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The Effects of PCSO-524®, a Patented Marine Oil Lipid derived from the New Zealand Green Lipped Mussel (*Perna canaliculus*), on Pulmonary and Respiratory Muscle Function in Non-asthmatic Elite Runners

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ABSTRACT

International Journal of Exercise Science 11(3): 669-680, 2018. Habitual endurance training may be associated with mild airway inflammation and subsequent deterioration in lung function. PCSO-524™ (Lyprinol®/Omega-XL®), a supplement extracted from the New Zealand green-lipped mussel (*Perna canaliculus*), has been shown to moderate airway inflammation in asthmatic subjects. The purpose of this study was to determine whether supplementation with PCSO-524™ improves pulmonary and respiratory muscle function in non-asthmatic elite runners. Sixteen male, non-asthmatic elite runners were randomly assigned to either a treatment (PCSO-524™; 1 capsule contains 50 mg *n*-3 polyunsaturated fatty acids and 100 mg olive oil, *n*=8) or placebo (1 capsule contains 150 mg olive oil; *n*=8) group. During the supplementation period, subjects ingested 8 capsules of either treatment or placebo per day for 12 weeks. Resting pulmonary and respiratory muscle function testing were assessed at baseline and every two weeks throughout the 12 week supplementation period. No significant between- or within-subjects main effects were observed in forced vital capacity, forced expiratory volume in 1-second, forced expiratory flow from 25-75% of lung volume (FEF_{25-75}), peak expiratory flow, maximal voluntary ventilation, maximal inspiratory mouth pressure, and closing volume ($p>0.05$). A significant within-subjects main effect was observed in maximal expiratory mouth pressure (P_{Emax}) ($p=0.024$) and lung diffusion capacity (D_{LCO}) ($p<0.0001$), but no significant between-subjects main effects were observed for P_{Emax} and D_{LCO} ($p>0.05$). A significant treatment by time interaction was observed in FEF_{25-75} ($p=0.026$) and D_{LCO} ($p=0.024$), but no other significant interactions were observed (all $p>0.05$). Supplementation with PCSO-524™ (Lyprinol®/Omega-XL®) does not improve pulmonary or respiratory muscle function in non-asthmatic elite runners.

KEY WORDS: Lung function; supplementation; fish oil; omega-3 fatty acids

INTRODUCTION

Regular training of endurance athletes in cold or temperate environments is associated with mild airway inflammation (5, 6, 13, 24, 32). Airway inflammation, coupled with acute immunosuppression during periods of high training volume, may put athletes at risk for upper respiratory tract infection and related performance decrements (4, 7, 11, 26). The precise mechanism behind this airway inflammation is unclear (31). While it may not be due to bronchial hyper-reactivity, post-exercise respiratory symptoms (5, 13), or immune cell activation (5, 6, 24), it may be associated with elevated airway neutrophil counts (5, 6, 8, 24), as well as insufficient conditioning of inspired air and osmotic changes that damage the epithelium (3). Mitigating airway inflammation has been shown to improve exercise tolerance in asthmatic populations (12); therefore, endurance athletes may benefit from interventions that can modify the severity of airway inflammation.

Current evidence suggests that the incorporation of supplements such as fish oil that contain *n*-3 polyunsaturated fatty acids may be beneficial in modifying the severity of airway inflammation patients with asthma (16-18). Fish oils containing high levels of the *n*-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to have a protective effect on airway function in elite athletes (20) and asthmatics (19), and may have an ergogenic effect on exercise performance (30). We have recently shown that PCSO-524™ (Lyprinol®/Omega-XL®), which is a patented marine oil extract of stabilized lipids from the New Zealand green-lipped mussel, *Perna canaliculus*, combined with olive oil and vitamin E (10), can attenuate airway inflammation and the severity of exercise-induced bronchoconstriction (EIB) in asthmatics (22) and reduce exercise-induced muscle damage and delayed-onset muscle soreness (21). This is likely through the action of furan fatty acids (34) and inhibition of the cyclooxygenase-2 and 5-lipoxygenase pathways, resulting in a significant reduction in pro-inflammatory prostanoids and leukotrienes, and a subsequent reduction in cytokine production from inflammatory cells (21, 35). Despite these potential benefits, the utility of such a nutritional approach has not been examined in non-asthmatic athletes.

PCSO-524™ is a mixture of the five main lipid classes including sterol esters, triglycerides, free fatty acids, sterols, and polar lipids (36), and has been shown to contain up to 91 fatty acids, with docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) accounting for 84% of the *n*-3 polyunsaturated fatty acid content (36). PCSO-524™ has been shown to exert potent anti-inflammatory effects through the action of furan fatty acids (34) and inhibition of the cyclooxygenase-2 and 5-lipoxygenase pathways, which normally metabolize arachidonic acid into pro-inflammatory prostanoids and leukotrienes (21, 35). The inhibitory effects of PCSO-524™ on these pathways therefore produce a significant reduction in pro-inflammatory prostanoids and leukotrienes, and a subsequent reduction in cytokine production from inflammatory cells.

Therefore, the purpose of this study was to evaluate the effects of PCSO-524™ supplementation on pulmonary and respiratory muscle function in non-asthmatic elite runners. Given that runners who undertake a period of high training volume may be susceptible to mild airway

inflammation, we hypothesized that PCSO-524™ supplementation would improve pulmonary and respiratory muscle function in elite non-asthmatic runners compared to placebo.

METHODS

Participants

Sixteen elite non-asthmatic male runners participated in the study. One additional participant enrolled in the study but dropped out due to reasons unrelated to the study (subject relocated to a different city and was no longer able to participate). Participant information is given in Table 1. In order to be enrolled in the study, subjects were required to be classified as "elite" (which we defined as a personal best competition time of < 15 min for 5 km or < 30 min for 10 km race, or equivalent for other running events) and be rated as low risk based on American College of Sports Medicine risk stratification criteria (15). Subjects were excluded if they had any history of pulmonary or cardiovascular disease, or allergies to fish, seafood, or shellfish, as assessed by questionnaire. All subjects entered the study on their normal diet and training regimen and were told to maintain both throughout the study period. Prior to each testing session, subjects were instructed to refrain from strenuous exercise and from consuming caffeine and alcohol for 6 hours prior to each testing session. All testing procedures and the informed consent statement were approved by the Indiana University Human Subjects Committee and conformed to the guidelines set out in the Declaration of Helsinki. Written informed consent was obtained before participants were enrolled in the study. Sample size was based on an *a priori* power analysis (G*Power 3.1.3, Franz Faul, Germany) of each dependent measure (22) at a power of 0.8 (d = 0.6). However, we acknowledge that the expected effect size in this healthy, highly-trained subject population could be expected to be smaller compared to the asthmatic subjects in the above-referenced study (22).

Table 1. Participant information. Data are presented as mean ± SE.

Variable	Mean ± SE
Age (y)	23.9 ± 0.6
Stature (cm)	179.6 ± 1.3
Body Mass (kg)	68.1 ± 1.1
Training History (months)	124.0 ± 10.6
5000m run Personal Best (s)	884.0 ± 8.9

Protocol

The study was conducted as a randomized, placebo-controlled, parallel group study over 12 weeks. Subjects visited the laboratory a total of 7 times, at baseline, and every two weeks thereafter throughout the study period. Following baseline testing, subjects were randomly assigned to either the treatment (PCSO-524™, Lyprinol®/Omega-XL®; n = 8) or placebo (olive oil; n = 8) group. Supplementation information is given below. Group assignment was double-blinded, randomized, and counterbalanced (by resting pulmonary and respiratory muscle function). Both groups were instructed to consume 8 of their assigned capsules per day, every day throughout the 12-week study period. Subjects were instructed to return any unconsumed pills every two weeks and compliance for all subjects was assessed by counting the unconsumed pills for each subjects and dividing by the prescribed number of pills. Figure 1 illustrates the

study timeline. Pulmonary and respiratory muscle function were measured during each visit (every 2 weeks).

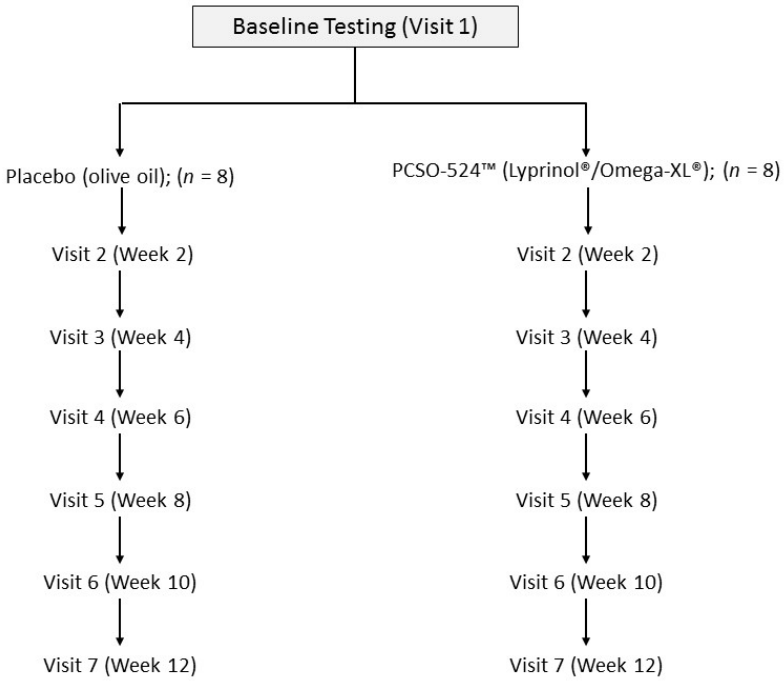

Figure 1. Study timeline. Pulmonary and respiratory muscle function was measured during each visit. Subjects were instructed to ingest 8 capsules of either placebo (olive oil) or PCSO-524™ per day throughout the 12 week supplementation period.

Supplementation: During the supplementation period, subjects ingested 8 capsules per day of the assigned supplement (PCSO-524™ or placebo) for 12 weeks. The treatment group (n = 8) received PCSO-524™ (Lyprinol®/Omega-XL®; Pharmalink International Ltd, Hong Kong), which contained 50 mg lipid extract (fatty acids), 7.3 mg (14% EPA), 5.5 mg (11% DHA), 100 mg olive oil, and 0.225 mg vitamin E (d-alpha tocopherol) per capsule. The prescribed supplementation equaled 800 mg olive oil, 400 mg lipid extract (~58 mg EPA and 44 mg DHA), and 1.8 mg vitamin E (d-alpha-tocopherol) per day for the treatment (PCSO-524™) group. The placebo group (n = 8) received capsules containing 150 mg olive oil per capsule. The prescribed supplementation equaled 1200 mg olive oil per day for the placebo group. The active capsules (PCSO-524™) were identical in size, color, texture, and taste to the placebo counterpart. The trial sponsor (Pharmalink) provided product specifications to the investigators. Analysis of the raw materials was conducted by an independent laboratory (Cawthron Laboratories, Nelson, NZ) and analysis of the final, finished PCSO-524™ product was conducted by a separate, independent laboratory (Chemisches Labor, Hannover, Germany). Placebo capsules were analyzed by Alpha laboratories (Auckland, NZ). Specific active product (PCSO-524™; Batch No. A6530-01) and placebo (Batch No. 7820) information is presented in Table 2. A full description of the composition of PCSO-524™ can be found elsewhere (21, 36).

Table 2. Fatty acid composition (%) of PCSO-524™, a patented marine oil extract of the New Zealand green-lipped mussel (*Perna canaliculus*)* and placebo (olive oil)** capsules.

Lipid Name	Common Name	PCSO-524™ (Weight, %)	Placebo (olive oil) (Weight, %)
14:0	Myristic acid	1.7	
16:0	Palmitic acid	13.4	9.2
16:1	Palmitoleic acid	3.6	3.0
18:0	Stearic acid	3.6	3.5
18:1	Oleic acid	58.2	81.0
18:2*n*-6	Linoleic aced	5.7	2.6
18:3*n*-3	Alpha-linolenic acid	0.9	0.4
18:4*n*-3	Octadecatetraenoic acid	1.0	
20:0	Arachidic acid	0.4	0.3
20:1	Eicosamonoenoic acid	0.7	
20:4*n*-6	Arachidonic acid	0.1	
20:4*n*-3	Eicosatetraenoic acid	0.3	
20:5*n*-3	Eicosapentaenoic acid	5.8	
22:5*n*-3	Docosapentaenoic acid	0.3	
22:6*n*-3	Docosaphexaenoic acid	3.0	
Others		1.3	

*Batch number: A6530-01; **Batch number: 7820. Lipid name given as: carbon chain length:number of double bonds, position of last double bond from methyl (omega) end

Pulmonary Function Testing: During each testing session, subjects completed pulmonary function (dynamic lung volumes, diffusing capacity of the lung, and closing volume) testing according to American Thoracic Society guidelines (1) as previously described in our laboratory (29). All pulmonary function testing was conducted with a calibrated metabolic cart (Vmax Encore system, CareFusion, Yorba Linda, CA, USA). In addition to these spirometry tests, subjects completed three maximal flow-volume maneuvers, which were initiated from residual lung volume (RV) after several resting tidal breaths. From RV, subjects were instructed to inspire as quickly and forcefully as possible to total lung capacity (TLC), and then expire as quickly and forcefully as possible back to RV. Forced vital capacity (FVC), forced expiratory volume in 1 s ($FEV_{1.0}$), peak expiratory flow (PEF), and forced expiratory flow from 25-75% of lung volume (FEF_{25-75}) were calculated from each maneuver. If either FVC or $FEV_{1.0}$ values varied by more than 0.15 L, additional trials were completed until three reproducible trials were achieved.

Pulmonary diffusing capacity of the lung (D_{LCO}) was assessed in duplicate using the single-breath method (25, 28) with a 5 minute washout period between measurements. Closing volume (CV) was assessed by a single-breath O_2 method as previously described (23, 27). CV measures were performed in duplicate with a 10 minute washout period between measurements.

Respiratory Muscle Function Testing: Respiratory muscle function testing (maximal inspiratory/expiratory mouth pressures and maximal voluntary ventilation) was completed during each testing session in a seated, upright position using the same calibrated metabolic cart according to published guidelines (ATS/ERS 2002). Maximal voluntary ventilation in 12-

seconds (MVV) was measured as an index of respiratory muscle endurance. Briefly, subjects were instructed to inspire and expire the highest volume of air possible during a 12-second time period following a period of normal tidal breathing. Breathing frequency and tidal volume were self-selected by subjects during the MVV test. Respiratory muscle strength was assessed by maximal inspiratory and expiratory mouth pressures (P_{Imax} and P_{Emax}, respectively). P_{Imax} and P_{Emax} tests were initiated from RV and TLC, respectively, then subjects were instructed it inspire/expire as forcefully as possible while the opening of the mouthpiece was occluded. The maneuver was sustained for a minimum of 1 s according to published guidelines (2). Subjects repeated the maneuvers until three trials within 10% of each other were observed. The highest values are reported.

Statistical Analysis

Data for all participants (n = 16) were analyzed using SAS 9.4 (SAS Institute, Cary, NC, USA) statistical software. Pulmonary and respiratory muscle function measures were compared between groups using a two-way (treatment x time), repeated-measures analysis of variance (ANOVA). Normality of data was assessed using the Shapiro-Wilk test. Sphericity of data was assessed using Mauchly's sphericity test. When sphericity was violated, the departure from sphericity (ε) was calculated. If ε was < 0.75, the Huynh-Feldt correction factor was applied and if ε was > 0.75, the Greenhouse-Geisser correction factor was applied. Where significant main effects were observed, *post-hoc* comparisons were made using Tukey's test. Statistical significance was accepted at $p < 0.05$. Data are presented as mean ± SE.

RESULTS

No significant between-subjects main effects were observed in pulmonary function (FVC, $FEV_{1.0}$, FEF_{25-75}, PEF, D_{LCO}, and CV) or respiratory muscle function (MVV, P_{Imax}, and P_{Emax}) (all $p > 0.05$). A significant within-subjects main effect was observed in P_{Emax} ($p = 0.024$) and D_{LCO} ($p < 0.0001$). No significant within-subject main effects were observed in FVC, $FEV_{1.0}$, FEF_{25-75}, PEF, MVV, P_{Imax}, and CV (all $p > 0.05$). A significant treatment by time interaction was observed in FEF_{25-75} ($p = 0.026$) and D_{LCO} ($p = 0.024$), but no other significant interactions were observed (all $p > 0.05$). Spirometry data are presented in Figure 2; respiratory muscle function, CV, and D_{LCO} data are presented in Table 3.

DISCUSSION

The main finding of this study was that supplementing with a marine oil lipid and *n*-3 PUFA blend did not improve resting pulmonary and respiratory muscle function in non-asthmatic elite runners compared to placebo. No differences were observed between groups in any pulmonary or respiratory muscle function measures, however a significant treatment by time interaction was observed in FEF_{25-75} and D_{LCO}. Therefore, non-asthmatic elite runners are unlikely to experience benefits in pulmonary and respiratory muscle function from supplementation with this marine oil extract during periods of heavy training.

Table 3. Respiratory muscle function, CV, and D_{LCO} data. TRT, treatment (PCSO-524™) group; PLA, placebo group; MVV, maximal voluntary ventilation (L min^{-1}); P_{Imax}, maximal inspiratory mouth pressure (cmH_2O); P_{Emax},

maximal expiratory mouth pressure (cmH_2O); CV, closing volume (% of forced vital capacity); D_{LCO}, pulmonary diffusion capacity (mL CO $\cdot min^{-1} \cdot mmHg^{-1}$). Data are presented as mean ± SE.

		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
					PCSO-524™ Treatment			
MVV	Mean	192.9	203.5	195.1	195	209.8	204.5	207.8
($L \cdot min^{-1}$)	(SE)	(7.6)	(13.7)	(12.3)	(9.5)	(9.9)	(10.9)	(10.8)
P_{Imax}	Mean	127.3	124.8	126.1	126.4	124.8	123.6	122.8
(cmH_2O)	(SE)	(9.0)	(9.6)	(11.5)	(10.9)	(10.5)	(10.8)	(9.0)
P_{Emax}	Mean	148.0	142.3	145.4	150.5	146.0	156.6	158.4
(cmH_2O)	(SE)	(7.8)	(5.4)	(6.9)	(5.5)	(9.3)	(9.3)	(9.6)
CV	Mean	10.5	8.9	9.9	10.1	10.0	9.6	9.6
(% FVC)	(SE)	(0.4)	(0.3)	(0.6)	(0.4)	(0.4)	(0.3)	(0.5)
D_{LCO}	Mean	38.8	40.6	39.4	38.8	38.5	37.5	36.6
($mL\ CO \cdot min^{-1} \cdot mmHg^{-1}$)	(SE)	(1.3)	(1.6)	(1.8)	(1.7)	(1.6)	(1.5)	(1.5)
					Placebo			
MVV	Mean	194.1	191.4	191.6	185.1	191.6	185.3	190.6
($L \cdot min^{-1}$)	(SE)	(6.1)	(6.6)	(7.5)	(6.2)	(8.8)	(5.4)	(5.4)
P_{Imax}	Mean	135.8	135.3	135.5	136.5	136.1	138.5	142.6
(cmH_2O)	(SE)	(14.8)	(12.5)	(12.6)	(12.6)	(12.2)	(11.8)	(12.1)
P_{Emax}	Mean	158.6	156.6	159.8	163.8	173.6	173.0	172.6
(cmH_2O)	(SE)	(15.7)	(15.7)	(12.6)	(10.2)	(11.0)	(11.4)	(12.4)
CV	Mean	10.5	9.5	9.0	9.0	9.5	9.6	9.8
(% FVC)	(SE)	(0.6)	(0.5)	(0.4)	(0.6)	(0.4)	(0.5)	(0.5)
D_{LCO}	Mean	40.9	39.3	39.1	38.5	39.5	34.7	35.7
($mL\ CO \cdot min^{-1} \cdot mmHg^{-1}$)	(SE)	(1.7)	(1.1)	(1.3)	(1.3)	(1.4)	(1.2)	(1.2)

Our significant finding included a treatment by time interaction was observed in D_{LCO} ($p = 0.024$) and FEF_{25-75} ($p = 0.026$), indicating the athletes decreased expiratory flow as well as diffusing capacity. A possible reason for decreased D_{LCO} could be pulmonary edema due to airway inflammation during a period of high training volume, as seen acutely following an endurance exercise bout (14, 23). This could occur as a negative result of high volume training, despite increased membrane diffusing capacity and thus higher DLCO found in trained populations versus controls (33). Possible reasons for decrease in FEF_{25-75} in the treatment group could be due to small airway obstruction or bronchoconstriction, although unlikely because our athletes were non-asthmatic. However, the increase in FEF_{25-75} observed in the placebo group is unexpected. It is unlikely to be a result of familiarity or a learning effect given that subjects completed the tests multiple times and were familiarized with the procedure. It is possible that external factors which modify airway caliber, such as training load or environmental exposures, could have influenced the flow rates produced by these subjects. However, these claims are merely speculative, as our data cannot give mechanistic insight into these hypotheses.

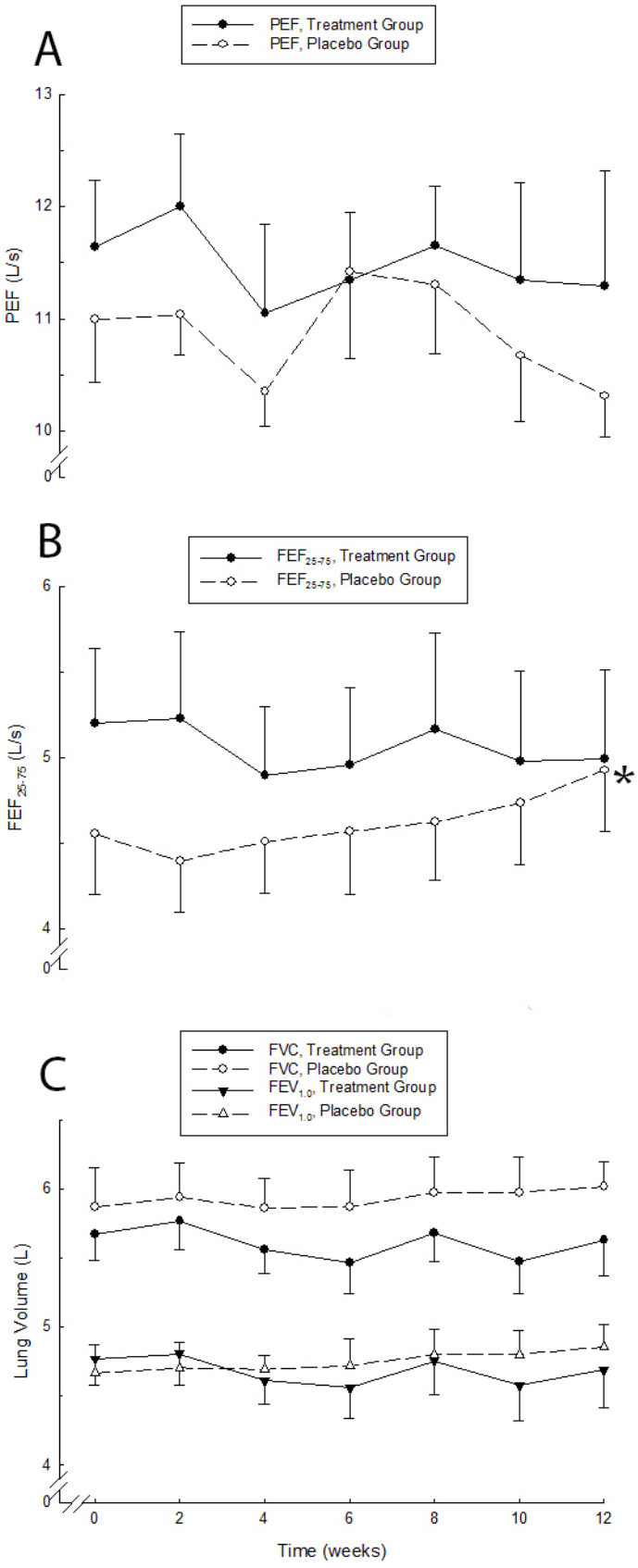

Figure 2. Spirometry data for both treatment (closed circles/triangles) and placebo (open circles/triangles) groups. No significant between-subject effects were observed (all $p > 0.05$). PEF, peak expiratry flow ($L \cdot s^{-1}$); FEF_{25-75}, forced expiratory flow from 25-75% of lung volume ($L \cdot s^{-1}$); FVC, forced vital capacity (L); $FEV_{1.0}$, forced expiratory volume in 1 s (L). *A significant treatment by time interaction was observed in FEF_{25-75} ($p = 0.026$).

Although this supplementation protocol did not improve resting lung function in elite athletes, it is unclear whether marine oil extract could improve the acute impairments in lung function that are found to occur after exercise. Even though elite athletes often have above-average resting lung function, the occurrence of a transient post-exercise narrowing of the airways, termed exercise-induced bronchoconstriction (EIB), is common after training or competition (4). Respiratory variables such as FVC, FEV_1, and D_{LCO} may be reduced, and variables such as RV and CV may be increased, after sustained running exercise, possibly due to perivascular or peribronchial edema or changes in pulmonary capillary blood flow (14, 23). We have shown that supplementation with marine oil extract mitigates these symptoms in asthmatics (22). We therefore propose that future investigations elucidate whether supplementation with this marine oil extract has a protective effect on lung function immediately post-exercise in non-asthmatic elite runners.

In addition to resting or post-exercise lung function, this supplement has been used to reduce markers of airway inflammation (cysteinyl leukotrienes, 11β-prostaglandin, Club cell proteins) in asthmatic populations (22). Similar airway inflammation may also be present during habitual endurance training in non-asthmatic athletes; however, it remains unclear whether this occurs consistently in elite runners. While acute exercise may induce short-term immune dysfunction, and intensified training may compromise immune function over a longer term, this does not represent clinical immunodeficiency in elite athletes (11). As such, Chimenti et al., (8, 9) demonstrated that neutrophil counts increased after running races, but did not find evidence of chronic airway inflammation in a longitudinal field study in healthy runners. Further, chronic endurance training has been shown to induce potent anti-inflammatory effects mediated through cytokines in tandem with downregulation of toll-like receptor expression (11). Thus, it is likely that the athletes in the present study had a high capacity to mitigate airway inflammation induced by running exercise and therefore did not experience any additional beneficial effects supplementing with the marine oil extract. Additionally, it is possible that healthy runners are not significantly affected by exposure to mild seasonal changes and airborne pollutants that cause bronchial hyper-reactivity in asthmatic populations, and therefore may not experience significant airway inflammation even during sustained exercise. We did not take any measures of inflammation or neutrophil counts, and therefore we are unable to confirm the presence or severity of airway inflammation in our subjects, and the subsequent effect of marine oil extract supplementation. Moreover, the effects of mild seasonal changes and airborne pollutants were not assessed, thus these factors could have influenced our results. Future studies should aim to confirm the presence and evaluate the severity of airway inflammation in elite runners during a period of intensified training, and the effect of marine oil extract supplementation on those symptoms and the resulting athletic performance.

In summary, we have demonstrated that there did not appear to be any positive effect of supplementation with PCSO-524™, a marine oil lipid extract derived from the New Zealand green lipped mussel (*Perna canaliculus*), on resting pulmonary and respiratory muscle function in non-asthmatic elite runners. Therefore, non-asthmatic elite runners aiming to optimize resting pulmonary function through nutraceutical supplementation are unlikely to obtain any benefits from using this marine oil extract.

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The authors declare no real or perceived conflicts of interest.

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