., . . . Carneado, J. (1999). Sodium transport kinetics, cell membrane lipid composition, neural conduction and metabolic control in type 1 diabetic patients. Changes after a low-dose n-3 fatty acid dietary intervention. *Ann Nutr Metab, 43*(2), 113-120. doi: 10.1159/000012775

Takarada, Y. (2003). Evaluation of muscle damage after a rugby match with special reference to tackle plays. *Br J Sports Med, 37*(5), 416-419

Tartibian, B., Maleki, B. H., & Abbasi, A. (2010). The effects of omega-3 supplementation on pulmonary function of young wrestlers during intensive training. *J Sci Med Sport, 13*(2), 281-286. doi: 10.1016/j.jsams.2008.12.634

Tavares, F., Healey, P., Smith, T. B., & Driller, M. (2017). The effect of training load on neuromuscular performance, muscle soreness and wellness during an in-

season non-competitive week in elite rugby athletes. *J Sports Med Phys Fitness*. doi: 10.23736/S0022-4707.17.07618-6

Tavares, F., Smith, T. B., & Driller, M. (2017a). Fatigue and Recovery in Rugby: A Review. *Sports Med, 47*(8), 1515-1530. doi: 10.1007/s40279-017-0679-1

Tavares, F., Smith, T. B., & Driller, M. (2017b). Fatigue and Recovery in Rugby: A Review. *Sports Med*. doi: 10.1007/s40279-017-0679-1

Tinsley, G. M., Gann, J. J., Huber, S. R., Andre, T. L., La Bounty, P. M., Bowden, R. G., . . . Grandjean, P. W. (2016). Effects of Fish Oil Supplementation on Postresistance Exercise Muscle Soreness. *J Diet Suppl*, 1-12. doi: 10.1080/19390211.2016.1205701

Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle, D., Jr., and Wolfe, R.R. (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* 276: E628-634.

Tsuchiya, Y., Yanagimoto, K., Nakazato, K., Hayamizu, K., & Ochi, E. (2016). Eicosapentaenoic and docosahexaenoic acids-rich fish oil supplementation attenuates strength loss and limited joint range of motion after eccentric contractions: a randomized, double-blind, placebo-controlled, parallel-group trial. *Eur J Appl Physiol, 116*(6), 1179-1188. doi: 10.1007/s00421-016-3373-3

Table Captions

Table 1: Study protocol outline

Figure Captions

Figure 1: Omega-3 Polyunsaturated acid (PUFA) concentrations (%) at baseline, Day 19 and End (Day 35) for Fish Oil and Placebo.

Figure 2: Countermovement jump performance, expressed as mean(SD) percent change from baseline for fish oil (FO) and placebo (P) conditions during the 35 day period of pre-season training.

Figure 3: Muscle soreness (A, upper body; B, lower body), expressed as mean(SD) change from baseline for fish oil (FO) and placebo (P) conditions during the 35 day pre-season training period.

Figure 4: Fatigue score, expressed as mean(SD) change from baseline for fish oil (FO) and placebo (P) conditions during the 35 day period of pre-season training.