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Article Title: Adding Fish Oil to Whey Protein, Leucine and Carbohydrate Over a 6 Week Supplementation Period Attenuates Muscle Soreness Following Eccentric Exercise in Competitive Soccer Players

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Running Head: n-3PUFA supplementation and recovery in soccer

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Adding fish oil to whey protein, leucine and carbohydrate over a 6 week supplementation period attenuates muscle soreness following eccentric exercise in competitive soccer players

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Abstract

Soccer players often experience eccentric exercise-induced muscle damage given the physical demands of soccer match-play. Since long chain n-3 polyunsaturated fatty acids (n3PUFA) enhance muscle sensitivity to protein supplementation, dietary supplementation with a combination of fish oil-derived n-3PUFA, protein and carbohydrate may promote exercise recovery. This study examined the influence of adding n-3PUFA to a whey protein, leucine and carbohydrate containing beverage over a 6 week supplementation period on physiological markers of recovery measured over 3 days following eccentric exercise. Competitive soccer players were assigned to one of three conditions (2 × 200mL): FO (n=10) contained n-3PUFA (1100mg DHA/EPA – approx. 550mg DHA, 550mg EPA), whey protein (15g), leucine (1.8g) and carbohydrate (20g); PRO (n=10) contained whey protein (15g), leucine (1.8g) and carbohydrate (20g) and CHO (n=10) contained carbohydrate (24g). Eccentric exercise consisted of unilateral knee extension/flexion contractions on both legs separately. Maximal force production was impaired by 22% during the 72 hour recovery period following eccentric exercise ($p < 0.05$). Muscle soreness, expressed as AUC during 72 hour recovery, was less in FO (1948 ± 1091 mm \times 72 h) than PRO (4640 ± 2654 mm \times 72h, $p < 0.05$) and CHO (4495 ± 1853 mm \times 72h $p = 0.10$). Blood concentrations of creatine kinase, expressed as AUC, were ~60% lower in FO compared to CHO ($p < 0.05$) and tended to be lower (~39%, $p = 0.07$) than PRO. No differences in muscle function, soccer performance or blood c-reactive protein concentrations were observed between groups. In conclusion, the addition of n-3PUFA to a beverage containing whey protein, leucine and carbohydrate ameliorates the increase in muscle soreness and blood concentrations of creatine kinase following eccentric exercise in competitive soccer players.

Key Words: Omega-3 polyunsaturated fatty acids, muscle damage, muscle recovery, soccer performance.

Introduction

Elite soccer players may be required to complete two competitive matches per week, interspersed with intense training sessions (Carling et al., 2015). Such intense scheduling, indicative of fixture congestion, places significant physiological stress on soccer players over the course of a season, as demonstrated experimentally by decrements in sprint speed, jump performance and distance covered at maximal intensity when soccer players completed two vs. one match per week over a 6 week to full season period (Lago-Penas et al., 2011; Rollo et al., 2014). The typical movement patterns performed by soccer players during match-play and training include sprints, explosive jumps and repeated changes in direction (Bloomfield et al., 2007). Repeated eccentric-based muscle contractions are required to execute these multidirectional and intermittent movements (Jones et al., 2009), but also are implicated in causing skeletal muscle fibre damage (Nédélec et al., 2012; Russell et al., 2015).

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

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

The multifactorial nature of recovery from high-intensity exercise includes the refuelling, repair and remodelling of skeletal muscle. It follows that optimising nutritional strategies for promoting muscle recovery should apply a multi-dimensional approach, encompassing the synergistic role of multiple nutrients. Protein is the key nutritional component to facilitate muscle protein remodelling following exercise (Witard et al., 2016) and carbohydrate ingestion is critical for replenishing muscle glycogen stores depleted following intense exercise (Bergstrom & Hultman, 1966). Therefore, the aim of the present study was to investigate the impact of adding fish oil-derived n-3PUFA to a whey protein, leucine and carbohydrate containing supplement over a 6 week period on acute recovery from eccentric muscle damage in competitive soccer players. Rationale for combining n-3PUFA with whey protein, leucine and carbohydrate is supported by previous literature that demonstrates n3PUFA enhances the muscle anabolic sensitivity to an amino acid source (Smith et al.,

2011). Therefore, we hypothesised that coingesting n-3PUFA with whey protein, leucine and carbohydrate over a 6 week supplementation period would reduce muscle soreness, attenuate the inflammatory response to exercise and improve soccer-specific performance during a 72 hour exercise recovery period compared to a protein control condition and a carbohydrate control condition.

Methods

Participants

Thirty young competitive male soccer players (age: 23 ± 1 yrs; body mass: 73.8 ± 5.9 kg; height: 178.9 ± 6.1 cm; Baseline Yo-Yo test score: 395 ± 158 m) were recruited from local soccer teams. All participants engaged in soccer training and/or match-play ≥ 3 times per week and were not taking n-3PUFA-containing supplements or any other supplement known to potential impact recovery from eccentric-based exercise. The study was approved by University of Stirling School of Sport Research Ethics Committee.

Experimental Design

The study design is summarized in Figure 1. Participants visited the laboratory on five separate occasions, including familiarisation. Based on their performance on the YoYo Intermittent Endurance Test Level 2, participants were assigned to one of three supplementation conditions; fish oil plus whey protein, leucine and carbohydrate (FO), a whey protein, leucine, carbohydrate placebo (PRO) or a carbohydrate only placebo (CHO). We previously demonstrated that as little as 2 wk of n-3PUFA supplementation (4.5g/day) significantly increased muscle lipid composition and that this response was further enhanced after 4 wk of supplementation (McGlory et al., 2014). Therefore, to elicit a pronounced increase in muscle lipid composition, in the present study we implemented a 6 wk n-3PUFA supplementation period. Following a 6 week supplementation period, participants visited the laboratory on four consecutive mornings to complete experimental trials in the fasted state with no pre-exercise meal provision. On the second laboratory visit, baseline testing was

followed by an eccentric-based exercise protocol. At each time point, measurements of muscle soreness and muscle function, as well as blood markers of muscle damage and inflammation were collected. Soccer-specific performance tasks were completed at baseline, 24 and 72 h post-exercise. All laboratory visits started at the same time of day (07:30) and measurements were always collected in the following order: blood sampling for measurements of serum creatine kinase (CK) and plasma CRP concentrations, muscle soreness, muscle function (completed in ~20 min), soccer skill tests (completed in ~30 min) and anaerobic endurance (completed in ~15 min).

Dietary supplementation

Within this double-blind, parallel group designed study, participants consumed 2 × 200mL volume juice-based drinks (1 × morning and 1 × evening) daily over the 6 week supplementation period and on each trial day (Smartfish Sports Nutrition, Ltd). All three supplements were matched for taste. Table 2 details the nutritional composition of each supplement.

Diet and Physical Activity Control

Participants were asked to maintain their exercise training and habitual diet routine throughout the 6 week supplementation period. Participants completed a 3 d food diary during the first 3 d of testing. Diet diaries were analysed for macronutrient and micronutrient content using Microdiet 2 (Downlee Systems Ltd).

Blood collection and treatment

On each laboratory visit and following an overnight fast, a blood sample was obtained from a forearm vein. Approximately 1 mL of blood was dispensed onto specialised Whatman 903 blood collection cards (GE Healthcare Ltd, Forest Farm Industrial Estate, Cardiff, CF 14 7YT, UK). The cards were left open and allowed to dry for 3 h after which the dried whole blood sample was detached from the collection device using forceps and placed into a screwcap vial containing 1 mL of methylating solution (1.25M methanol/HCl). The vials were

placed in a hot block at 70°C for 1 h. The vials were allowed to cool to room temperature before 2 mL of distilled water and 2 mL of saturated KCl solution were added. Fatty acid methyl esters

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

Eccentric exercise protocol

We utilised a laboratory-controlled eccentric exercise protocol that isolated the hamstring muscles to elicit a physiological state of local muscular stress. Using an isokinetic dynamometer (Biodex Corporation, New York), participants completed 12 sets of an individualised workload on each leg (total of 24 sets), alternating every four sets. In order to calculate an individualised workload, each participant performed 3 sets of 3 repetitions of the eccentric exercise protocol, each separated by 1 min. The peak eccentric and concentric forces were determined and the sum was multiplied by an estimated number of total repetitions to complete each set (e.g. 10) as per (Kennedy et al., 2017). This figure was then multiplied

by 1.2 to ensure the muscles were maximally worked. Once the workload was reached, the set was completed and the participants had a 1 min rest prior to engaging in the subsequent set. If a participant was unable to complete consecutive sets in less than 30 repetitions, the workload was reduced by 40% to enable the participant to complete the next set in ~20 repetitions. The number of repetitions, the rate of perceived exertion (RPE) and peak force achieved was recorded for each set.

Muscle Soreness

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

Muscle Function

A single leg isokinetic/eccentric MVC of the knee flexors was used to assess muscle function. Participants were seated on the dynamometer with their upper body, hips and exercising thigh securely strapped into the seat. The lower leg was attached to the arm of the dynamometer 1 cm above the lateral malleolus ankle joint with the axis of rotation of the dynamometer arm aligned with the lateral femoral condyle. The dynamometer arm was set to start and stop at angles 90° and 0° respectively at the knee joint. Each participant performed 3x3 sets/reps of the MVC protocol.

Soccer Skill Test

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

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

Anaerobic Endurance

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

Blood Analysis

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

Data Presentation and Statistical Analysis

Data were analysed using Statistical Package for Social Sciences 21 (IBM SPSS, Chicago, IL). Differences across time for muscle soreness, muscle function, performance and blood markers of muscle damage and inflammation were analysed by a mixed-design, two way ANOVA with three between-subject (FO, PRO, and PLA) and 3/4 within-subject (0,24,48 and 72 hour time-points) variables. Where a significant main effect was detected, Bonferroni post-hoc tests were performed to distinguish differences between supplement groups. Muscle soreness, CK and CRP data were expressed as raw values and as area under the curve (AUC) to determine the cumulative response over 72 hours. AUC data were analysed using 1 way ANOVA. Statistical difference was assumed at the level of ≤ 0.05 . Cohen's effect size (d) were calculated to compare differences between conditions. Effect sizes of 0.2 were

considered small, 0.5 considered medium and >0.8 were considered large (Cohen 1988). All data are expressed as means \pm SEM, with the exception of participant characteristics and the nutritional composition of supplements (mean \pm SD).

Results

Dietary intake

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

Blood n-3PUFA composition

Baseline (pre) % n-3PUFA/total PUFA composition in blood was similar between participants in all three groups ($p=0.246$). Whereas blood n-3PUFA composition increased by 58% following 6 weeks of supplementation in FO ($p<0.001$), no changes were observed in PRO ($p=0.611$) or CHO ($p=0.93$) (Figure 2A). The % n-3PUFA/total PUFA blood composition increased in all 10 participants after supplementation in FO group (Figure 2B).

Muscle soreness

Perceived ratings of general muscle soreness measured at baseline in both dominant and non-dominant legs was similar in all three conditions. Muscle soreness in both legs was elevated above baseline at 24, 48 and 72 hour time points. Dominant leg soreness was lower in FO compared with PRO ($p=0.016$) and CHO ($p=0.006$) after 24 hours (Figure 3A). Likewise, soreness was lower in FO compared with PRO ($p=0.003$) and CHO ($p=0.032$) after 48 hours and lower in FO than PRO after 72 hours ($p=0.008$). General soreness of the dominant leg, expressed as AUC over the entire 72 hour recovery period, was 58% lower in FO compared with CHO ($p=0.018$) ($d = 1.7$). Non-dominant leg soreness was lower in FO compared with CHO after 24 hours ($p=0.012$). Likewise, soreness was lower in FO compared with PRO ($p=0.005$) and CHO ($p=0.029$) after 48 hours and lower than PRO ($p=0.02$) after 72 hours (Figure 3B). General soreness of the non-dominant leg, expressed as AUC over the entire 72

hour recovery period, was lower in FO compared with PRO (58%, $d = 1.3$, $p=0.007$) and CHO (57%, $d = 1.6$, $p=0.011$). The pattern of muscle soreness scores with the knee joint in a flexed and extended position mimicked that of general soreness.

Muscle function

MVC of both dominant and non-dominant legs was reduced below baseline for the entire 72 hour recovery period in all groups ($p<0.05$) (Figure 4). However, no statistically significant differences in MVC of either leg were observed between groups in either leg ($p>0.05$).

Soccer-specific performance tasks

Performance on the LSPT and Nike spark Yoyo intermittent recovery test level 2 did not change statistically from baseline during the 72 hour recovery period in any condition (Figure 5).

Blood creatine kinase concentrations

Serum CK concentrations increased from baseline after 24 hours of exercise recovery and remained elevated at 48 and 72 hour time points ($p>0.05$) (Figure 6). CK concentrations, expressed as AUC over the entire 72 hour recovery period, were lower in FO compared with CHO ($d = 1.1$, $p=0.02$) and there was a tendency ($d = 0.9$, $p=0.07$) for CK concentrations were lower in FO compared to PRO.

Blood c-reactive protein concentrations

Serum CRP concentrations increased from baseline after 24 hour of exercise recovery, but returned to baseline at 48 hours and remained at baseline for the remainder of the recovery period (Figure 7). No statistical differences in CRP concentrations were detected between groups when expressed as time or as AUC over the entire 72 hour recovery period ($p>0.05$).

Discussion

This study demonstrated a decrease in perceived feelings of muscle soreness and serum CK concentrations under fasting conditions in response to eccentric exercise when n3PUFA were added to whey protein, leucine and carbohydrate over a 6 week supplementation period. However, the addition of n-3 PUFA to the multi-ingredient beverage did not appear to modulate the systemic inflammatory response or attenuate the decline in muscle function and soccer-specific performance during exercise recovery in competitive soccer players. Taken together, these data suggest that adding n-3PUFA to a whey protein, leucine and carbohydrate containing supplement over a 6 week period may elicit a protective role in maintaining the structural integrity of the muscle cell membrane, thereby reducing the severity of muscle soreness experienced following eccentric-based muscle damaging exercise.

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

test drinks. Carbohydrate intake has previously been shown to suppress the production of glucocorticoids, such as cortisol, which is known to reduce inflammation (Yeager et al., 2011). Since all test drinks contained a similar dose of carbohydrate, it is possible that attenuating the cortisol response blunted the systemic inflammatory response in all conditions. Therefore, refuting our original hypothesis, the present data suggest that the reduced muscle soreness with the addition of n-3PUFA to a mixed ingredient supplement was not mediated by an attenuated systemic inflammatory response.

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

A more likely mechanism for the reduced muscle soreness with the addition of n3PUFA to a multi-ingredient supplement may relate to a protective effect of n-3PUFA in maintaining the structural integrity of the muscle cell membrane. Previous work demonstrates that fish oil derived n-3PUFA are incorporated into phospholipid membrane of muscle cells (Smith et al., 2011). The presence of n-3PUFA within the muscle membrane also is thought to improve membrane integrity and thus reduce the leakage of intramyocellular proteins, such as CK (Clarkson & Hubal, 2002). We speculate that the incorporation of n-3PUFA into the muscle phospholipid membrane over 6 weeks of supplementation enhanced the structural integrity of the muscle cell membrane prior to the eccentric exercise protocol and, as such, protected the

muscle fibres of the active muscles from the mechanical stress induced by eccentric exercise. Consistent with this theory, in the present study we report a greater percentage contribution of n-3PUFA in whole blood (Figure 2) and an attenuated increase in serum CK concentrations (Figure 6) during eccentric exercise recovery when fish oil was added to the multi-nutrient supplement. Given that the incorporation of n-3PUFA into red bloods cells with fish oil supplementation follows a similar, albeit earlier, time course as profiled in skeletal muscle (McGlory et al., 2014), the attenuated CK response reported in the present study implies an enhanced maintenance of cell membrane integrity when n-3PUFA was added to a multi-ingredient beverage.

Muscle soreness following eccentric-based exercise previously has been shown to impair both muscle function (Legault et al., 2015) and sport-specific performance (Eston et al., 1996). In the present study, although eccentric exercise failed to initiate any change in performance of soccer-specific tests, hamstring MVC was impaired over the entire recovery period, indicating that the protocol successfully elicited symptoms of muscle damage. Interestingly, the reduced perceived feeling of muscle soreness reported when adding n3PUFA to a multi-ingredient beverage did not translate into the better maintenance of muscle function or soccer-specific performance during exercise recovery. This observation, combined with the attenuated increase in serum CK concentrations following eccentric exercise in FO vs. PRO and CHO suggests that ultrastructural damage occurred in FO, but without disruption to the phospholipid membrane of the muscle cell. Whilst there is no definitive reason for this apparent disconnect between measurements of muscle soreness and muscle function/soccer performance obtained in the present study, the most likely explanation may relate to methodological considerations. For logistical reasons, we did not measure soccer-specific performance 48 h following eccentric exercise when soccer players reported peak muscle soreness scores and when soccer-specific performance scores likely reached their nadir. Thus, we potentially missed any benefit of FO on soccer-specific performance at the peak of muscle soreness. Moreover, the practical application of our study findings are limited to a

single muscle damage stimulus over an acute recovery period. In a real-world setting, during periods of fixture congestion, soccer players typically experience several muscle damage stimuli. Rollo et al. (2014) demonstrated that 2 games vs. 1 game per week impaired soccer specific performance in soccer players. Therefore, future studies should examine the impact of adding n-3PUFA to a multi-ingredient supplement on performance indices during recovery from multiple muscle damage stimuli.

To conclude, our results suggest that adding fish oil to a multi-ingredient supplement effectively protects the muscle from damaging eccentric-based exercise, resulting in reduced muscle soreness during exercise recovery in soccer players.

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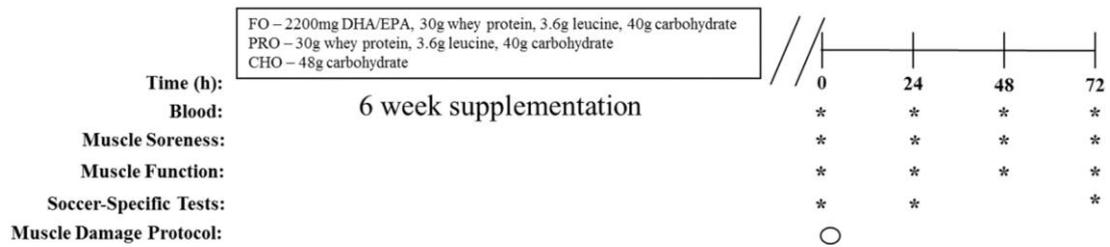
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Figure 1 – Schematic overview of study design



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

Figure 2 – Percentage of n-3PUFA/totalPUFA composition in blood before (Pre) and after (Post) 6 week of supplementation. A, group data expressed as means ± SEM. B, individual data values. * significant difference versus Pre in corresponding supplement condition. Abbreviations as of Figure 1.

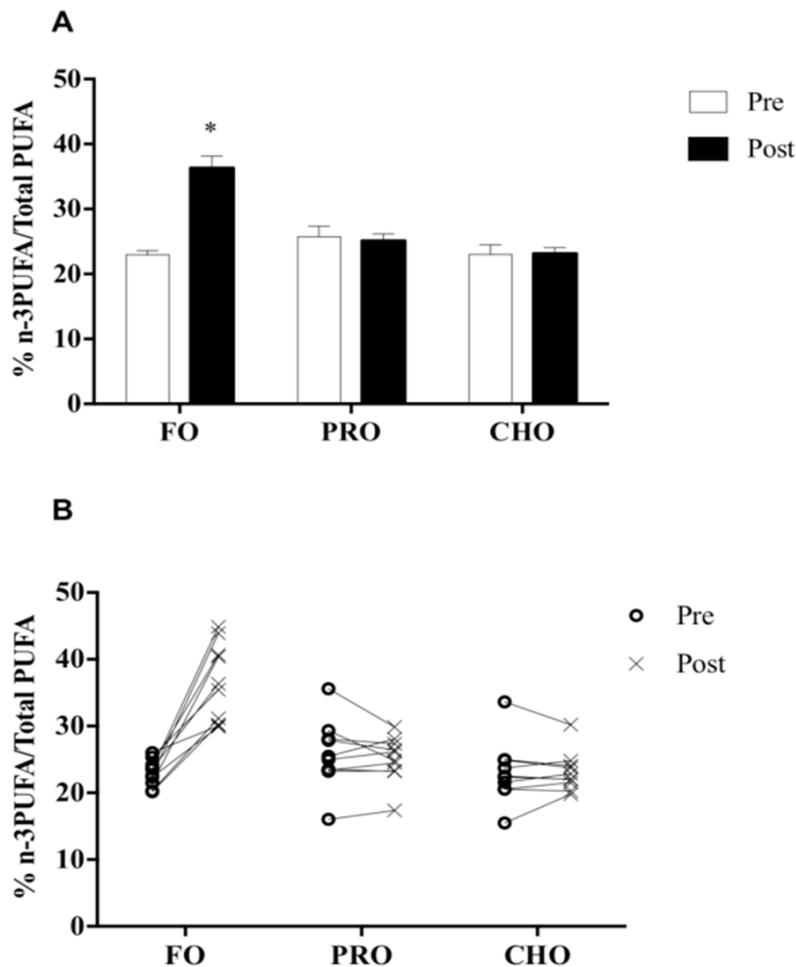
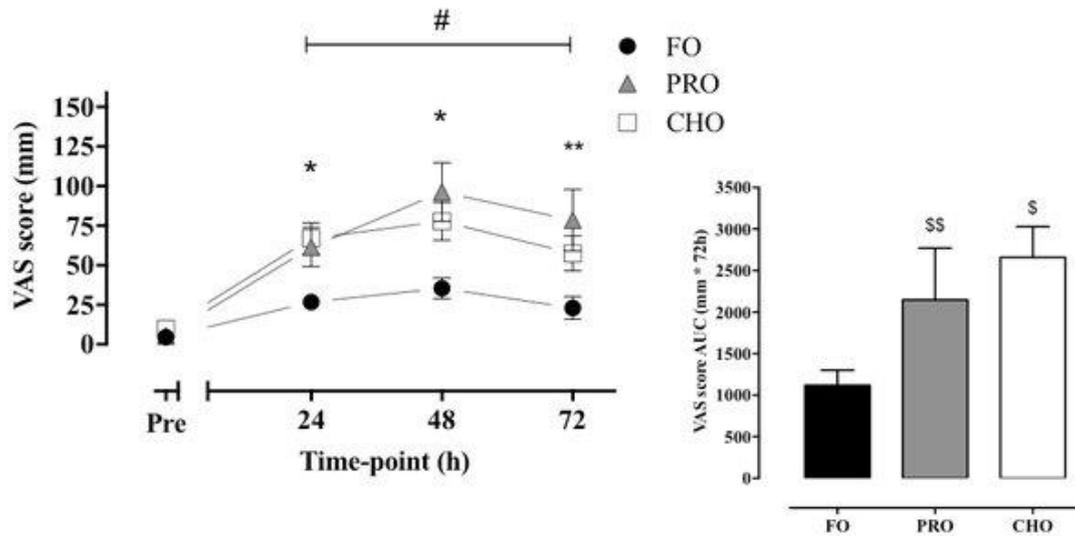


Figure 3 – Muscle soreness, expressed as raw values over time and area under the curve during the overall 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means \pm SEM. Insert shows area under the curve (AUC). A and B, general soreness of the dominant and non-dominant leg respectively. * FO significantly different versus PRO and CHO. ** FO significantly different versus PRO. *** FO significantly different versus CHO. #Significantly different versus PRE in corresponding supplement condition. \$Significantly different versus FO. \$\$Tendency to be different versus FO. Abbreviations as of Figure 1.

A



B

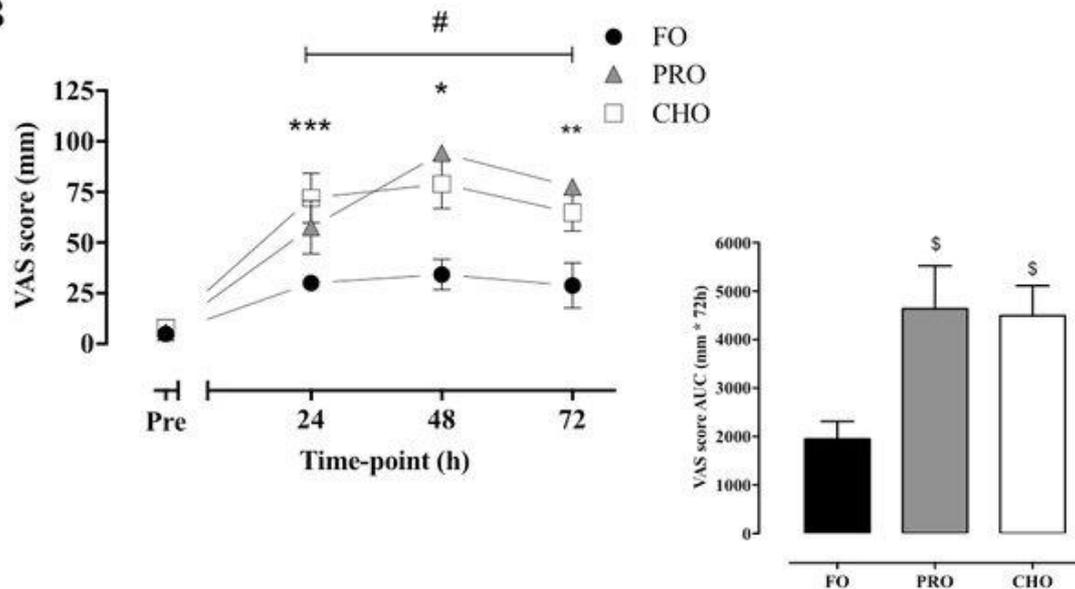
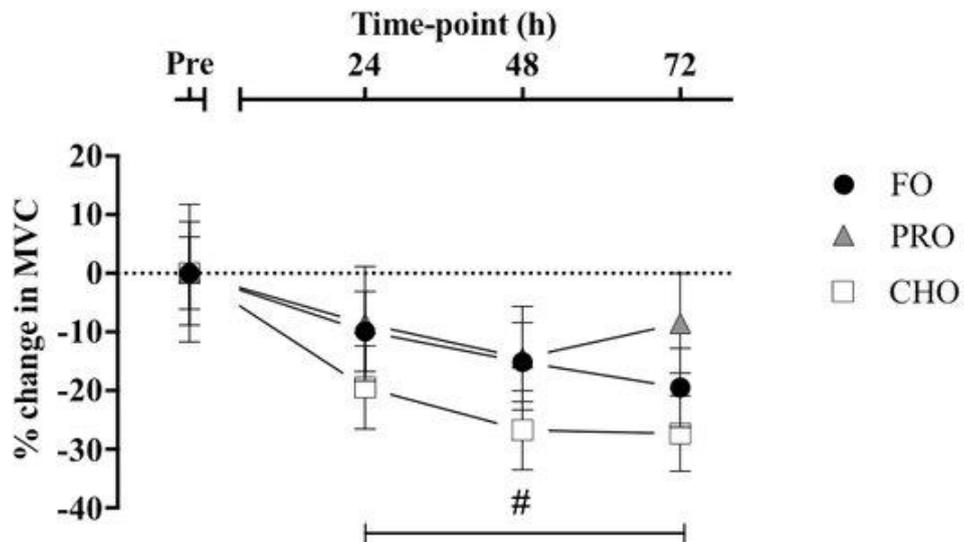


Figure 4 - Percentage change in muscle maximum voluntary contraction over the entire 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means \pm SEM. A, MVC changes in the dominant leg. B, MVC changes in the non-dominant leg. #Significantly different versus Pre. Abbreviations as of Figure 1.

A



B

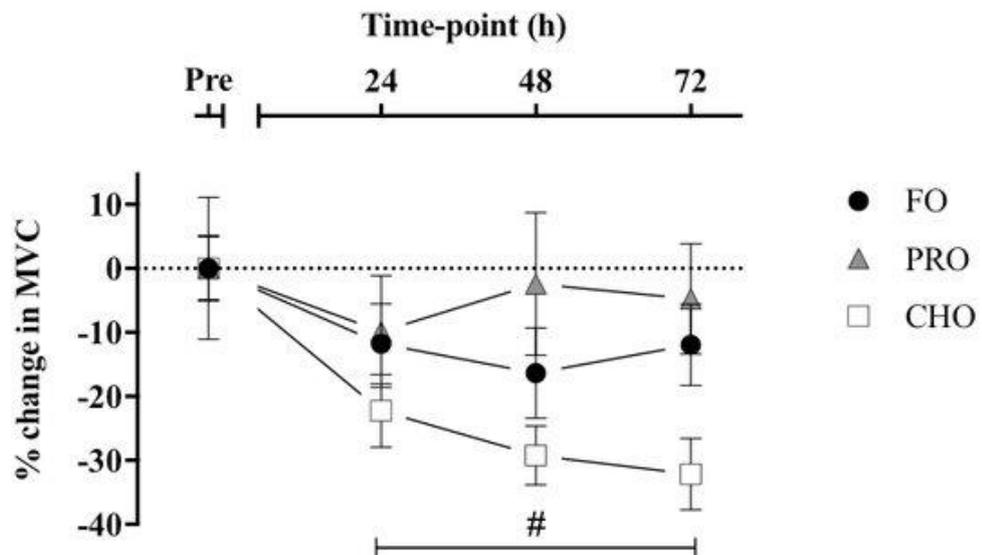
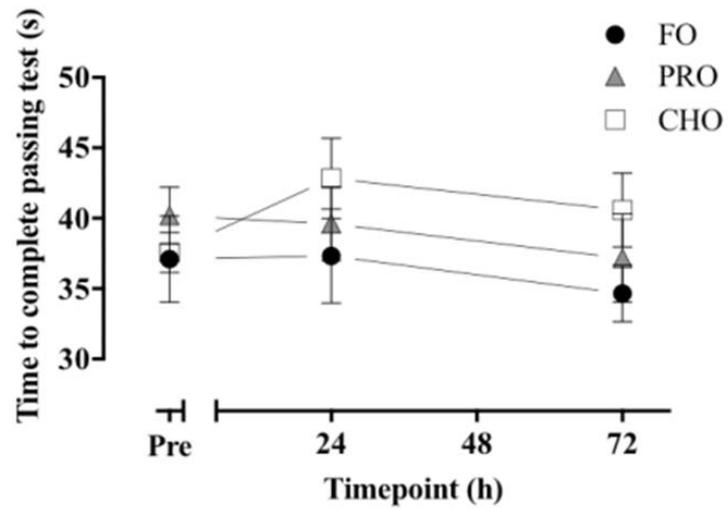


Figure 5 – Performance on soccer-specific tasks measured over the entire 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means \pm SEM. A, Counter movement jump test; B, Loughborough Soccer Passing Test; C, Yoyo intermittent recovery Test level 2; Abbreviations as of Figure 1.

A



B

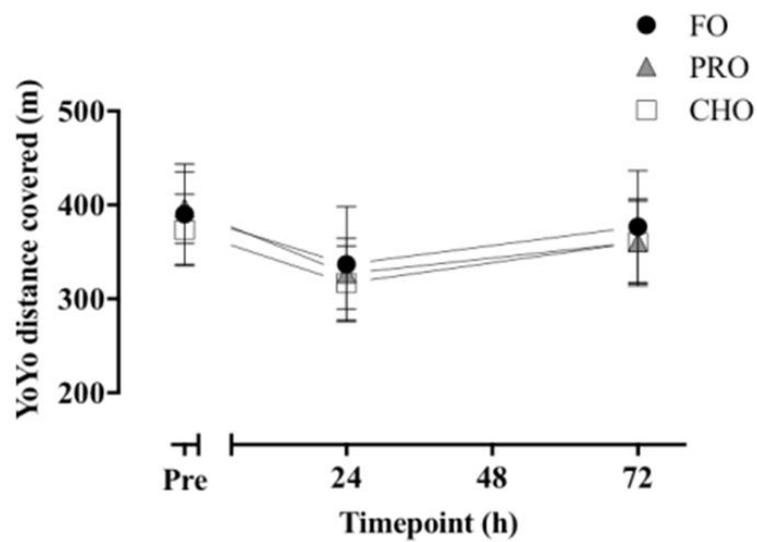


Figure 6 – Serum creatine kinase concentrations over the entire 72h recovery period following an intense bout of eccentric exercise. Data are expressed as means \pm SEM. Insert shows area under the curve (AUC). *Significantly different versus FO. **Tendency to be different versus FO. #Significantly different versus Pre. Abbreviations as of Figure 1.

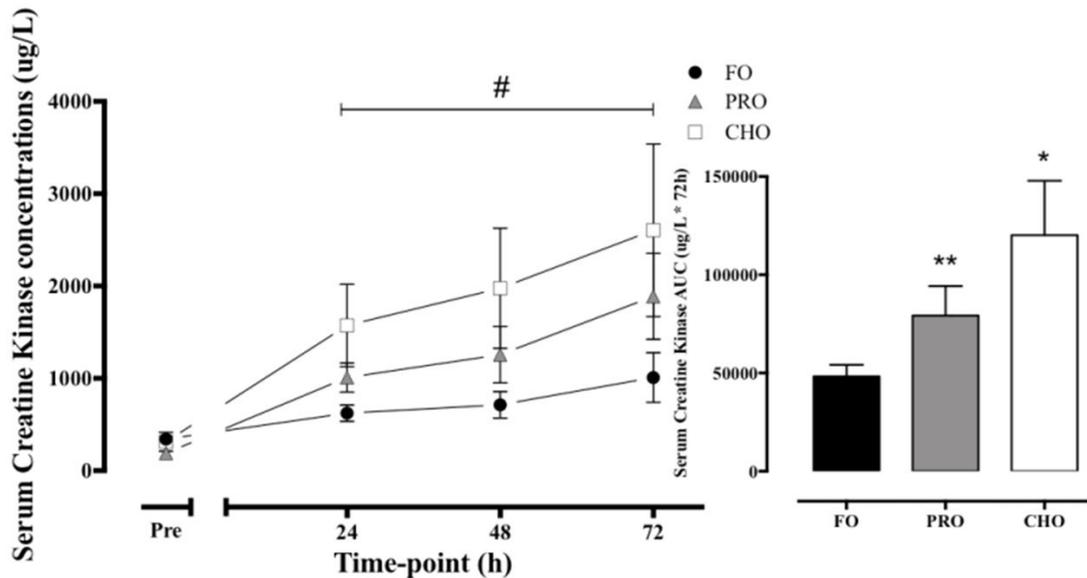


Figure 7 – Serum C-reactive protein concentrations over the entire 72 h recovery period following an intense bout of eccentric exercise. Data are expressed as means \pm SEM. Insert shows area under the curve (AUC). # Significantly different versus Pre. Abbreviations as of Figure 1.

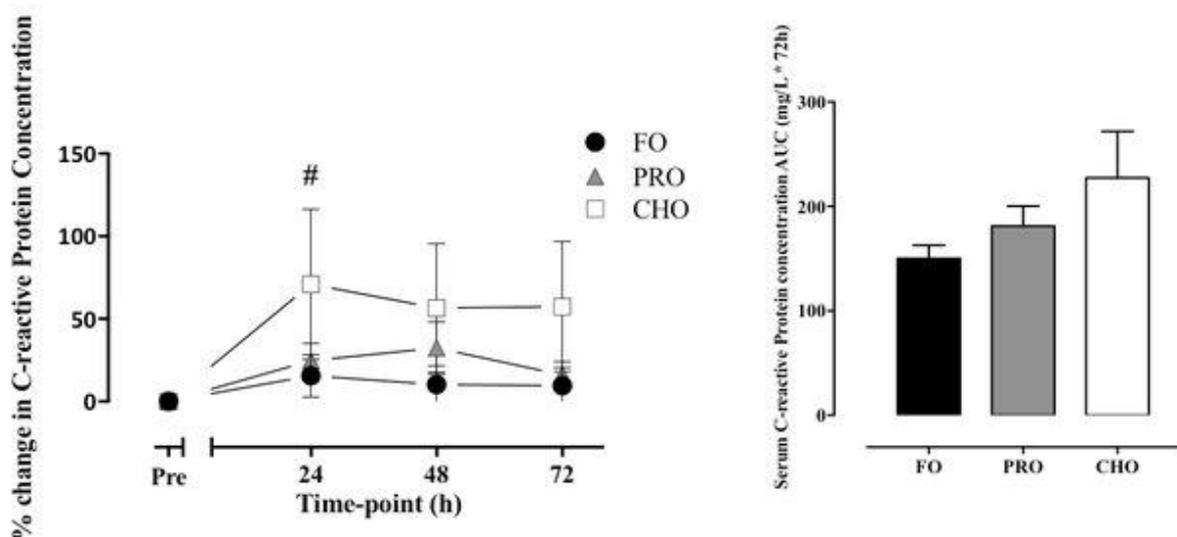


Table 1 – Nutrition composition of supplements

	FO	PRO	CHO
Volume (mL)	200	200	200
Energy Value (kcal)	200	150	200
EPA (mg)	550		
DHA(mg)	550		
Whey Protein (g)	15	15	
Leucine (g)	1.8	1.8	
Carbohydrate (g)	20	20	24
Vitamin D (μ g)	3	4	

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