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ARTICLE

Effectiveness of a combined New Zealand green-lipped mussel and Antarctic krill oil supplement on markers of exercise-induced muscle damage and inflammation in untrained men

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ABSTRACT

Green-lipped mussel oil (PCSO-524^{VR}) has been shown to attenuate signs and symptoms of exercise-induced muscle damage (EIMD), and krill oil has been shown to have a protective effect against cytokine-induced tissue degradation. The purpose of this study was to com-pare the effects of PCSO-524^{VR} and ESPO-572^{VR} (75% PCSO-524^{VR} and 25% krill oil) on signs and symptoms of EIMD. Fifty-one untrained men consumed 600 mg/d of PCSO-524^{VR} ($n = 24$) or ESPO-572^{VR}($n=27$) for 26 d prior to and 72h following a downhill running bout. Delayed onset muscle soreness (DOMS), pressure pain thresh-old, limb swelling, range of motion (ROM), isometric torque, and blood markers of inflammation and muscle damage were assessed at baseline, 24, 48 and 72 h post-eccentric exercise. ESPO-572^{VR} was 'at least as good as' PCSO-524^{VR} and both blends were superior ($p < 0.05$) to placebo in lessening the increase in DOMS at 24, 48, 72 h. ESPO-572^{VR} and PCSO-524^{VR} were protective against joint ROM loss compared to placebo ($p < 0.05$) at 48 and 72 h. Notably, at 24 and 48 h, joint ROM was higher in the ESPO-572^{VR} compared to the PCSO-524^{VR} group ($p <0.05$). No differences between the two blends for the other markers were found. ESPO-572^{VR} is 'at least as good' as PCSO-524^{VR} in reducing markers of muscle damage and soreness fol-lowing eccentric exercise and was superior to PCSO-524^{VR} in protect-ing against the loss in joint ROM during recovery. Our data support the use of ESPO-572^{VR}, a combination of green-lipped mussel and krill oil, in mitigating the deleterious effects of EIMD.

KEYWORDS

Omega-3 fatty acids; delayed onset muscle soreness; eccentric; green-lipped mussel oil; krill oil

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Introduction

Exercise-induced muscle damage (EIMD) is generally a transient event that can be the consequence of an individual engaging in novel (unaccustomed), eccentric type, long-duration or vigorous high-intensity exercise (Jang et al. 2018; Harty et al. 2019). This type of exercise results in a primary response of myofibrillar disruption (Yu et al. 2013), and a secondary pro-inflammatory response that can result in tissue damage and low-grade systemic inflammation and oxidative stress (Clarkson and Hubal 2002; Hirose et al. 2004). The signs and symptoms of EIMD, such as delayed onset muscle soreness (DOMS), increased pain and tenderness, and reduced range of motion (ROM) are evident a few hours following exercise, and can continue for up to 96 h during the recovery period (Bongiovanni et al. 2020). Optimal recovery from strenuous exercise is crucial for both professional and recreational athletes, as EIMD has been shown to hinder performance in activities ranging from basic physical activity to athletic training and competition (Trost et al. 2011; Doma et al. 2018).

Since there appears to be no effective treatment available to ameliorate the signs and symptoms related to EIMD, nutritional supplementation strategies have become of interest (Bongiovanni et al. 2020). Since inflammation and oxidative stress are evident following EIMD, supplementing the diet with long-chain omega (n)-3 polyunsaturated fatty acids (PUFAs) may prove advantageous in accelerating the recovery process fol-lowing muscle damaging exercise (Bongiovanni et al. 2020). PCSO-524^{VR} (Lyprinol^{VR}/ Omega-XL^{VR}), is a patented marine oil extract of stabilized lipids from the New Zealand green-lipped mussel, *Perna canaliculus*, and is a mixture of the five main lipid classes including sterol esters, triglycerides, free fatty acids, sterols, and polar lipids, and has been shown to contain up to 91 fatty acids, with docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) accounting for 84% of the n-3 PUFA content (Wolyniak et al. 2005). PCSO-524^{VR} has been shown to exert potent anti-inflammatory effects through the action of furan fatty acids (Wakimoto et al. 2011) and inhibition of the cyclooxygenase-2 and 5-lipoxygenase pathways, which normally metabolize arachidonic acid into pro-inflammatory prostanoids and leukotrienes (Whitehouse et al. 1997). The inhibitory effects of PCSO-524^{VR} on these pathways therefore produce a significant reduction in pro-inflammatory prostanoids and leukotrienes, and a subsequent reduc-tion in cytokine production from inflammatory cells.

Our group (Mickleborough et al. 2015) has previously shown that supplementing the diet of untrained males with 1,200 mg/d of a green-lipped mussel (PCSO-524^{VR}) oil blend for 26 days significantly reduces functional and blood markers of EIMD following muscle damaging exercise. In support of our findings, Baum et al. (2013) found 11 weeks of PCSO-524^{VR} supplementation to reduce DOMS induced by a 30 km run in men and women distance runners. Thus, the data on PCSO-524^{VR} seem to support its use as a therapeutic nutraceutical for mitigating muscle damage and inflammation fol-lowing strenuous exercise in untrained as well as in trained individuals.

Krill oil is an increasingly popular source of marine n-3 PUFAs. Krill oil (*Euphausia superba*) is rich in long-chain n-3 PUFAs, EPA, and DHA, which have been found to have positive effects on inflammation (Xie et al. 2019). In krill oil, n-3 PUFAs are bound to phospholipids, whereas in other marine oils (e.g. fish oil) the majority of n-3 PUFAs are bound to triacylglycerol. Greater bioavailability of n-3 PUFAs from krill oil

in comparison to fish oil has been suggested based on lower doses of krill oil needed to result in a similar bloodstream level of EPA and/or DHA (Ramprasath et al. 2013; Sung et al. 2018). Krill oil contains astaxanthin, a red carotenoid pigment and strong antioxi-dant that naturally occurs in salmon, shrimp, krill, crustaceans, or certain types of algae, giving krill its reddish color. The antioxidant activity of astaxanthin is superior to other antioxidants, including A-tocopherol (Miki 1991), and has been reported to improve functional capacity and athletic performance, prevent against exercise induced free-rad-ical production, and facilitate recovery from maximal aerobic exercise (Earnest et al. 2011; Djordjevic et al. 2012; Fleischmann et al. 2019). In addition, the ample amounts of phospholipids found in krill oil (phosphatidylcholine, phosphatidylserine, and phosphatidic acid) have anabolic properties, which may be conducive to enhanced recovery following muscle damage inducing exercise (Georges et al. 2018).

Therefore, a potent nutritional strategy to enhance recovery from muscle damaging exercise would be to combine krill oil with PCSO-524^{VR}. The rationale for combining krill oil with PCSO-524^{VR} is supported by data that shows krill oil to be superior to green-lipped mussel oil in its ability to upregulate anabolic genes and protect against cytokine-induced tissue degradation (Buddhachat et al. 2017). In light of the evidence supporting the use of krill oil (Skarpa~nska-Stejnborn et al. 2015; Georges et al. 2018), and our previous work (Mickleborough et al. 2015) demonstrating the efficacy of PCSO-524^{VR} in mitigating the muscle damaging effects of eccentric exercise, the primary aim of this study was to evaluate the potential of a novel marine oil lipid blend (ESPO-572^{VR}) that contains a PCSO-524^{VR}/krill oil blend (75%/25% respectively) to enhance recovery from muscle damage inducing exercise. We hypothesized that ESPO-572^{VR} will infer greater protection than PCSO-524^{VR} alone against signs and symptoms of EIMD that develop following eccentric exercise.

Methods

Subjects

From December 2018 through May 2019, a total of 59 untrained men were recruited to participate in the present study (age, 21.7 ± 2.4 years; height, 183.4 ± 5.7 cm; weight, 80.7 ± 11.6 kg; body mass index, 24.0 ± 3.6 kg/m^2) (Figure 1. CONSORT dia-gram). Subjects were included if they regularly exercised or had a history of exercising for <30 min for <3 times per week and were excluded if they 1) had a history of sig-nificant pain in the lower extremities, 2) engaged in a strength training program within 60 days prior to enrollment (Ochi et al. 2018), 3) regularly used nutritional supplements (i.e. multivitamins, creatine, whey protein, beta-alanine, turmeric, fish oil) or over-the-counter and/or prescription anti-inflammatory medications, or 4) were determined to be at greater than low level of risk for experiencing medical complications while participating in maximal exercise testing (ACSM 2013). Eligible subjects were instructed to maintain their usual exercise habits and to refrain from anti-inflammatory nutraceut-icals and medications as well as avoid downhill running, trail running, plyometric or other modes of exercise that could potentially induce eccentric muscle damage for the duration of their participation in the study. Procedures were approved by the Indiana University Institutional Review Board and the study protocol was registered under

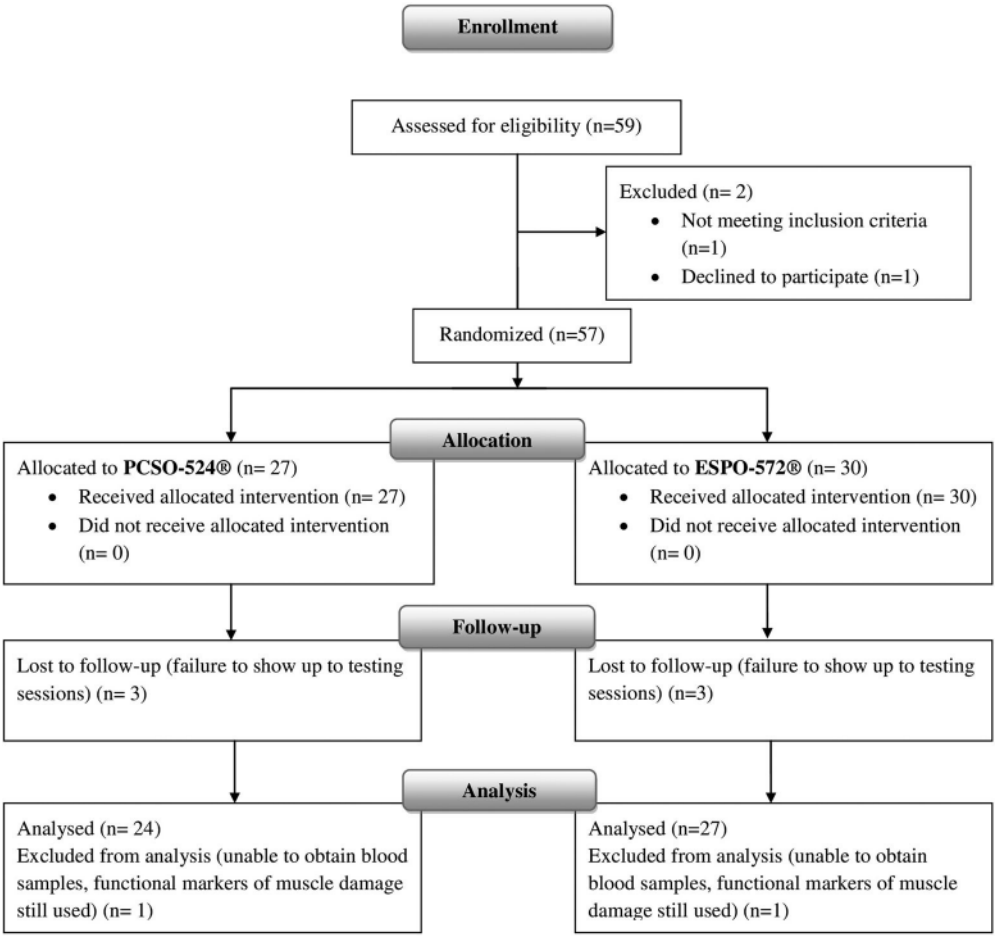

Figure 1. CONSORT diagram.

ClinicalTrials.gov identifier: NCT03760757 and written informed consent was obtained from all subjects prior to enrollment.

Study design

A randomized, double-blind, and parallel group study design at a single university setting was employed in which subjects were randomly assigned *via* coin flip to a green-lipped mussel oil (PCSO-524^{VR}, *n* 1/4 24) or a 75/25% PCSO-524^{VR}/krill oil blend (ESPO-572^{VR}; *n* 1/4 27) supplementation group. A parallel design, rather than a cross-over design, was chosen to avoid the repeated-bout effect which can occur as a consequence of muscle damaging exercise (Nosaka et al. 2005; Hyldahl et al. 2017). Familiarization of functional measures of muscle damage occurred during visit 1. Subjects began the supplementation protocol 26 d prior to an eccentric exercise bout (downhill treadmill running) and continued taking the supplement for 72 h following the eccentric exercise bout. The justification for using the 26-day dietary intake protocol and dosage was based on our previous study which showed that a supplementation period of 26 day

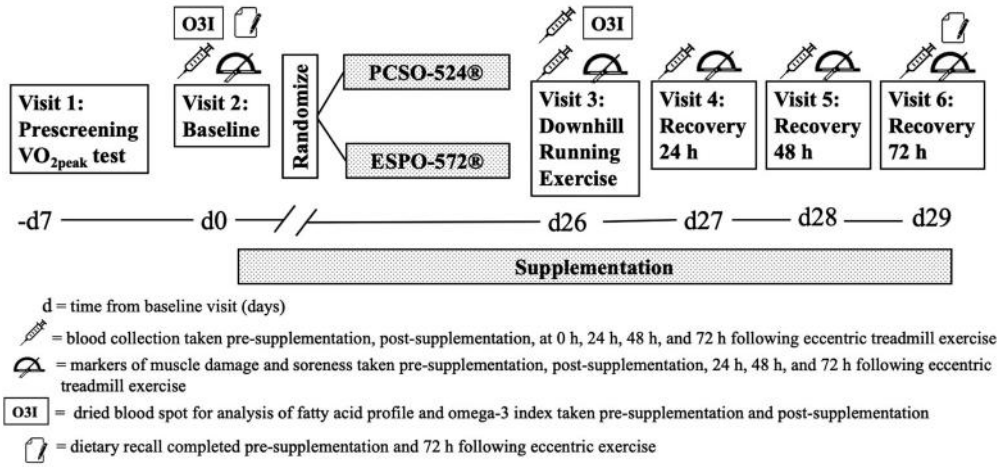

Figure 2. Schematic of the randomized, double-blind, and parallel group study design.

with eight capsules per day of PCSO-524^{VR} was effective in attenuating EIMD and inflammation in untrained men (Mickleborough et al. 2015). Blood was drawn (for the assessment of muscle damage and inflammation), and functional measures of muscle damage [i.e. isometric torque, knee flexion (joint ROM), thigh circumference (swelling), muscle soreness, and muscle pain] were taken prior to supplementation, as well as immediately before, 24 h, 48 h, and 72 h after muscle-damaging exercise. Figure 2 provides a schematic of the study design.

Compliance to supplementation was assessed *via* a dried blood spot sample at the beginning of visit 2 and 3 (Omega Quant, Sioux Falls, SD) to quantify the Omega-3 index (O3I) (Harris and Polreis 2016). All subjects were reminded *via* email, as well as in person at each study visit, when to consume the supplements. A third party who was unaffiliated with the study separated the two supplements into identical plastic prescription bottles that were then allocated to subjects in each of the appropriate treatment groups

Supplementation protocol

The PCSO-524^{VR} or ESPO-572^{VR} supplement were supplied in capsule form, with two capsules taken upon waking (with or without food) and the other two capsules taken at least 12 h later, which equated to a total of four capsules taken per day. The daily dose of PCSO-524^{VR}(PCSO-524^{VR}/Omega XL^{VR}; Pharmalink International Ltd, Hong Kong) contained 400mg olive oil, 200mg green-lipped mussel lipid extract (~18% EPA and 14% DHA), and ~0.9 mg vitamin E (d-alpha-tocopherol). The ESPO-572^{VR} (Lyprinol Advanced^{VR}, Pharmalink International Ltd, Hong Kong) contained 400mg olive oil, 200mg of New Zealand green-lipped mussel and Antarctic krill oil blend (~5.7% EPA and 3.7% DHA), and 0.34 mg vitamin E (d-alpha-tocopherol). The 'lipid extract' portion of the green-lipped mussel oil blend has been shown to contain up to 91 different fatty acids (including EPA and DHA) (Wolyniak et al. 2005). The PCSO-524^{VR} and ESPO-572^{VR} capsules were identical in size, color, texture, smell, and taste. Supplement nutrient analysis composition information was provided by Cawthron Analytical Science (Nelson 7010, New Zealand).

Peak oxygen uptake $_{(V\_O2peak)}$ treadmill test

Subjects performed an incremental exercise test, adapted from a previously published protocol from our laboratory (Duke et al. 2014) on a motorized treadmill (A.R. Young Company, Indianapolis, IN) for determination of peak oxygen uptake $_{(V\_O2peak).}$ A heart rate correspond-ing to 70% $_{V\_O2peak}$ for each subject was subsequently prescribed as the intensity for the down-hill running protocol. Subjects were outfitted with a Polar H10 heart rate monitor (Polar Electro Inc., Lake Success, NY) and oronasal face mask (7450 Series V2, Hans Rudolph, Shawnee, KS) for direct measurement of expired gases. Following a 5-minute warm-up at a self-selected running speed and 0% grade, the belt of the treadmill was adjusted to a speed of 1.0 mph less than the selected (warm-up) speed for one minute. After which, the speed was increased to the selected speed for two minutes at 0% grade. For the remainder of the exercise test, the speed remained constant while the grade was increased to 4% for the following two minutes, and then increased incrementally by 2% every two minutes until volitional exhaus-tion. Ventilatory and metabolic data were collected breath-by-breath using a metabolic cart (Vmax Encore System, CareFusion, Yorba Linda, CA). $_{V\_O2peak}$ was taken as the highest VO_2 value averaged over 30-seconds.

Eccentric muscle damaging treadmill exercise

To induce eccentric muscle damage, subjects ran downhill at a —16% grade on a motor-ized treadmill (A.R. Young Company, Indianapolis) at a speed eliciting 70% of heart rate at $_{V\_O2peak}$ for 20 min. This protocol has previously been shown to elicit changes in markers used to quantify muscle damage such as myoglobin, CK-MM, skeletal muscle slow troponin I, tumor necrosis factor-a, and maximum voluntary isometric torque at 24 h, 48 h, and 72 h following exercise (Sorichter et al. 1997; 1998; Mickleborough et al. 2015). Once the downhill running began, subjects were verbally encouraged to continue until the 20-minute downhill running protocol was complete. The treadmill speed was adjusted as needed throughout exercise so that the subjects maintained a heart rate that corresponded to 70% $_{V\_O2peak}$ for the entirety of the exercise bout.

Delayed onset muscle soreness and pain pressure threshold

Subjective measures of DOMS were determined using a numeric visual analog scale (0-10) to assess lower limb soreness following a squat motion performed at a knee angle of 90˚. Ratings of “0” corresponded with “no soreness,” while a “10” indicated “unbearably painful” (Mickleborough et al. 2015). DOMS were repeated three times and reported as an average of the three trials.

Pain pressure threshold (PPT), a marker of muscle tenderness, was quantified by the same investigator throughout the study using a digital algometer (Force One, Wagner Instruments, Greenwich, CT) at five specific sites on the quadriceps. Our previous work has demonstrated significant elevations in the ratings of PPT at these five specific sites following muscle damaging exercise (Mickleborough et al. 2015). Subjects laid supine on an athletic training table and were instructed to indicate the point at which sensations of pressure were felt as pain to the investigators. The amount of force applied by the algometer

was recorded in Newtons (N) at the threshold of pain. This procedure was repeated a total of three times, and an average force was recorded for each location as the PPT.

Range of motion, swelling and maximum voluntary contraction

A digital protractor goniometer (Medigauge, Columbia, MO) was used to measure the range of unassisted knee flexion in degrees. Subjects were instructed to maximally flex their left knee while lying in a prone position. The angle between the lateral malleolus and greater trochanter was measured three times and reported as an average for knee flexion ROM.

Measures of right thigh circumference (swelling) were taken using anthropometric measurement tape (Idass, Glastonbury, UK) with subjects standing in the anatomical position and placing all their weight on the left leg. The circumference of the thigh was assessed midway between the anterior superior iliac spine and the base of the patella three times. The site of measurement was marked with a semi-permanent marker to ensure consistent measurements and an average was recorded. Reliability data using this technique from our laboratory has demonstrated a technical error of measurement (TEM) of <0.2% (i.e. <0.1 cm) (Mickleborough et al. 2015).

A dynamometer (Cybex Isokinetic System, Medway, MA) was used to assess peak isometric torque of the right quadriceps muscle. Subjects were seated with their right knee at an angle of 80˚ and distal shin secured to the dynamometer arm using a Velcro strap. Subjects performed a series of three warm-up contractions (two sub-maximal, one maximal) separated by 10s of rest to allow familiarization with the equipment. The warm-up trials were followed by two minutes rest; after which they were instructed and verbally encouraged by the researchers to give full effort to perform three maximum voluntary contractions (MVCs) of the quadriceps separated by 10s of recovery (Nunan et al. 2010). The highest peak torque (Newton-meter) measure from the three trials was recorded as peak isometric torque. Maximal voluntary contraction provides a reliable measure of muscle injury resulting from eccentric contractions, and is considered to be one of the best indirect markers of EIMD (Damas et al. 2016).

Blood sampling and analysis

The 6.0 mL samples of blood were obtained from the antecubital vein after subjects rested comfortably in the supine position for 10 min. Samples of blood were taken prior to supplementation, as well as immediately before and after, 24 h, 48 h, and 72 h after muscle-damaging exercise and collected into EDTA-coated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). The samples were immediately centrifuged at 4˚C at 1,300 RCF for 15 min (model X-22R; Beckman, St. Louis, MO). Plasma was aliquoted into 0.5 mL cryogenic storage vials (CryoKING^{VR}, Biologix, Jinan, Shandong, China) and stored immediately at —80 ˚C until later analysis of muscle damage and inflammatory markers using enzyme-linked immunoassay techniques with detection by spectropho-tometry (Powerwave XS^{TM} Spectrophotometer, Bio-Tek Instruments, Winooski, VT).

Skeletal muscle damage and inflammatory stress blood markers

Plasma creatine kinase (CK-MM), tumor necrosis factor-o (TNF- o), and interleukin 1-*fl* (IL-*fl*) concentrations were assessed according to manufacturer instructions. The kit specifications were as follows: CK-MM; sensitivity: 0.014 U/L; detection range: 0.03–2 U/L (Ab185988, Abcam Inc., Cambridge, MA), TNF- o; sensitivity: 6.23 pg/ml; detection range: 15.6–1,000 pg/ml, (DTA00D, R&D Systems, Inc., Minneapolis, MN), and IL-*fl*; sensitivity: 1.0 pg/ml; detection range: 3.9–250 pg/ml (DLB50, R&D Systems, Inc., Minneapolis, MN). Samples were run in duplicate. Intra-assay coefficients of variation were 6.4% for CK-MM, 9.9% for TNF-o, and 12.9% for IL-*fl*.

Fatty acid profile analysis and the omega-3 index

Samples were obtained by the investigators at two time points during the study (pre- and post-supplementation), by using a lancet on the finger of choice of each subject in order to acquire 50uL of blood on pretreated antioxidant cocktail filter paper provided by Omega Quant (Omega Quant, Sioux Falls, SD), and who performed the fatty acid profile analyses. Samples were then stored at —80˚C and sent off for analysis at a later time. Utilizing capillary gas chromatography as previously described (Harris and Polreis 2016), levels of EPA þ DHA (omega-3 index) were measured along with 22 other fatty acids using whole blood. Data are expressed as a percent of total blood fatty acids, and the O3I, the sum of EPA and DHA levels in erythrocyte membranes, is expressed as a percentage of total erythrocyte fatty acids (Harris and Polreis 2016).

Nutrient intake

Subjects were asked to record all food, beverages, and supplements consumed during a 24 h period twice (one weekday and one weekend) before supplementation, and twice during the supplementation period (one weekday and one weekend) for a total of four dietary recalls to ensure that the dietary habits of subjects did not change throughout the duration of the supplementation intervention. Dietary intake recall data was col-lected and analyzed using the Web-based Automated Self-Administered 24-Hour (ASA24^{VR}), Dietary Assessment Tool, version 2019, developed by the National Cancer Institute, Bethesda, USA (Subar et al. 2012). The ASA24^{VR} tool has been shown to be more accurate at assessing dietary intake compared to other commonly used methods (i.e. food frequency questionnaires) as well as provide consistent data in regards to the National Health and Nutrition Examination Survey (Freedman et al. 2014; Thompson et al. 2015). Subjects completed the ASA24^{VR} themselves but had the option to meet with a registered dietitian to receive training on the ASA24^{VR} tool; however, no dietary counseling was provided. The ASA24^{VR} provides an automatic analysis of nutrient com-position. Due to their ability to alter EIMD (Taghiyar et al. 2013) and the O3I (Jackson et al. 2019), nutrients of interest obtained from the ASA24^{VR} analysis included macronu-trient composition, antioxidants (o-tocopherol, fl-carotene, lycopene, vitamin C), certain minerals (magnesium, sodium, zinc), and types of dietary fatty acids (omega-3, total polyunsaturated fatty acids, saturated fatty acids).

Primary outcome measures

The primary outcome measures of the study were tests of non-inferiority between PCSO-524^{VR} and ESPO-572^{VR} on various markers of muscle damage (CK-MM, DOMS, PPT, ROM, MVC, and muscle swelling) and inflammation (IL-b, TNF-a).

Statistical analyses

For data analysis purposes, we included placebo group (n 1/4 16) data generated from our previously published work that followed an identical study design (Mickleborough et al. 2015). To analyze the data, linear mixed models were performed using the SAS system for Windows 9.4 statistical software (SAS Institute Inc., Cary, NC, USA) on each measure to compare the outcomes across five time points [(pre-supplementation, post-supplementation, and 24 h, 48 h and 72 h following downhill treadmill running)] for three groups (ESPO-572^{VR}, PCSO-524^{VR}, and placebo), including an interaction of time x group, with repeated measures for subjects across time. Mixed models allowed for the analysis of unbalanced data with different numbers of subjects at each time point (i.e. for missing data), while accounting for the correlation of data within subject across time. Within the mixed models, pairwise comparisons were per-formed between groups at each time point with estimated difference, confidence interval, and p-value, as well as pairwise comparisons between time points within groups.

To evaluate whether the new treatment (ESPO-572^{VR}) is at least as beneficial as the reference treatment (PCSO-524^{VR}), non-inferiority tests were applied to ensure that the new treatment is significantly better than the placebo by the equivalence margin. The margin was selected as the lower bound of the 95% confidence interval (Schumi and Wittes 2011) of PCSO-524^{VR} compared to placebo (Mickleborough et al. 2015). When the reference treatment (PCSO-524^{VR}) was not significantly better than the placebo data (Mickleborough et al. 2015), non-inferiority tests for the new treatment (ESPO-572^{VR}) could not be performed. Statistical significance was set at $p < 0.05$. Calculation of sample size *via* G*Power (Faul et al. 2007) was computed using serum skeletal muscle slow troponin I (sTnI) as the variable stated in our previous study (Mickleborough et al. 2015). Fifty-one total subjects ((27 ESPO-572^{VR} and 24 PCSO-524^{VR}), provided 80% power for tests of superiority between the two treatments (alpha 1/4 0.05) where differences are large (Cohen's d 1/4 0.80). For tests of non-inferiority, a sample size of 50 (25 per group) was needed to show that the 95% CI for the difference between the new treatment (ESPO-572^{VR}) vs. the reference treatment (PCSO-524^{VR}) does not reach the selected margin of –4.36, as based on the upper limit of the 95% CI for PCSO-524^{VR} vs placebo for sTnI concentration reported in our previous work (Mickleborough et al. 2015). While sTnI is not presented in this study, a sample size of 25 per group is sufficient for the same level of precision of confidence intervals for the non-inferiority tests for each dependent variable.

Results

Subject characteristics

Table 1 provides details of subject characteristics taken at baseline. A total of 59 untrained men were assessed for eligibility (Figure 1. CONSORT diagram), and 57 met

Table 1. Subject characteristics at baseline.

	ESPO-572® (*n* = 27)	PCSO-524® (*n* = 24)	Placebo (*n* =16)	p-value
Age (years)	22.2±2.2	21.4 ±2.6	21.5±2.4	0.446
Height (cm)	182.7± 6.1	184.3 ± 5.2	174.2±6.7	<0.001*
Body mass (kg)	81.4 ± 11	80±12.5	66.6±9.7	<0.001*
BMI (kg/m^2)	24.5±3.6	23.5 ± 3.5	21.9±2.8	0.059
V_O$_{2peak}$ (L)	3.9±0.5	3.8 ±0.7	3.0±0.6	<0.001*
V_O$_{2peak}$ (ml/kg/min)	47.7± 6.5	47.6 ±6.5	45.6±6.1	0.538

*Significantly different ($p < 0.05$) between groups.
Values are expressed as mean ±SD.
BMI= body mass index, V_O$_{2peak}$ = peak aerobic exercise capacity.
Placebo subjects from our previous work (12).

inclusion criteria to participate. Data from six subjects were excluded from the final analysis due to missed testing sessions (incomplete data). Of the remaining 51 subjects included in the final analysis, investigators were unable to obtain adequate blood sam-ples from two, resulting in 49 subjects with analyzable blood marker data. There were no significant differences ($p > 0.05$) for age, BMI, and $_{V\_O2peak}$ (mL/kg/min) between subjects in the ESPO-572®, PCSO-524®, and placebo group. However, significant differ-ences were detected ($p < 0.05$) for ESPO-572® vs. placebo and PCSO-524® vs. placebo for height, body mass, and $_{V\_O2peak}$ (L). During the downhill treadmill run, where the goal was to have each subject elicit a heart rate of 70% $_{V\_O2peak}$ (obtained during visit 1), the subjects maintained an average 73.5% of $_{V\_O2peak}$ for the duration of the down-hill run. Subjects' percentage of average heart rate of 70% $_{V\_O2peak,}$ at the 5-, 10-, 15-, and 20-minute intervals were 65.7, 72.8, 76.8, and 78.7%, respectively.

Delayed onset muscle soreness and pain pressure threshold

Refer to the panel, Figure 3, which displays difference scores for DOMS (arbitrary unit) and PPT (newtons) following eccentric exercise. There were no significant differences ($p > 0.05$) in absolute values of DOMS between groups prior to supplementation (base-line) and after 26-days of nutraceutical supplementation before muscle damaging exer-cise. Muscle soreness significantly increased ($p < 0.05$) in all three groups after the muscle damaging exercise protocol, peaking between 24 h and 48 h and then declining at 72 h. Significant effects for time were found in all three groups ($p < 0.05$). Pairwise comparisons between groups at each time point indicated significantly lower DOMS in the ESPO-572® and PCSO-524® groups compared to placebo, at 24 h [ESPO-572® vs. placebo: diff= -1.64, 95% CI (-2.69, -0.60), p = 0.002; PCSO-524® vs. placebo: diff= -1.86, 95% CI (-2.93, -0.79), p = 0.001], 48h [ESPO-572® vs. placebo: $p < 0.001$; diff= -1.93, 95% CI (-2.98, -0.89); PCSO-524® vs. placebo: p = 0.001; diff= -1.75, 95% CI (-2.82, -0.68)] and 72 h [ESPO-572® vs. placebo: $p < 0.001$; diff= -1.88, 95% CI (-2.93, -0.82); PCSO-524® vs. placebo: $p < 0.001$; diff= -2.20, 95% CI (-3.27, -1.13)] following muscle damaging exercise. ESPO-572® was shown to be non-inferior to PCSO-524® in DOMS at 24 h, 48 h and 72 h after muscle damaging exercise.

For percent change in PPT, the tests of between-subject effects indicated that there were no significant effects ($p > 0.05$) between the three groups at any time point. Non-inferiority tests were not performed as the equivalence margin could not be computed

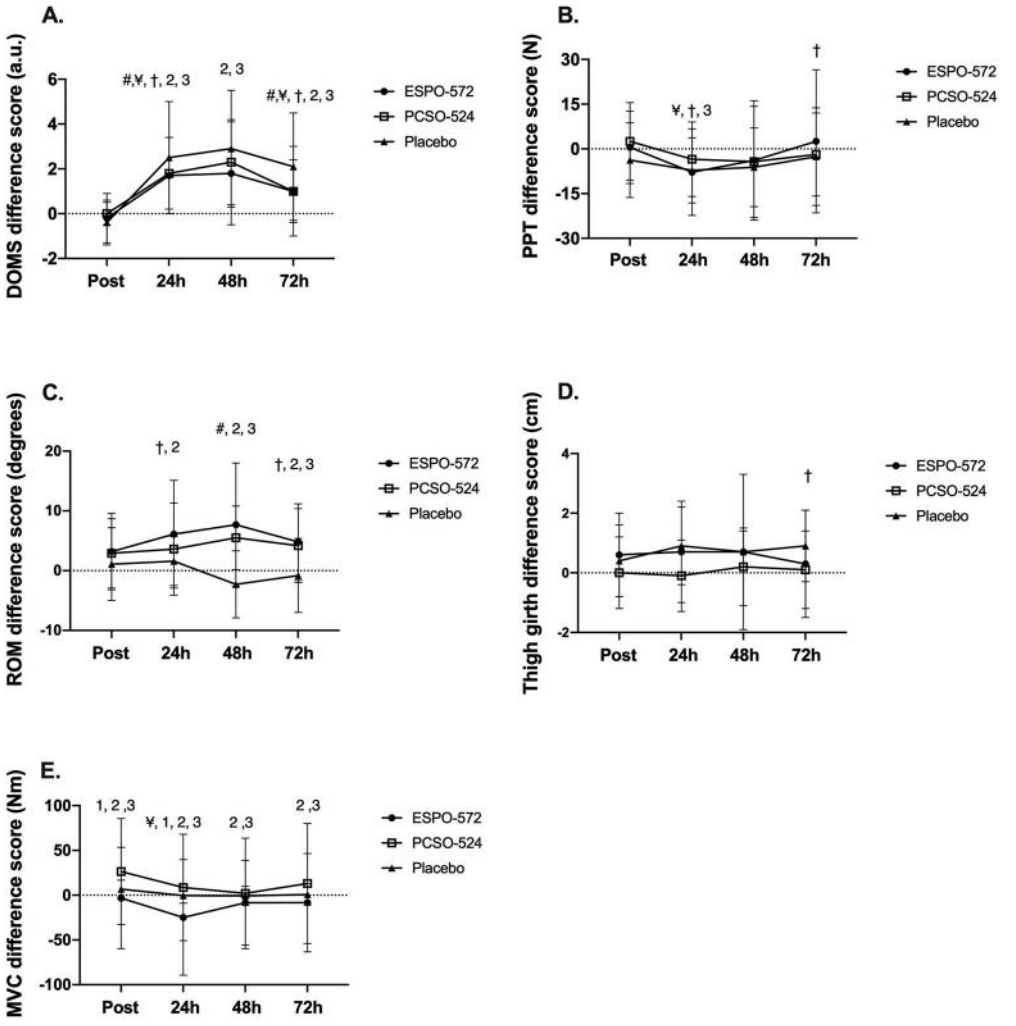

Figure 3. Difference scores for A.) delayed onset muscle soreness (DOMS), B.) pain pressure threshold (PPT), C.) knee flexion (ROM), D.) thigh girth (swelling), and E.) isometric torque (MVC) from pre-sup-plementation to post-supplementation prior to eccentric exercise (Post), 24 h, 48 h, and 72 h following eccentric exercise for groups supplementing with 600 mg/d of either a New Zealand green-lipped mussel (PCSO-524^{VR}), Antarctic krill oil (ESPO-572^{VR}) oil blend, or placebo. Values are mean ± SD. #, sig-nificantly different ($p < 0.05$) from previous time point within placebo group; ¥, significantly different from previous time point within PCSO-524^{VR} group; †, significantly different from previous time point within ESPO-572^{VR} group; 1, significant difference between PCSO-524^{VR} and ESPO-572^{VR}; 2, significant difference between PCSO-524^{VR} and placebo; 3, significant difference between ESPO-572^{VR} and placebo.

when the reference treatment (PCSO-524^{VR}) was not better than placebo (Mickleborough et al. 2015). Pairwise comparisons between time points within-group revealed significant increases ($p < 0.05$) in PPT from post-supplementation to 24 h post-muscle damaging exercise in all three groups. For the ESPO-572^{VR} group, the change in PPT increased sig-nificantly from 48 h to 72 h after muscle damaging exercise (p 1/4 0.015).

Range of motion, swelling, and maximum voluntary contraction

The difference scores for joint ROM (degrees), thigh girth (centimeters), and MVC (Newton-meter) from baseline to recovery from eccentric exercise are displayed in the panel of Figure 3. The tests of within-subject effects indicated a significant effect ($p < 0.05$) of time on ROM within each group. Within the ESPO-572® group, ROM significantly increased at 24 h post-muscle damaging exercise compared to before muscle damaging exercise (AROM = 2.89, 95% CI (0.20, 5.58), p = 0.035); however, it significantly decreased from 48 h to 72 h post-muscle damaging exercise (AROM = —3.10, 95% CI (-5.82, —0.38), p = 0.026). Within the placebo group, ROM was significantly reduced from 24 h to 48 h post-muscle damaging exercise (AROM = —3.88, 95% CI (-7.37, —0.38), p = 0.030). Significant differences were found for ROM at post-supplementation, 24 h, 48 h, and 72 h post-muscle damaging exercise compared to baseline within the ESPO-572® and PCSO-524® groups ($p < 0.05$).

ESPO-572® showed significantly higher ROM than PCSO-524® at 24 h (diff= 6.29, 95% CI (1.12, 11.47), p= 0.017) and 48 h after muscle damaging exercise (AROM = 5.91, 95% CI (0.73, 11.08), p =0.025). Both ESPO-572® and PCSO-524®showed ROM improvement over placebo. PCSO-524® had significantly higher ROM than placebo at 48 h (AROM = 9.22, 95% CI (3.26, 15.17), p =0.003) and 72 h after muscle damaging exercise (AROM = 6.37, 95% CI (0.42, 12.32), p = 0.036); while ESPO-572® had significantly higher ROM than placebo at post-supplementation (AROM = 7.18, 95% CI (1.37, 13.00), p = 0.016), 24 h (AROM = 9.64, 95% CI (3.82, 15.46), p =0.001), 48 h (AROM = 15.12, 95% CI (9.30, 20.94), $p < 0.001$), and 72 h after muscle damaging exercise (AROM = 10.53, 95% CI (4.69,16.36), $p < 0.001$). While ESPO-572® was superior to PCSO-524® at 24 h and 48 h in ROM, there was no evi-dence of superiority or non-inferiority at 72 h.

For thigh girth, a significant decrease was found in the ESPO-572® group at 72 h after muscle damaging exercise compared to 48 h. No significant differences were observed for the percent change from baseline within or between groups at any other time points. Non-inferiority tests were not performed for thigh girth because PCSO-524® was not significantly better than placebo, and no margin could be determined.

For MVC, the test of within-subject effects revealed that there was a significant decrease from before muscle damaging exercise to 24 h after exercise within the PCSO-524® group. Pairwise comparisons between groups for each time point revealed signifi-cant differences in the ESPO-572® and PCSO-524® groups compared to placebo at post supplementation, 24 h, 48 h, and 72 h after eccentric exercise.

Skeletal muscle damage and inflammatory blood markers

Mean values for TNF-oc (pg/mL), CK-MM (U/L), IL-fl (pg/mL) taken at all time points of the intervention are displayed in Figure 4. Mean plasma TNF-oc concentration did not significantly differ ($p > 0.05$) at any time point within or between ESPO-572® and PCSO-524® groups. However, in the placebo group, plasma TNF-oc concentration sig-nificantly increased from baseline to 24, 48, and 72 h post-muscle damaging exercise. Plasma TNF-oc level peaked at 24 h following muscle damaging exercise in the placebo group and stayed elevated through 72 h. The ESPO-572® and PCSO-524® groups were

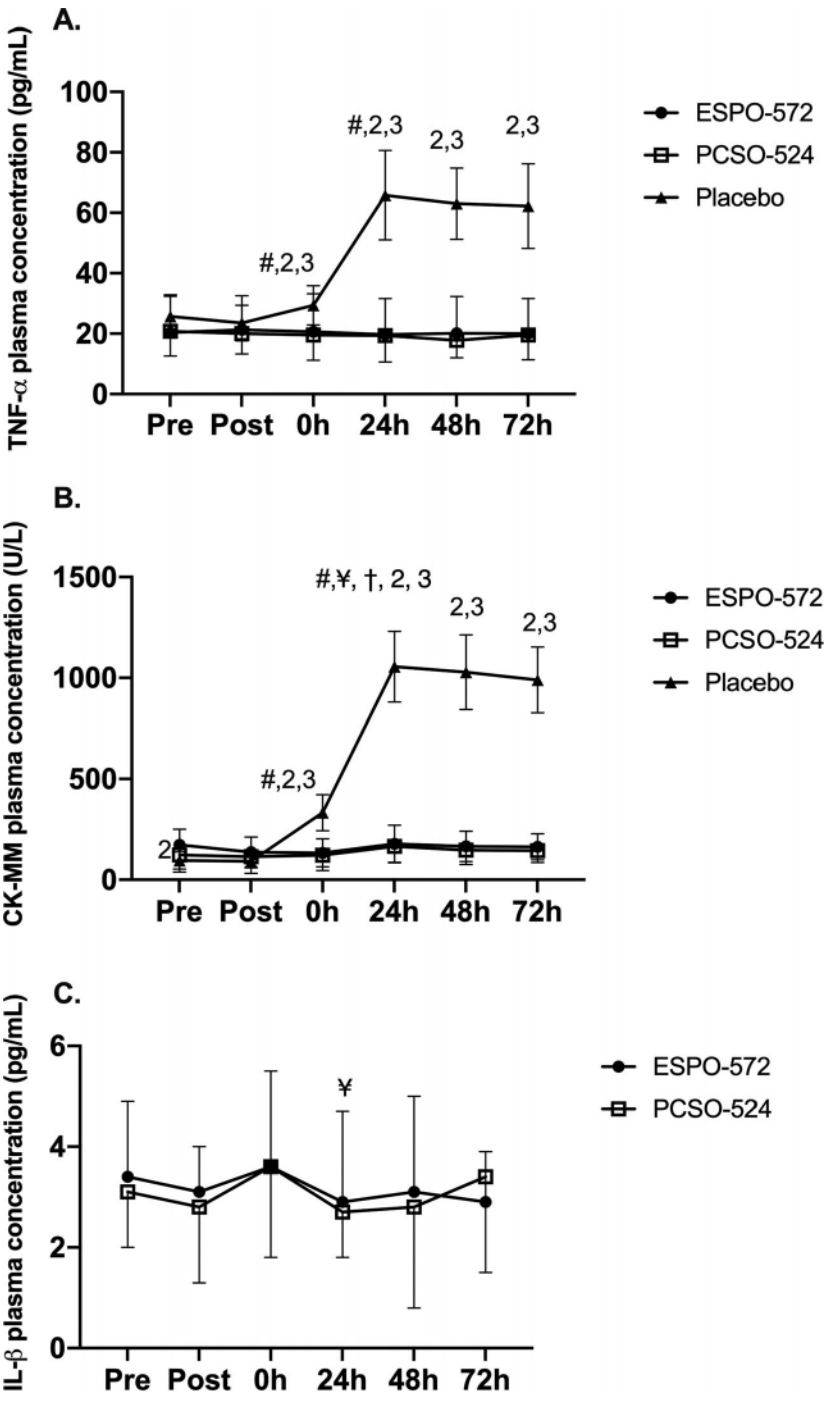

Figure 4. Mean values for A.) tumor necrosis factor-O (TNF-O), B.) creatine kinase (CKMM), and C.) interleukin-fl (IL-fl) from pre-supplementation to 26 d post-supplementation (Post) with 600 mg/d of either a New Zealand green-lipped mussel (PCSO-524^{VR}), Antarctic krill oil (ESPO-572^{VR}) oil blend, or placebo immediately following (0h) and 24 h, 48 h, and 72 h after muscle damage inducing downhill running exercise. Measures of IL-fl were not taken in the Mickleborough *et al.* trial. As such, a placebo group is not included for IL-fl. Values are mean ± SD. #, significantly different ($p < 0.05$) from previous time point within placebo group; ¥, significantly different from previous time point within PCSO-524^{VR} group; †, sig-nificantly different from previous time point within ESPO-572^{VR} group; 2, significant difference between PCSO-524^{VR} and placebo; 3, significant difference between ESPO-572^{VR} and placebo.

significantly different from placebo at 0 h [ESPO-572® vs. placebo: ΔTNF-α = -8.78, 95% CI (-15.23, -2.33), p = 0.008; PCSO-524® vs. placebo: ΔTNF-α = -9.88, 95% CI (-16.49, -3.27), p = 0.004], 24 h [ESPO-572® vs. placebo: ΔTNF-α = -46.14, 95% CI (-52.59, -39.69), p < 0.001; PCSO-524® vs. placebo: ΔTNF-α = -46.42, 95% CI (-53.03, −39.81), p < 0.001], 48h [ESPO-572® vs. placebo: ΔTNF-α = -42.83, 95% CI (-49.28, −36.38), p < 0.001; PCSO-524® vs. placebo: ΔTNF-α = -45.12, 95% CI (-51.73, -38.51), p < 0.001] and 72 h [ESPO-572® vs. placebo: ΔTNF-α = -42.17, 95% CI (-48.62, −35.72); PCSO-524® vs. placebo: p < 0.001; ΔTNF-α = -42.67, 95% CI (-49.28, -36.06), p < 0.001] after muscle damaging exercise. ESPO-572® was non-inferior to PCSO-524®in TNF- α concentration at all time-points.

Interleukin-1β (IL-1β) was not significantly different between ESPO-572® and PCSO-524® at any time point. A significant decrease was observed in the PCSO-524® group at 24 h compared to 0 h after muscle damaging exercise (ΔIL-1β = -0.88, 95% CI (-1.73, −0.03), p = 0.042).

Percent change in plasma CK-MM concentrations were not significantly different (p > 0.05) immediately prior to muscle damaging exercise within or between groups. Significant increases (p < 0.05) in percent changes, compared to previous time points, were observed at 0 and 24 h post-muscle damaging exercise within the placebo group. For all three groups, percent change of plasma CK-MM concentration reached a peak at 24 h following muscle damaging exercise where it stayed elevated. No significant differ-ences were observed between ESPO-572® and PCSO-524® groups across time. Significant differences in percent change were detected in ESPO-572® and PCSO-524®groups compared to placebo at 0 h, 24 h, 48 h and 72 h after muscle damaging exercise. ESPO-572® was non-inferior to PCSO-524® in CK-MM concentration at all-time point's post downhill running.

Nutrient intake

Mean daily nutrient intake, including total calories, protein, saturated and polyunsatur-ated fat, magnesium, zinc, vitamin C, α-carotene, β-carotene, choline, vitamin D, DHA, EPA, alpha-linolenic acid, α-tocopherol, lycopene, sodium, and potassium did not differ significantly (p > 0.05) between ESPO-572® and PCSO-524® during the course of the study. Total caloric intake (kcal/day) consumed by the ESPO-572® and PCSO-524®groups before muscle damaging exercise was 2,430.1 ± 1,040.6 and 2,594.9 ± 930.6 (p = 0.513) respectively, while post downhill run caloric consumption averaged 2,199.9 ± 784.6 and 2,285.5 ± 773.6 (p = 0.784) respectively. Total protein intake (grams/ day) consumed by the ESPO-572® and PCSO-524® groups before muscle damaging exercise was 123 ± 67.8 (1.5 g/kg of body mass) and 109.2 ± 46.8 (1.4 g/kg of body mass) (p = 0.353) respectively, while post downhill run protein consumption averaged 113.2 ± 48 (1.4 g/kg of body mass) and 96.2 ± 36.7 (1.2 g/kg of body mass)(p = 0.172) respectively. Total fat intake (grams/day) consumed by the ESPO-572® and PCSO-524®groups before muscle damaging exercise was 106.4 ± 65.4 (1.3 g/kg of body mass) and 103.5 ± 46 (1.3 g/kg of body mass)(p = 0.835) respectively, while post downhill run pro-tein consumption averaged to 93.4 ± 38.6 (1.1 g/kg of body mass) and 89.6 ± 36.2 (1.1 g/ kg of body mass) (p = 0.798) respectively. Total carbohydrates (grams/day) consumed

by the subjects significantly differed between the treatment groups [ESPO-572^{VR} group (232.3 ± 82.1 g/day (2.9 g/kg of body mass) versus PCSO-524^{VR} group (287.8 ± 99.9 g/day (3.6 g/kg of body mass)] before muscle damaging exercise ($p = 0.033$) with no significant difference being evident after muscle damaging exercise between groups [ESPO-572^{VR}group = 217.7 ± 80.7 g/day (2.7 g/kg of body mass); PCSO-524^{VR} group = 251.3 ± 97 g/day (3.1 g/kg of body mass)($p = 0.220$). Complete nutrient intake data is available in Supplementary Table 1, online supplementary information (OSM).

Fatty acid profile analysis

The fatty acid composition, including myristic, palmitic, stearic, alpha-linolenic, oleic, linoleic, arachidonic, DHA, EPA, docosapentaenoic (DPA), and the O3I did not differ between ESPO-572^{VR} and PCSO-524^{VR} during the course of the study. However, within both groups, DPA increased significantly compared to baseline. The mean differences between the two groups were significant at both time points (baseline: $p = 0.047$; post supplementation: $p = 0.043$). Complete fatty acid profile analysis is available in Supplementary Table 2, OSM.

Discussion

The purpose of this double-blind, randomized controlled trial was to examine whether a novel nutraceutical (ESPO-572^{VR}) containing a mixture of marine lipids from the New Zealand green-lipped mussel and krill oil (ESPO-572^{VR}) has the potential to enhance recovery from muscle damage inducing exercise. We hypothesized that ESPO-572^{VR}would infer a superior protection than PCSO-524^{VR} (green-lipped mussel oil only) against signs and symptoms of EIMD. This hypothesis was partially supported.

The data from the present study has shown that ESPO-572^{VR} was '*at least as good as*' PCSO-524^{VR} at attenuating several indirect functional and blood markers of muscle damage and soreness following eccentric exercise. Measures taken during the 72 h recovery period following eccentric exercise indicate ESPO-572^{VR} was '*at least as good as*' PCSO-524^{VR} in attenuating the increase in DOMS and moderating the rise in CK-MM and TNF-a observed in the placebo group. Importantly, ESPO-572^{VR} was found to be super-ior to PCSO-524^{VR} for preserving joint ROM at 24 h and 48 h following muscle damage inducing exercise. To our knowledge, this is the first study to date, which has demon-strated the efficacy of combining a blend of New Zealand green-lipped mussel and krill oil in mitigating indirect makers of muscle damage and inflammation following eccen-tric exercise.

Effect of ESPO-572^{VR} compared to PCSO-524^{VR} on delayed onset muscle soreness and pain threshold

DOMS in all groups was found to peak between 24-48 h following the downhill run-ning protocol, which is a common feature following eccentric exercise (Clarkson and Hubal 2002; Mickleborough et al. 2015). At every time point during recovery, the pla-cebo group had higher DOMS ratings compared to ESPO-572^{VR} and PCSO-524^{VR}. These

data add to previous evidence supporting the consumption of PUFAs for the relief of DOMS (Jouris et al. 2011; Tsuchiya et al. 2019). Polyunsaturated fatty acids have been shown to inhibit nociception by downregulating various crucial factors known to stimulate pain (i.e. neurodegeneration, inflammation, reactive oxygen species, and nociceptive fiber sprouting) (Figueroa et al. 2013). Importantly, DOMS has been shown to interfere with activities of daily living in adult populations and to impair neuromuscular function in athletes, and elevating the risk of injury (Cheung et al. 2003; Trost et al. 2011). Thus, a reduction in DOMS following chronic supplementation with n-3 PUFA dense oil blends may importantly encourage more spontaneous physical activity by accelerating recovery from previous physical activity and/or provide an injury protective effect in athletes.

Pain pressure threshold increased in all three groups 24 h after downhill running, with no difference between groups. This finding agrees with our previous work (Mickleborough et al. 2015) which demonstrated no change in PPT following eccentric exercise with PCSO-524^{VR} supplementation. Although PPT measurements have demonstrated consistent test-retest reliability, the response magnitude of young subjects has been shown to steadily decline with consecutive measures taken across 4 d (Jones et al. 2007). As such, it is possible due to the repeated pressure of the algometer head over consecutive days (similar to the well-established repeated-bout effect) subject anticipatory cues or microtrauma at the PPT locations (Sand et al. 1997; Jones et al. 2007) may have confounded any potential differences observed in PPT following the muscle damage inducing exercise.

Effect of ESPO-572^{VR} compared to PCSO-524^{VR} on knee joint range of motion (knee flexion), limb swelling (thigh girth), and isometric strength (torque)

Knee flexion ROM was reduced from 24 to 48 h following eccentric exercise in the placebo group, with no loss in ROM occurring in the ESPO-572^{VR} and PCSO-524^{VR} groups. Notably, the ROM in the ESPO-572^{VR} group was greater than the PCSO-524^{VR} group at 24 and 48 h following muscle damaging exercise. Evidence suggests that joint ROM is diminished for up to 5 d after an eccentric exercise bout (Nosaka et al. 2001). Given the link between joint ROM of the lower limbs and walking/running economy and jump performance (Chen et al. 2007; Driller and Overmayer 2017), preserving ROM following muscle damage inducing exercise is important for subsequent athletic performance (Pizzuto et al. 2019). Specifically, astaxanthin (a carotenoid in krill oil) has been shown to inhibit the expression of matrix metalloproteinases which, when uncontrolled, can impair joint ROM (Anandacoomarasamy et al. 2009). This finding is reinforced by others, who have found krill oil consumption to ameliorate mild joint pain in healthy adults (Suzuki et al. 2016), and joint pain, stiffness, and functional impairment in indi-viduals with chronic inflammatory disease (Deutsch 2007).

The MVC data demonstrated that peak torque decreased at 24 h following eccentric exercise in the PCSO-524^{VR} group. While MVC remained unchanged in the ESPO-572^{VR}group at 24 h, the same result was evident in the placebo group, indicating neither n-3 PUFA blend conferred protection against the acute loss in muscular strength/power typically observed with recovery from muscle damaging exercise (Warren et al. 1999).

There were no differences between the two blends in the remainder of the recovery period. Comparably, other studies have shown n-3 PUFA supplementation to have no effect on MVC following recovery from eccentric exercise (Pumpa et al. 2011; VanDusseldorp et al. 2020). Dynamic muscular power findings *via* vertical jump are found to be positively impacted by n-3 PUFA supplementation (Jakeman et al. 2017; VanDusseldorp et al. 2020), which suggests that static muscular strength is less beneficially impacted than dynamic muscular strength by n-3 supplementation. Even whey protein, well-known to facilitate the restoration of contractile function (Davies et al. 2018) oftentimes is unable to offer a protective effect in the decline in MVC (Buckley et al. 2010). Lastly, when sufficient protein is consumed, supplementation with n-3 supplements seems to promote no additional increase in muscle protein synthesis in response to resistance training (Rossato et al. 2020). In the present study the subjects consumed 1.4 and 1.2 g/kg of body weight of protein each day during the recovery period in the ESPO-572^{VR} and PCSO-524^{VR} group, respectively. These quantities are higher than the Recommended Dietary Allowance of 0.8 g protein/kg (Medicine I 2002), which suggests sufficient muscle protein synthesis was achieved during the recovery period; this may partially explain the null findings of the MVC data.

In accordance with our previous findings (Mickleborough et al. 2015), thigh girth was unaffected by downhill running during recovery in both groups. It has been sug-gested (Mickleborough et al. 2015) that the time frame of 72 h may be too short in order to observe any potential effects of EIMD on muscle swelling. Observed changes in muscle fiber size have been shown to occur at 7-8 d, but not 2-3 d, post- eccentric exer-cise in young men (Yu et al. 2013).

Effect of ESPO-572^{VR} compared to PCSO-524^{VR} on blood markers of muscle damage and inflammation

The two oil blends ESPO-572^{VR} and PCSO-524^{VR} attenuated the rise in serum CK-MM and TNF- concentration, compared to placebo, following downhill running. The serum TNF- concentration in both supplement groups was reduced compared to placebo at 24, 48 and 72 h after eccentric exercise, suggesting ESPO-572^{VR} is equally effective as PCSO-524^{VR} in moderating the inflammatory response following eccentric exercise. These findings are similar to others who have found n-3 PUFA dense blends to alleviate various inflammatory cytokine and eicosanoid levels (TNF- , interleukin-6, prostaglan-din E_2) after eccentric exercise in healthy subjects (DiLorenzo et al. 2014; Tsuchiya et al. 2019). Further, ESPO-572^{VR} and PCSO-524^{VR} similarly mitigated the characteristic rise in CK-MM plasma concentration commonly seen after a bout of downhill running (F~easson et al. 2002; Peake et al. 2005).

Intriguingly, IL-fl, a cytokine released from blood monocytes in response to an inflammatory stimuli (i.e. downhill running) is normally significantly elevated post-eccentric exercise (Cannon et al. 1989; Fielding et al. 1993). This is in contradiction of our current study findings as neither group saw a significant rise in IL-fl post exercise, possibly indicating the effectiveness of inflammation suppression of the two marine oil blends. However, it is important to note that Kanda *et al.* (Kanda et al. 2013) found similar findings to ours in that lower body eccentric exercise can induce DOMS and

muscle damage without adversely affecting IL-fl levels. Along with monocytes, a strenuous exercise stimulus results in the elevation of various cytokines (i.e. IL-fl) that assist in controlling the development of systemic (i.e. cortisol secretion) and local (i.e. muscle pain in worked muscle) acute phase effects with the goal of returning the body back to homeostasis. Since muscle biopsies was not conducted in the current study, it is possible that we may have missed the peak IL-fl plasma value following the downhill run, since Cannon *et al.* found this marker to peak within 9 h and return to baseline within 24 h of exercise (Cannon et al. 1986). However, the role of IL- fl for tissue remodeling is clear in EIMD as muscle biopsies have shown an elevation five days post-exercise (Cannon et al. 1989).

Potential mechanisms underlying the effectiveness of PCSO-524^{VR} and ESPO-572^{VR}in attenuating exercise-induced muscle damage and inflammation

Our group (Mickleborough et al. 2015) and others (Baum et al. 2013; Zawadzki et al. 2013) have shown that PCSO-524^{VR} to be an effective nutraceutical for reducing functional indices of EIMD and inflammation in various subject groups from untrained (Mickleborough et al. 2015) to trained (Baum et al. 2013), as well as alleviating symptoms of various clinical conditions (i.e. osteoarthritis and asthma) (Cho et al. 2003; Mickleborough et al. 2013). The anti-inflammatory effects of PCSO-524^{VR} are likely in part attributed to the action of furan fatty acids which have been shown to exhibit more potent anti-inflammatory activity than EPA in animal models (Wakimoto et al. 2011), and to effectively inhibit non-enzymatic lipid peroxidation and minimize oxida-tive stress (Teixeira et al. 2013; Xu et al. 2017). In addition to furan fatty acids, ESPO-572^{VR} contains a novel ingredient, Antarctic krill oil (*Euphausia superba*). Similar to green-lipped mussel oil blends, krill oil is dense in n-3 PUFAs, and contains various other promising, although less-studied, compounds that include astaxanthin (not found in PCSO-524^{VR}) and high amounts of phospholipids, which may further attenuate indir-ect markers of EIMD (Djordjevic et al. 2012; Baralic et al. 2015). While our findings confirm those of others who have demonstrated the beneficial effects of n-3 PUFAs on recovery from muscle damaging inducing exercise (Baum et al. 2013; Mickleborough et al. 2015), not all have found PCSO-524^{VR} to favorably reduce EIMD (Pumpa et al. 2011).

Astaxanthin a carotenoid found in krill oil, and therefore exclusively found in the ESPO-572^{VR} blend, exhibits remarkable antioxidant properties that are reportedly 10-100 times more active than other common antioxidants such as O-tocopherol, lutein, and fl-carotene (Miki 1991). A myriad of findings in animal models supports its utility in facilitating muscle recovery through the suppression of oxidative radicals, inflammation, and matrix metalloproteinases (Aoi et al. 2003; Kochi et al. 2014; Park et al. 2020). It has been suggested that due to its antioxidant/anti-inflammatory properties, astaxanthin has been shown to improve muscle function and recovery from exercise in humans (Earnest et al. 2011; Liu et al. 2018; Fleischmann et al. 2019). Baralic *et al.* (Baralic et al. 2015) and Djordjevic *et al.* (Djordjevic et al. 2012) presented findings in support of astaxanthin as a muscle recovery agent in young male soccer players. Across a 90 d competitive season, astaxanthin was found to attenuate markers of muscle damage,

oxidative stress, and inflammation, which remained elevated in a control group (Djordjevic et al. 2012). Therefore, it is possible that the astaxanthin component of ESPO-572^{VR}, may facilitate muscle recovery by inhibiting proinflammatory and prooxidant physiologic pathways contributing to cytokine-induced tissue damage (Buddhachat et al. 2017).

In addition to its antioxidant effects, the unique phospholipids found in ESPO-572^{VR}may further enhance muscle recovery by facilitating muscle protein synthesis. The pri-mary phospholipids in krill oil are phosphatidylcholine, phosphatidylserine, and phosphatidic acid, and approximately 40-80% of the n-3 LC PUFAs in krill oil are bound to phospholipids (Xie et al. 2019). Phosphatidic acid has been shown to stimulate an increase in mammalian target of rapamycin (mTOR), a key factor in regulating skeletal muscle protein synthesis (Joy et al. 2014). Phosphatidylserine has been found to reduce muscle soreness as well as reduce exercise-induced elevations in the catabolic hormone cortisol (Fahey and Pearl 1998; Starks et al. 2008). Furthermore, recent data suggest krill oil can stimulate mTOR signaling in humans (Georges et al. 2018), and that astaxanthin increases mTOR expression in mice (Liu et al. 2018). The n-3 PUFAs, phosphatidic acid, phosphatidylserine, and/or astaxanthin, or a synergistic combination of these phospholipids and antioxidants may be responsible for the increase in mTOR signaling, DOMS reduction and anti-inflammatory effects seen with krill oil consumption (Georges et al. 2018; Xie et al. 2019). An important question that needs to be resolved is whether chronic supplementation with nutraceuticals known to have potent antioxi-dant and anti-inflammatory properties blunts the training response, since oxidative stress and inflammation are key aspects in modulating training adaptations (Pastor and Tur 2019). However, important to this study, it has been found that n-3 supplementa-tion for three weeks (Lewis et al. 2015), and even up to 10 weeks, did not dampen either aerobic or anaerobic training adaptations in well-trained athletes (Raastad et al. 1997). In addition, it has been shown that professional athletes consuming an n-3 nutraceutical for five weeks during pre-season training experienced numerous benefits such as main-tenance of explosive power (Black et al. 2018). Therefore, we feel, that the type of nutra-ceuticals, supplementation period and dosage used in the present study to be trivial, if any on training adaptations as the krill oil component of the ESPO-572^{VR} oil blend, may in part be responsible for the increase in knee flexion ROM documented in this group compared to the PCSO-524^{VR} group following the eccentric exercise bout. This is an important finding since knee flexion ROM is considered one of the best tools for quan-tifying muscle damage (Warren et al. 1999).

Although every effort was made to implement a rigorously designed study, the present study does have several limitations that we would like to address. While the fatty acid profile analysis was intended to serve as an objective measure to assess compliance with supplementation, no discernable differences were observed in the relevant blood markers of n-3 PUFA status (i.e. DHA, EPA, ALA or the O3I) with supplementation of either blend. The fact that the O3I, which is indicative of the erythrocyte fatty acid profile, did not change as a result of supplementation is not surprising as human erythrocytes survive for 115 days (Franco 2012). It is plausible that the present 30 d supplementation period was insufficient to allow for the detection of meaningful changes in the fatty acid profile of erythrocytes. Additionally, Arterburn and colleagues

found that DHA did not reach a steady state in RBC membranes until after at least 4 months of supplementation (Arterburn et al. 2006). However, we are confident that subject compliance to the supplementation protocol was achieved since blood levels of docosapentaenoic acid (DPA), an n-3 PUFA, did increase as result of supplementation with both oil blends. In addition, the increase in plasma DPA observed in the present study is not unusual as other groups have found that both krill and fish oil supplementation significantly elevate DPA concentrations (Ramprasath et al. 2013). DPA has been found to be more anti-inflammatory than EPA or DHA in some studies (Khaddaj-Mallat et al. 2016; Tian et al. 2017), and this finding may partially explain the nutra-ceuticals' beneficial effects on inflammation in the absence of a change in O3I. Further evidence suggests that in healthy men, those with higher levels of erythrocyte DPA have significantly lower levels of inflammatory markers such as TNF- and C-reactive protein (Labont~e et al. 2014).

The placebo group data used for analysis was obtained from our previous work (Mickleborough et al. 2015), and therefore subjects were not randomized into three separate groups. However, the primary outcome of interest was to compare the equivalency or superiority of ESPO-572^{VR} with PCSO-524^{VR}, since the efficacy of PCSO-524^{VR} versus placebo has previously been established (Mickleborough et al. 2015). Nevertheless, there were no differences in age, BMI, or relative $_{V\_O2peak}$ between all three groups. It should be noted that data were collected using an identical protocol and the same equipment as used in our previous study (Mickleborough et al. 2015).

The recruitment of only untrained men is another recognized limitation of this study, as our findings may not be generalizable to other populations (i.e. trained athletes, women). Untrained subjects were included for the purpose of maximizing the effect size of the functional indices and blood markers of EIMD in response to the downhill running protocol, since it has been shown that trained subjects experience smaller changes in muscle damage and inflammation after eccentric exercise (Newton et al. 2008). Therefore, it is reasonable to assume that trained athletes would experience less alleviation of muscle damage and inflammation with this type of supplementation. Nevertheless, the use of n-3 PUFA dense oil blends has been shown to lessen muscle soreness and improve neuromuscular function in professional athletes (Lewis et al. 2015; Black et al. 2018).

Conclusion

The current study demonstrates that EPSO-572^{VR}, containing a mixture of marine lipid oil from the New Zealand green-lipped mussel and krill oil, is '*at least as good as*' PCSO-524^{VR}, a proven anti-inflammatory and muscle damage-mitigating nutraceutical containing New Zealand green-lipped mussel oil only, at ameliorating DOMS, TNF- , IL- /3, and importantly superior to PCSO-524^{VR} in preserving joint ROM following eccentric-type exercise in untrained men. Our data support the use of ESPO-572^{VR}, a novel nutraceutical, in mitigating the deleterious effects of EIMD in untrained men. Although the data from the present study is encouraging, future investigations using ESPO-572^{VR} to reduce EIMD and inflammation should look at various other demo-graphics such as trained athletes and the elderly.

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Disclosure statement

The authors report no conflicts of interest.

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Ms. Kadie Freeman, MS, is a medical student at St. George University. She received her BS and MS degrees from Indiana University Bloomington. She has served in a research support role within the exercise physiology department during her time at Indiana University.

Ms. Xiwei Chen, MS, received her MS degree in Statistics from Indiana University, Department of Statistics in December 2017. She previously earned her bachelor's degree in Mathematics at Indiana University, which was followed by working as a Data Analyst in Information Technology. In addition to statistical consulting at IU, she performed an internship at a technol-ogy company in Beijing working in Machine learning techniques. Since June 2018 she has been working for the Biostatistics Consulting Center at Indiana University.

Ms. Stephanie L. Dickinson, MS, received a Master of Applied Statistics degree from The Ohio State University in 2001, and worked as a Senior Consultant at OSU's Center for Biostatistics after graduation. After a short time in Washington DC doing biostatistics consulting with Statistics Collaborative, she moved to Indiana University, where she worked at the Indiana Statistical Consulting Center (ISCC) for eight years. In Fall 2015, she returned to a focus on bio-statistics by transitioning to the Department of Epidemiology and Biostatistics in the School of

Public Health, where she consults on any health-related research at IU and helps coordinate collaborations with Epi-Bio faculty.

Dr. Alyce D. Fly, PhD, CFS, is Professor in the Department of Nutrition and Health Science at Ball State University. She earned her doctorate in Nutritional Sciences at University of Illinois and went to work for Indiana University Bloomington for many years where she achieved the rank of Professor, retiring as Professor Emeritus in July of 2020. Professor Fly has conducted nutrition research on the following topics: Measurement of food intake in children, dietary fatty acids and immunity, antioxidants and oxidant stressors, including oxidative stress in response to a high fat meal, bioavailability of minerals and carotenoids, measurement of biological markers, understand factors related to mothers' decisions to exclusively breastfeed, and generally, obesity and health. She continues to work collaboratively with her IU colleagues in research and her new colleagues at Ball State University as the Department chair.

Dr. Keisuke Kawata, PhD, is a clinical neuroscientist and an assistant professor in the Department of Kinesiology and Program in Neuroscience at Indiana University. His B.S. from Henderson State University focused on sports medicine/athletic training, and he worked in various sports settings, such as NFL Detroit Lions, MLS Sporting Kansas City, ESPN Wide World of Sports, and MLB Atlanta Braves. In his M.S. at Temple University, he studied molecular aspect of brain health using animal models and neuronal cells, coupled with exercise and trauma com-ponents. In his Ph.D., also at Temple University, he conducted a series of clinical neuroscience studies to study the effects of repetitive head impacts (sub-concussion) on brain health. At Indiana University, his team is currently conducting a wide array of sub-concussion research in both field and laboratory settings, aiming to understand the short- and long-term effects of sub-concussive brain damage, and to establish clinical guidelines to prevent chronic brain deficits in athletes and military service members.

Dr. Robert F. Chapman, PhD, FACSM, is an associate professor in the Department of Kinesiology at Indiana University. An integrative exercise physiologist by training, Dr. Chapman has over 25 years of research history with a common theme of physiological limitations which limit human performance during exercise and sport. His research has focused on the effects of altitude and hypoxia on exercise performance. The application of physiological principles to sport performance has been at the heart of his work, first as the Head Cross Country / Assistant Track & Field Coach at Indiana University (1998–2007), where he coached multiple NCAA and USA national champions. Dr. Chapman also serves as the Director of Sport Science & Medicine for USA Track & Field since 2010.

Dr. Timothy D. Mickleborough, PhD, is a Professor in the Department of Kinesiology at Indiana University-Bloomington. His main research focus is integrative (whole-body) human exercise physiology; in particular the interaction between nutrition and the cardiorespiratory system in health and disease, with emphasis on intervention studies in healthy trained/untrained individu-als, the pathophysiology of respiratory disorders in athletes such as asthma and expiratory flow limitation, and the potential cardiorespiratory limitations to exercise tolerance and performance. Research related to respiratory muscle function is directed at the respiratory system determinants of fatigue in health and disease and encompasses respiratory and circulatory mechanics, neuro-physiology, muscle physiology, perception of effort and biochemistry. An additional research interest is assessing the relative contributions of central and peripheral factors in human muscle fatigue, and the physiological basis for pacing strategies, during exercise.

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