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**VITAMIN D AND OMEGA-3 POLYUNSATURATED FATTY ACID  
SUPPLEMENTATION IN ATHLETES WITH EXERCISE-INDUCED  
BRONCHOCONSTRICTION: A PILOT STUDY**

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## **ABSTRACT**

**Objective:** The aim of this pilot study was to determine the combined effect of vitamin D and omega-3 PUFA supplementation on airway function and inflammation in recreational athletes with exercise-induced bronchoconstriction (EIB). **Methods:** Ten recreational athletes with EIB participated in a single blind, placebo-controlled trial over six consecutive weeks. All subjects attended the laboratory on three occasions. Each visit was separated by a period of 3 weeks; visit 1 (usual diet), visit 2 (placebo) and visit 3 (SMARTFISH® NutriFriend 2000; 30µg vitamin D3 - 3000mg EPA, 3000mg DHA) consumed once daily for a period of 3-weeks. Venous blood was collected at the beginning of each trial to determine vitamin D status. Spirometry was performed pre and post eucapnic voluntary hyperpnea (EVH). **Results:** The  $\Delta FEV_{1max}$  post EVH was not different between visits (usual diet:  $-15.9 \pm 3.6\%$ ; placebo:  $-16.1 \pm 6.1\%$ ; vitamin D + omega-3 PUFA:  $-17.8 \pm 7.2\%$ ). Serum vitamin D remained unchanged between visits. **Conclusion:** Vitamin D and omega-3 PUFA supplementation does not attenuate the reduction in lung function post EVH. These findings should be viewed as preliminary until the results of randomised controlled trials are made available.

**Key words:** Airway dysfunction, Exercise-induced bronchoconstriction, Inflammation, Omega-3 polyunsaturated fatty acids, Vitamin D.

## 2 INTRODUCTION

3 Exercise-induced bronchoconstriction (EIB) describes the phenomenon of acute, transient  
4 airway narrowing in association with physical activity [1] and is highly prevalent in both  
5 recreational and elite level athletes [2,3]. Although the precise pathogenesis of EIB is not  
6 completely understood, it is generally acknowledged that exercise hyperpnea initiates  
7 bronchoconstriction by inducing osmotic changes at the distal airway surface [4]. This  
8 precipitates the release of pro-inflammatory mediators including histamine, neuropeptides,  
9 cytokines, cysteinyl leukotrienes and prostaglandins, ultimately resulting in airway smooth  
10 muscle contraction [5]. In the chronic setting, repeated, prolonged periods of exercise  
11 hyperpnea have been associated with injury-repair cycling of the airway epithelium resulting  
12 in smooth muscle remodelling [6,7] and the development of EIB in athletes [2].

13 The mainstay of treatment for EIB consists of pharmacological medication (e.g. short acting  
14 inhaled beta-2 agonists (SABA)) [1]. However, there is accumulating evidence that non-  
15 pharmacological interventions, such as dietary modification, may have utility in the treatment  
16 of EIB in athletes [8]. This is pertinent given the possible side effects of chronic beta-2  
17 agonist therapy (e.g. development of tachyphylaxis and degenerative changes in lung function)  
18 [9]. One of the most promising dietary interventions is fish oil supplementation. Specifically,  
19 omega-3 polyunsaturated fatty acids (PUFA) (eicosapentaenoic acid (EPA) and  
20 docosahexaenoic acid (DHA)) have previously been shown to attenuate airway inflammation  
21 and the bronchoconstrictor response to exercise hyperpnea [10,11]. The purported therapeutic  
22 effect of omega-3 PUFA for the treatment of EIB in athletes is biologically plausible;  
23 however the findings to date remain equivocal [10-16]. The proposed mechanism of omega-3  
24 PUFA protecting against EIB consists of EPA and DHA competitively inhibiting arachidonic  
25 acid metabolism and therefore reducing the generation of pro-inflammatory leukotrienes,  
26 prostaglandins and cytokine production from inflammatory cells [17].

27 Indeed, other dietary interventions may also be important. Recently, epidemiological studies  
28 have highlighted a direct association between vitamin D deficiency and the incidence and  
29 severity of asthma [18]. Although the evidence is sparse, low serum vitamin D levels have  
30 previously been associated with reduced lung function and increased airways hyper-reactivity  
31 to exercise in asthmatic children with EIB [19]. Mechanisms by which vitamin D may  
32 prevent EIB are likely multifactorial. The vitamin D receptor is expressed in most tissues and  
33 it has been proposed that vitamin D deficiency may result in an increase in mast cells,  
34 histamine release and apoptosis [20,21]. Furthermore, a reduction in the expression of pro-  
35 inflammatory interleukins (i.e. interleukin (IL)-13) associated with bronchoconstriction has  
36 been observed [22]. Vitamin D receptors in respiratory epithelial cells and bronchial smooth  
37 muscle have also been reported to regulate the expression of genes implicated in the  
38 pathogenesis of asthma [23] and smooth muscle proliferation (i.e. airway remodelling) [24].  
39 Consequently, as vitamin D deficiency may play a role in the pathogenesis of lung disease,  
40 supplementation may present a novel preventative and/or therapeutic strategy for athletic  
41 individuals with EIB.

42 The principal aim of this pilot study was to evaluate the combined effect of a commercially  
43 available vitamin D and omega-3 PUFA supplement (SMARTFISH® NutriFriend 2000), on  
44 airway function in recreational athletes with EIB. We hypothesised that lower levels of  
45 vitamin D would be associated with reduced lung function, and that vitamin D and omega-3  
46 PUFA supplementation would attenuate airway inflammation and bronchoconstriction  
47 following an indirect bronchoprovocation challenge. Eucapnic voluntary hyperpnea (EVH)  
48 was selected as the bronchoprovocation challenge since it is the test currently favoured by the  
49 International Olympic Committee-Medical Commission (IOC-MC) for diagnosing EIB in  
50 elite athletes [25].

51

## 52 **METHODS**

### 53 **Preliminary screening**

54 One hundred and one endurance trained recreational athletes (mean  $\pm$  SD: 6  $\pm$  1 hours  
55 training/week) were recruited and subsequently tested for EIB via a EVH challenge  
56 (described below). Sixteen athletes (17%) were positive for EIB (i.e.  $\geq$ 10% fall in FEV<sub>1</sub> post  
57 EVH) and thus considered eligible for participation.

### 58 **Study population**

59 Ten athletes (runners, cyclists and triathletes) (male:  $n = 9$ ) with EIB (63%) agreed to take  
60 part in the study. All subjects were non-smokers, free from respiratory, cardiovascular,  
61 metabolic and psychiatric disease, and any other significant medical condition except mild  
62 asthma. Four subjects had a previous physician-based diagnosis of clinical asthma and were  
63 prescribed a SABA; two of the four were also prescribed maintenance-inhaled corticosteroid.

### 64 **Experimental design**

65 The study was conducted as a single blind placebo-controlled trial over six consecutive  
66 weeks (June – September, United Kingdom). A randomised double-blind crossover design  
67 was not practical due to the half-life (~15 days) of vitamin D [26] (i.e. approximately 6-  
68 month wash-out period) and the effect of seasonal variation on airway calibre in atopic  
69 individuals [27]. All subjects were required to attend the laboratory on three occasions. Each  
70 visit was separated by a period of 3 weeks; visit 1 (usual diet), visit 2 (placebo; matching the  
71 treatment beverage for appearance, taste, quantity and packaging) and visit 3 (treatment;  
72 vitamin D + omega-3 PUFA consisting of a 600 ml fruit and berry flavoured beverage -  
73 SMARTFISH<sup>®</sup> NutriFriend 2000; 30 $\mu$ g vitamin D3 i.e. cholecalciferol, 3000mg EPA,  
74 3000mg DHA) consumed once daily for a period of 3-weeks. SMARTFISH<sup>®</sup> provided

75 documented evidence (i.e. quality assurance) of the content of both placebo and experimental  
76 beverages.

77 Subjects arrived at the laboratory 1 h postprandial at a similar ( $\pm$  1 h) time of day following  
78 their usual diet. At visit 1 an assessment of respiratory health and evaluation of allergy status  
79 was determined via completion of the Allergy Questionnaire for Athletes (AQUA) and  
80 aeroallergen skin prick testing. For all visits, venous blood was collected at the beginning of  
81 each trial to determine serum vitamin D status. Spirometry was performed pre- and post-EVH  
82 provocation. Airway inflammation was determined via fractional exhaled nitric oxide (FE<sub>NO</sub>)  
83 (indirect marker for up-regulation of airway inflammation) pre- and 30 min post-EVH. Urine  
84 samples were obtained pre- and 60 min post-EVH for cysteinyl leukotriene (LTE<sub>4</sub>) and  
85 prostaglandin (9 $\alpha$ , 11 $\beta$ - prostaglandin F<sub>2</sub>) quantification (markers of airway inflammation and  
86 mast cell activation, respectively). With the exception of AQUA and aeroallergen skin prick  
87 testing, all visits were replicated precisely on subsequent visits (Figure 1).

88 Subjects were excluded from follow-up assessment if changes in training and/or health status,  
89 respiratory tract infection, allergen or sunlight exposure were reported between visits.  
90 Subjects were asked to abstain from dietary supplements (e.g. vitamins and anti-oxidants)  
91 throughout the duration of the study and SABA and inhaled corticosteroid medication for 24  
92 and 72 h, respectively, prior to each visit. Northumbria University ethics committee approved  
93 all tests and procedures, and all subjects provided written informed consent for  
94 experimentation with human subjects.

## 95 **Atopic Status**

96 Sensitivity to seven common airborne allergens (early blossom tree, mid blossom tree, grass,  
97 weed, mould, cat and dust mite) were assessed via skin prick testing [28]. A subject was  
98 classified as atopic if, in the skin prick test, at least 1 allergen caused a wheal of at least 3 mm

99 in diameter, in the presence of a negative saline control and positive histamine. Subjects also  
100 completed AQUA to assess allergic symptoms [29]. An athlete was considered to be allergic  
101 if they presented with a positive skin prick test and a positive AQUA score  $\geq 5$ .

## 102 **Pulmonary function**

### 103 **Spirometry**

104 Lung function was assessed by forced flow-volume spirometry (MicroLoop ML3535;  
105 Cardinal Health, UK) [30].

### 106 **Eucapnic voluntary hyperpnea**

107 Bronchoprovocation challenge testing with EVH was performed as described previously  
108 [31,32]. In brief, subjects were required to inhale a mixture of dry compressed gas (21% O<sub>2</sub>,  
109 5% CO<sub>2</sub>, balance N<sub>2</sub>) at a ventilation rate equivalent to approximately 85% maximal  
110 voluntary ventilation (MVV)—calculated as  $30 \cdot FEV_1$  for a period of 6 min. Subjects viewed  
111 their ventilatory volume in real-time in order to ensure they maintained the target level. A  
112 positive diagnosis for EIB was defined by a post-EVH reduction in FEV<sub>1</sub> of  $\geq 10\%$  compared  
113 to resting spirometry.

### 114 **Airway inflammation**

115 Fraction of exhaled nitric oxide (FE<sub>NO</sub>) was the first test performed during each visit and  
116 measured using a hand-held measuring device (NIOX MINO<sup>®</sup>) (Aerocrine AB, Stockholm,  
117 Sweden). FE<sub>NO</sub> levels were obtained in accordance with international guidelines [33].

### 118 **Vitamin D status**

119 The Elecsys Total 25-hydroxyvitamin D assay (Roche Diagnostics GmbH, Germany) was  
120 used for the quantitative determination of total serum 25-hydroxyvitamin D (25(OH)D)  
121 (nmol/L) [34]. Intra-assay coefficient of variation was  $< 10\%$ . Vitamin D status was classified

122 according to previous recommendations as sufficient: 75 – 100 nmol/L; insufficient: 50-75  
123 nmol/L; deficient: < 50 nmol/L [19,35].

#### 124 **Urinary inflammatory markers**

125 Enzyme immunoassays of LTE<sub>4</sub> and 9 $\alpha$ , 11 $\beta$ - prostaglandin F<sub>2</sub> were performed in serially  
126 diluted urine (Cayman Chemical Company, Ann Arbor, MI) as previously described [36,37].  
127 Inter- and intra-assay coefficient of variation was <10%. All data were normalised and  
128 presented as nanograms of excreted mediator per millimole of creatinine. Creatinine analyses  
129 were performed using a modification of Jaffe's creatinine protocol [38].

#### 130 **Nutrient intake and compliance**

131 Subjects were instructed to maintain their usual diet (maximum of one fish meal per week)  
132 and physical activity levels throughout the duration of the study. Adherence to treatment  
133 regimens was monitored by athletes documenting the time and date of consumption and  
134 returning any supplements that were not consumed. In accordance with comparable research  
135 a compliance of  $\geq 90\%$  was considered acceptable [36].

#### 136 **Statistical analysis**

137 Normality of data was assessed using a Kolmogorov-Smirnov test and Levene's test to check  
138 for homogeneity of variance between groups. A two-way repeated measures analysis of  
139 variance (ANOVA) was used to analyse within subject effects. Mauchly's test was conducted  
140 to determine if sphericity was violated. If sphericity was violated, the repeated measures  
141 ANOVA was corrected using a Greenhouse-Geisser adjustment factor. A Bonferroni *post hoc*  
142 analysis was employed for multiple comparisons ( $P < 0.05$ ). A one way repeated measures  
143 ANOVA was employed where relevant and relationships between variables were determined  
144 via liner regression analysis (Pearson correlation coefficients). AUC<sub>0-20min</sub> was calculated by  
145 the trapezoidal method and expressed as percentage fall in FEV<sub>1</sub>. Data was analysed using

146 PASW Statistics 21 statistical software package (SPSS Inc., Version 21, Chicago, IL) and  
147 GraphPad Prism Version 5.0 (GraphPad Software, San Diego, California, USA). Data are  
148 expressed as mean ( $\pm$  SD) and significance was set at  $P < 0.05$ .

149

## 150 **RESULTS**

### 151 **Baseline characteristics, allergy and pre-challenge lung function**

152 Ten recreational athletes (male:  $n = 9$ ) completed the study. Subjects' characteristics are  
153 presented in Table 1. Eight athletes were atopic to skin prick testing and eight had a positive  
154 ( $\geq 5$ ) AQUA questionnaire. Seven athletes with a positive AQUA questionnaire were also  
155 atopic and therefore considered allergic. Five subjects reported respiratory symptoms (e.g.  
156 cough, wheeze, dyspnea etc.) in association with exercise. All pulmonary function measures  
157 were within normal predicted limits with no evidence of airflow obstruction. In addition, no  
158 difference in resting lung function was observed between visits ( $P > 0.05$ ) (Table 2).

### 159 **Compliance to treatment regimens**

160 Excellent adherence to treatment regimens was reported for placebo and vitamin D + omega-  
161 3 PUFA ( $99.5 \pm 1.1\%$  and  $98.5 \pm 3.4\%$ ) diets, respectively ( $P > 0.05$ ).

### 162 **Airway response to eucapnic voluntary hyperpnea**

163 Similar ventilation rates were achieved between all visits (usual diet:  $105 \pm 25 \text{ L}\cdot\text{min}^{-1}$ ;  
164 placebo:  $101 \pm 17 \text{ L}\cdot\text{min}^{-1}$ ; vitamin D + omega-3 PUFA:  $100 \pm 15 \text{ L}\cdot\text{min}^{-1}$ ) ( $P = 0.854$ ). All  
165 athletes maintained  $>60\%$  MVV throughout EVH thus achieving test validation [39]. The  
166  $\Delta\text{FEV}_{1\text{max}}$  post-EVH was no different between visits (usual diet:  $-15.9 \pm 3.6\%$ ; placebo:-  
167  $16.1 \pm 6.1\%$ ; vitamin D + omega-3 PUFA:  $-17.8 \pm 7.2\%$ ) ( $P = 0.719$ ). No difference was  
168 observed in the reduction in  $\text{FEV}_1$  between conditions at any time point ( $P > 0.05$ ) (Figure 2)  
169 (Table 3). Furthermore, no difference was observed for  $\text{AUC}_{0-20 \text{ min}} \% \text{ fall in FEV}_1$  between  
170 visits (usual diet:  $198.0 \pm 75.9\%$ ; placebo:  $239.7 \pm 99.4\%$ ; vitamin D + omega-3 PUFA:  
171  $256.9 \pm 135.5\%$ ) ( $P = 0.455$ ).

172 **Vitamin D status**

173 At visit one (usual diet), three athletes (30%) had sufficient levels of vitamin D, five were  
174 insufficient, and two were deficient. At visit two (placebo), two athletes were sufficient, six  
175 were insufficient and two were deficient. At visit three (vitamin D + omega-3 PUFA), three  
176 were sufficient, six were insufficient and one was deficient. No difference in serum vitamin D  
177 was observed between visits (usual diet:  $64.2 \pm 17.4$  nmol.L<sup>-1</sup>; placebo:  $65.1 \pm 16.5$  nmol.L<sup>-1</sup>;  
178 vitamin D + omega-3 PUFA:  $69.0 \pm 16.9$  nmol.L<sup>-1</sup> ( $P = 0.798$ ). In addition, change in serum  
179 vitamin D status between visits did not correlate with  $\Delta$ FEV<sub>1</sub>max ( $r = 0.11$ ;  $P = 0.559$ ).

180 **Airway inflammation**

181 No difference in FE<sub>NO</sub> was observed pre-EVH between visits (usual diet:  $28 \pm 16$ ppb;  
182 placebo:  $31 \pm 23$ ppb; vitamin D + omega-3 PUFA:  $37 \pm 27$ ppb) ( $P = 0.182$ ) or post-EVH  
183 between visits (usual diet:  $27 \pm 19$ ppb; placebo:  $25 \pm 19$ ppb; vitamin D + omega-3 PUFA:  $28$   
184  $\pm 18$ ppb) ( $P = 0.834$ ). However, a reduction in FE<sub>NO</sub> post-EVH was observed within  
185 condition for placebo (-20.1%) and vitamin D + omega-3 PUFA (-28.9%), respectively  
186 ( $P < 0.05$ ) (Figure 3).

187 **Urinary inflammatory markers**

188 ***Cysteinyl leukotriene LTE<sub>4</sub>***

189 LTE<sub>4</sub> was higher pre-EVH following vitamin D + omega-3 PUFA:  $104.1 \pm 26.7$  ng/mmol  
190 creatinine compared to both usual diet:  $72.6 \pm 16.6$  ng/mmol creatinine and placebo:  $72.6 \pm$   
191  $22.9$  ng/mmol creatinine ( $P < 0.05$ ). No difference was observed between usual diet and  
192 placebo ( $P > 0.05$ ). LTE<sub>4</sub> was higher post-EVH following vitamin D + omega-3 PUFA:  $99.1 \pm$   
193  $29.2$  ng/mmol creatinine compared to placebo:  $61.0 \pm 13.7$  ng/mmol creatinine ( $P = 0.007$ ).  
194 No difference was observed between usual diet and placebo or usual diet and vitamin D +

195 omega-3 PUFA respectively ( $P>0.05$ ) (Figure 4). LTE<sub>4</sub> did not correlate with  $\Delta$ FEV<sub>1</sub>max ( $r =$   
196  $0.30$ ;  $P = 0.107$ ).

197 ***9 $\alpha$ , 11 $\beta$ - prostaglandin F<sub>2</sub>***

198 No difference in 9 $\alpha$ , 11 $\beta$ - prostaglandin F<sub>2</sub> was observed pre-EVH between visits (usual diet:  
199  $88.9 \pm 59.1$  ng/mmol creatinine; placebo:  $82.8 \pm 37.6$  ng/mmol creatinine; vitamin D +  
200 omega-3 PUFA:  $79.2 \pm 43.7$  ng/mmol creatinine) or post-EVH between visits (usual diet:  
201 (usual diet:  $104.0 \pm 41.7$  ng/mmol creatinine; placebo:  $101.1 \pm 56.8$  ng/mmol creatinine;  
202 vitamin D + omega-3 PUFA:  $90.3 \pm 48.0$  ng/mmol creatinine) ( $P>0.05$ ) (Figure 4). A  
203 correlation was observed between 9 $\alpha$ , 11 $\beta$ - prostaglandin F<sub>2</sub> post-EVH and  $\Delta$ FEV<sub>1</sub>max ( $r =$   
204  $0.45$ ;  $P = 0.017$ ).

205

206 **DISCUSSION**

207 This study has shown, contrary to our hypothesis, that the combination of vitamin D and  
208 omega-3 PUFA supplementation over a 3-week period does not reduce markers of airway  
209 inflammation or attenuate the reduction in lung function post EVH in recreational athletes  
210 with EIB. Furthermore, serum vitamin D status does not appear to correspond directly to the  
211 severity of bronchoconstriction following indirect bronchoprovocation. The study design and  
212 intervention of the present study was based on the premise that dietary modification with a  
213 commercially available self-administrated supplement would be pragmatic and overall  
214 applicable to ‘real-life’.

215 Vitamin D deficiency (serum 25-hydroxyvitamin D <50 nmol.L<sup>-1</sup>) has previously been  
216 associated with a reduction in lung function and increased reactivity to exercise in asthmatic  
217 children with EIB [19]. However, the precise role of vitamin D in the pathogenesis of EIB  
218 has yet to be determined. In the current study 20% (2/10) of athletes presented with vitamin  
219 D deficiency following their usual diet. This is in contrast to previous findings where 51%  
220 (23/45) of asthmatic children with EIB were vitamin D deficient [19]. The dissociation  
221 between studies is somewhat surprising, however supports the notion that physical activity is  
222 directly related to the level of sun light exposure [40]. However, it is important to  
223 acknowledge that the comparison of prevalence estimates of vitamin D deficiency between  
224 studies may be confounded by the population studied (i.e. adults versus children). In addition,  
225 as the current study was conducted in the summer months (June – September, United  
226 Kingdom), this may, in part, explain the limited number of athletes presenting with vitamin D  
227 deficiency. However, it must be acknowledged that the long half-life of vitamin D [26]  
228 combined with controlling environmental factors (e.g. sunlight exposure and diet) limits the  
229 standardisation of vitamin D trials *in vivo* (i.e. human studies). Nevertheless, further work is

230 required to fully determine the extent of vitamin D deficiency and thus requirement of  
231 supplementation in athletic individuals.

232 In the present study adherence to the treatment regimens was high, however no difference  
233 was observed in serum vitamin D following supplementation. Previous epidemiological  
234 studies have highlighted a positive correlation between lung function and serum vitamin D  
235 levels [19,41], whereas others have shown no association [42]. However, observational  
236 studies do not confirm causality. Our findings show a poor relationship between vitamin D  
237 status and severity of bronchoconstriction, thus disputing a direct association. These findings  
238 are supported by a recent comparable study demonstrating no effect of vitamin D  
239 supplementation in children with mild asthma [43]. However, a general consensus regarding  
240 the optimal vitamin D dose has yet to be established (see recent review by Owens et al. [44]).  
241 It is therefore reasonable to speculate that the dose employed within the current study (30  
242  $\mu\text{g}/\text{day}$ ) or indeed length of supplementation was not sufficient to elicit a therapeutic effect.  
243 Thus, the optimal level of vitamin D supplementation remains elusive and clinical trials are  
244 required before informed recommendations can be employed.

245 Mickleborough et al. [10,11] previously reported that omega-3 PUFA (3.2g/day EPA and  
246 2.2g/day DHA) derived from fish oil results in a reduction in markers of airway inflammation  
247 (e.g.  $\text{LTE}_4$  and  $9\alpha, 11\beta$ - prostaglandin  $\text{F}_2$ ) and an attenuated bronchoconstrictor response  
248 following exercise in EIB and asthmatic patients, respectively. More recently, similar  
249 findings have been reported by the same group following EVH bronchoprovocation [12,36].  
250 Although Arms et al. [16] also observed a 50% inhibition of total leukotriene count in  
251 peripheral blood in mild asthmatics following 10 weeks of daily fish oil supplementation  
252 (3.2g EPA and 2.2g DHA), in agreement with our findings no change was observed in  
253  $\Delta\text{FEV}_{1\text{max}}$  post indirect bronchoprovocation. In further support of this concept, Brannan et  
254 al. [15] recently found that a 3-week period of omega-3 supplementation (4.0g/day EPA and

255 2.0g/day DHA) does not improve bronchial hyper-responsiveness to mannitol or inhibit  
256 urinary excretion of mast cell mediators in adults with mild-moderate asthma.

257 This observation is comparable with findings from the present study where no difference was  
258 observed in urinary  $9\alpha$ ,  $11\beta$ - prostaglandin  $F_2$  between visits. Although urinary  $LTE_4$   
259 increased pre and post EVH following vitamin D + omega-3 PUFA, the majority of athletes  
260 within our cohort were atopic (80%) and allergic (70%), and thus any potential anti-  
261 inflammatory effect of vitamin D and omega-3 PUFA may have been counteracted by the  
262 variation in allergen exposure (e.g. pollen count, house dust mite etc.) between visits [27]. In  
263 keeping with our findings however, Moreira et al. [45] observed no difference in  $FE_{NO}$   
264 following short-term dietary supplementation with omega-3 PUFA in woman with stable  
265 asthma.

266 Our finding of a correlation between  $\Delta FEV_{1max}$  and urinary excretion of  $9\alpha$ ,  $11\beta$ -  
267 prostaglandin  $F_2$  ( $P < 0.05$ ) further supports the role of mast cells in EIB [37]. Although the  
268 urine sampling time-points post challenge were not identical, similar to Kippelen et al. [37]  
269 no association existed between  $\Delta FEV_{1max}$  and urinary excretion of  $LTE_4$ . This observation  
270 could suggest that  $9\alpha$ ,  $11\beta$ - prostaglandin  $F_2$  is a more sensitive marker of EIB in atopic  
271 individuals than  $LTE_4$ , which warrants further investigation.

272 Although Mickleborough and Rundell [17] have highlighted statistical limitations to explain  
273 the inconsistency in results between studies [17], the majority of trials have consisted of a  
274 comparable sample size to the present study [10,11,16]. However, it should be acknowledged  
275 that the diagnostic methodology used to quantify the extent of bronchoconstriction often  
276 varies between studies [10-12,15]. Furthermore, it has previously been shown that a poor  
277 relationship exists between indirect bronchoprovocation challenges (i.e. exercise and EVH)  
278 [46,47]. It is therefore possible that the purported therapeutic effect of treatment varies  
279 according to the specific bronchoprovocation challenge employed.

280 Nonetheless, the disparities in findings are still somewhat surprising given the similarities in  
281 study design, population, sample size and similar dose of the respective interventions  
282 [10,11,16]. Whilst the form of vitamin D and omega-3 PUFA administration in the present  
283 study differed from previous research, there is currently no consensus in the literature to  
284 suggest that the absorption or indeed effect of supplementation significantly varies according  
285 to the form of consumption (i.e. encapsulated supplement versus commercially available  
286 nutritional beverage). However, it should be acknowledged that in contrast to previous work  
287 [6,10,14,19,40,41] equal quantities of EPA and DHA (3.0g/day) were employed in the  
288 current study. It is therefore possible that EPA may be more important than DHA in  
289 attenuating EIB. This theory is consistent with a previous pilot study by Head et al. [13]  
290 where supplementation with 4.0g/day of DHA did not attenuate bronchoconstriction or  
291 airway inflammation in asthmatic patients following EVH. Moreover, a recent mouse model  
292 of asthma observed pro-inflammatory effects following the consumption of DHA over a six  
293 week period [48].

294 Overall however, the results of the present study support the current recommendation by the  
295 American Thoracic Society that the evidence is not currently strong enough to confirm that  
296 omega-3 PUFA's are effective in the large majority of patients with EIB [1].

297 Pertinent to the present study and previous research [10-12,16,36], poor short-term test re-test  
298 clinical reproducibility of indirect bronchoprovocation (i.e. exercise and EVH) [49,50] has  
299 recently been observed in patients with mild EIB. Therefore, although the combination of  
300 vitamin D and omega-3 PUFA does not appear to attenuate the  $\Delta FEV_{1max}$  post  
301 bronchoprovocation, the inherent variability of a test employed to determine changes in lung  
302 function should be considered when advocating the efficacy of a treatment intervention to  
303 avoid masking or overestimating the proposed therapeutic benefit. Likewise, the use of  $FE_{NO}$

304 as a marker of airway inflammation may be confounded given the high ventilatory demand of  
305 EVH (i.e. exhaled nitric oxide often falls from baseline values even when EIB is confirmed).

### 306 **Methodological considerations / future research**

307 Although this study is the first interventional trial to address the impact of combining vitamin  
308 D and omega-3 PUFA supplementation in athletic individuals with EIB, there are a number  
309 of important considerations. Firstly, given the small sample size of the cohort, the results  
310 should be viewed with some caution. Whilst we are confident that false negative results (i.e.  
311 type II error) have not been reported, further work with a larger sample size is still required to  
312 provide a definitive answer. Secondly, the optimal level of vitamin D supplementation  
313 remains elusive and clinical trials are required before informed recommendations can be  
314 employed. Once established, randomised controlled trials are required to determine the  
315 individual and combined efficacy of vitamin D and omega-3 PUFA for the treatment of EIB  
316 in athletes. Whilst highly speculative, the possibility exists that the lipophilic properties of  
317 vitamin D may compete with omega-3 PUFA by an unknown mechanism. Thirdly, to  
318 understand the mechanism of action of specific interventions, future studies should assess  
319 nutritional deficiencies (i.e. vitamin D and omega-3 PUFA status) prior to study entry and  
320 recruit homogenous cohorts of athletes according to severity of disease and specific clinical  
321 phenotypes (e.g. asthma, EIB, airway hyper-responsiveness, atopy etc.) rather than ‘pooling’  
322 heterogeneous cohorts. Finally, the longitudinal impact of vitamin D and/or omega-3 PUFA  
323 supplementation has yet to be established. Conducting randomised double-blind crossover  
324 design studies (acknowledging the limitations of vitamin D washout) may provide value in  
325 this setting.

326 **Conclusion**

327 In conclusion, this pilot study has shown that a 3-week period of vitamin D and omega-3  
328 PUFA supplementation does not reduce markers of airway inflammation nor attenuate the  
329 reduction in lung function post EVH. In addition, vitamin D status does not appear to  
330 correspond directly to the severity of bronchoconstriction in recreational athletes with EIB.  
331 However, these findings should be viewed as preliminary until the results of randomised  
332 controlled trials are made available.

333

334 **KEY ISSUES**

335 • Vitamin D deficiency has previously been associated with the development and  
336 severity of asthma, with low serum vitamin D levels associated with reduced lung  
337 function and increased reactivity to exercise in children with EIB.

338

339 • Omega-3 PUFA supplementation has been shown to attenuate airway inflammation  
340 and bronchoconstriction following indirect bronchoprovocation.

341

342 • The aim of this pilot study was to determine the combined effect of acute vitamin D  
343 and omega-3 PUFA supplementation on airway function in recreational athletes with  
344 EIB.

345

346 • The combination of vitamin D and omega-3 PUFA supplementation does not reduce  
347 markers of airway inflammation nor attenuate the reduction in lung function  
348 following EVH.

349

350 • Serum vitamin D status does not appear to directly correspond to the severity of  
351 bronchoconstriction.

352

353 • The inherent variability of a test (i.e. indirect bronchoprovocation) employed to  
354 determine changes in lung function should be considered when advocating the  
355 efficacy of a treatment intervention to avoid masking or overestimating the proposed  
356 therapeutic benefit.

357

358 • Further work is required to determine the individual and combined effect of omega-3  
359 PUFA and vitamin D as a non-pharmacological treatment for EIB. The findings of the  
360 present study should be viewed as preliminary until the results of randomised  
361 controlled trials are made available.

362

363

364

365 **TABLE HEADINGS**

366 **Table 1:** Subject clinical characteristics.

367

368 **Definitions of abbreviations: BMI**, body mass index.

369

370 **Table 2:** Baseline pulmonary function.

371

372 **Definitions of abbreviations: FEV<sub>1</sub>**, forced expiratory volume in 1<sup>s</sup>; **FVC**, forced vital  
373 capacity; **PEF**, peak flow rate.

374

375 **Table 3:** Baseline lung function and response to eucapnic voluntary hyperpnea.

376 **Definitions of abbreviations: FEV<sub>1</sub>**, forced expiratory volume in 1<sup>s</sup>

377 **Table 1.**

<b>Subject</b>	<b>Sex (M:F)</b>	<b>Age (years)</b>	<b>Height (cm)</b>	<b>Weight (kg)</b>	<b>BMI (kg•m<sup>-2</sup>)</b>	<b>Training (hrs•wk<sup>-1</sup>)</b>	<b>Physician diagnosed asthma</b>	<b>Medication</b>	<b>Self-report symptoms</b>	<b>Allergy</b>
1	M	42	177.7	90.3	28.6	6	No	Nil	Asymptomatic	No
2	M	27	185.6	87.4	25.4	6	No	Nil	Asymptomatic	No
3	M	36	178.5	72.5	22.8	6	No	Nil	Asymptomatic	Yes
4	M	28	181.3	79.4	24.2	6	No	Nil	Asymptomatic	Yes
5	M	48	173.7	75.6	25.1	6	No	Nil	Asymptomatic	Yes
6	M	28	177.0	78.8	25.2	6	Yes	SABA + ICS	Symptomatic	Yes
7	F	42	166.6	64.2	23.1	6	Yes	SABA	Symptomatic	No
8	M	39	177.9	88.7	28.0	6	Yes	SABA + ICS	Symptomatic	Yes
9	M	34	181.1	72.7	22.2	6	Yes	SABA + ICS	Symptomatic	Yes
10	M	24	183.3	84.5	25.1	4.5	No	Nil	Symptomatic	Yes
<b>Total</b>	9:1	35 ± 8	178.3 ± 5.5	79.4 ± 8.4	25.0 ± 2.1	6 ± 1	4/10	4/10	5/10	7/10

378 **Table 2.**

<b>Baseline pulmonary function</b>			
	<b>Visit 1</b>	<b>Visit 2</b>	<b>Visit 3</b>
	Usual diet	Placebo	Vitamin D + Omega-3
FEV <sub>1</sub> (L)	4.04 ± 0.85	4.12 ± 0.77	4.00 ± 0.80
FEV <sub>1</sub> (% predicted)	96.5 ± 15.4	98.4 ± 12.0	95.4 ± 12.2
FVC (L)	5.61 ± 0.81	5.69 ± 0.78	5.61 ± 0.86
FVC (% predicted)	111.6 ± 10.7	113.1 ± 9.5	111.2 ± 10.4
FEV <sub>1</sub> /FVC (%)	71.4 ± 5.4	71.9 ± 4.2	71.0 ± 4.7
PEF (L/min)	552.4 ± 103.3	569.5 ± 85.6	556.1 ± 107.5
PEF (% predicted)	97.7 ± 13.7	100.6 ± 7.9	97.9 ± 11.5

379 Data presented as Mean ± SD. *n* =10.

**Table 3.**

<b>Subject</b>	<b>Baseline</b>	<b><math>\Delta</math>FEV<sub>1</sub>max</b>		
	Visit 1: FEV <sub>1</sub> (% predicted)	Visit 1: Usual diet	Visit 2: Placebo	Visit 3: Vitamin D + Omega-3 PUFA
1	87.0	-19.6	-12.5	-17.5
2	104.9	-17.2	-20.8	-20.5
3	102.6	-11.5	-20.1	-16.5
4	95.2	-12.9	-13.2	-14.7
5	89.8	-12.1	-12.1	-7.5
6	130.0	-13.6	-9.0	-12.0
7	80.2	-14.4	-17.6	-14.7
8	95.8	-16.8	-9.4	-25.1
9	104.4	-18.2	-16.9	-16.1
10	75.4	-22.6	-28.9	-33.4
<b>Mean <math>\pm</math> SD</b>	96.5 $\pm$ 15.4	-15.9 $\pm$ 3.6	-16.1 $\pm$ 6.1	-17.8 $\pm$ 7.2

381 **FIGURE LEGENDS**

382 **Figure 1.** Schematic depicting the experimental design.

383 **Definitions of abbreviations:** AQUA, The Allergy Questionnaire for Athletes; **EIB**,  
384 exercise-induced bronchoconstriction; **FEV<sub>1</sub>**, forced expiratory volume in 1<sup>-s</sup>; **EVH**;  
385 Eucapnic voluntary hyperpnea; **FE<sub>NO</sub>**, fractional exhaled nitric oxide.

386

387 **Figure 2.** Percentage change in FEV<sub>1</sub> post EVH between visits. Usual diet (*open circles*);  
388 placebo (*closed circles*); vitamin D + omega-3 PUFA (*closed triangles*). Broken horizontal  
389 line represents abnormal lung function (i.e.  $\geq 10\%$  fall in FEV<sub>1</sub>). Placebo SD error lines  
390 omitted to improve clarity of graph.

391

392 **Figure 3.** Fractional exhaled nitric oxide (FE<sub>NO</sub>) concentration (ppb) pre-EVH (*closed bar*)  
393 and 30 min post-EVH (*open bar*) between visits. \* denotes significant difference within  
394 condition between pre- and post-EVH ( $P < 0.05$ )

395

396 **Figure 4.** Panel a). Urinary LTE<sub>4</sub> concentration pre EVH (*closed bar*) and 60 min post EVH  
397 (*open bar*) between visits. Panel b). Urinary 9 $\alpha$ , 11 $\beta$ - prostaglandin F<sub>2</sub> pre EVH (*closed bar*)  
398 and 60 min post EVH (*open bar*) between visits. \* denotes significant difference pre-EVH  
399 between condition ( $P < 0.05$ ). # denotes significant difference post-EVH between condition  
400 ( $P < 0.05$ ).

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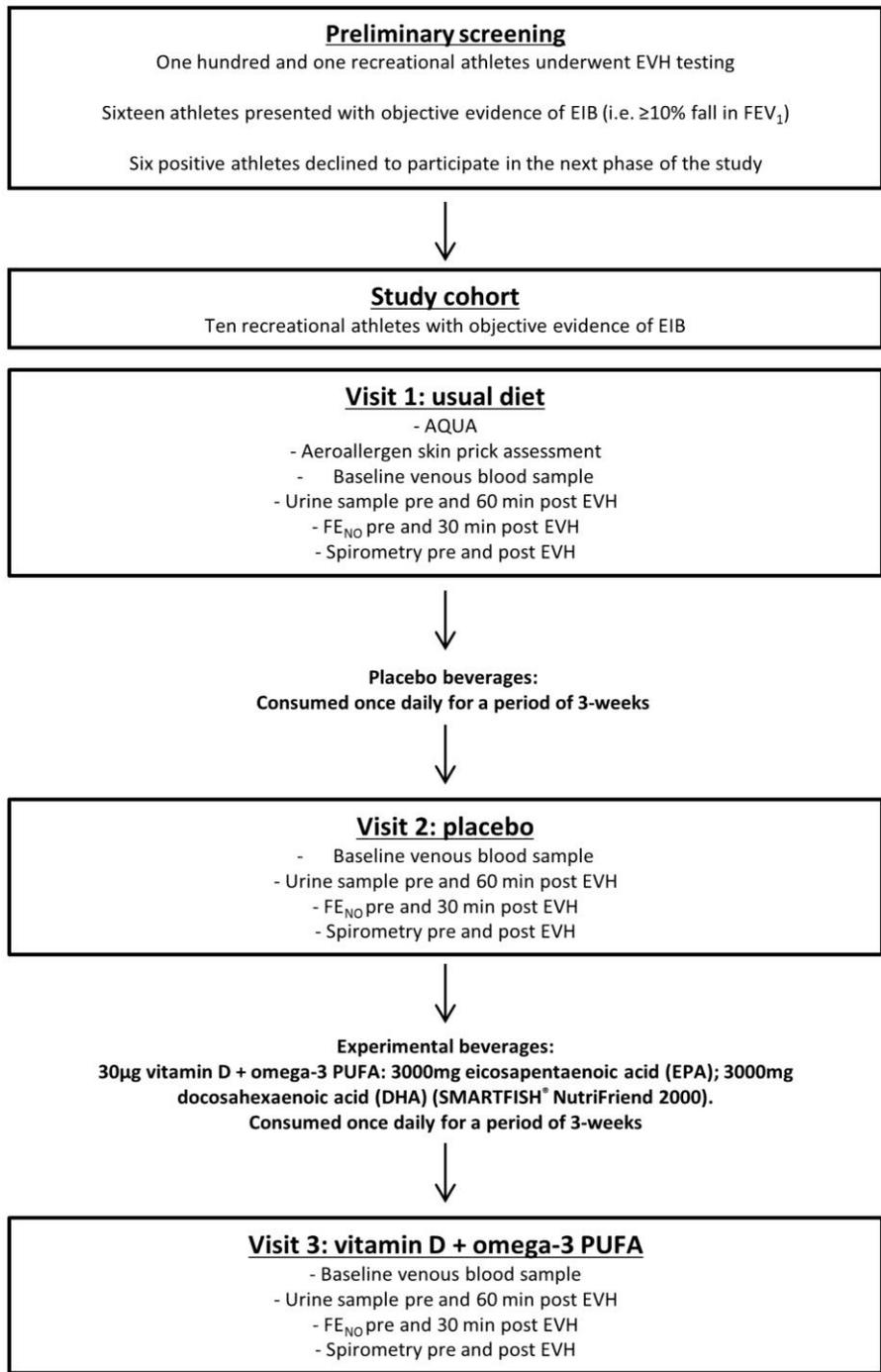
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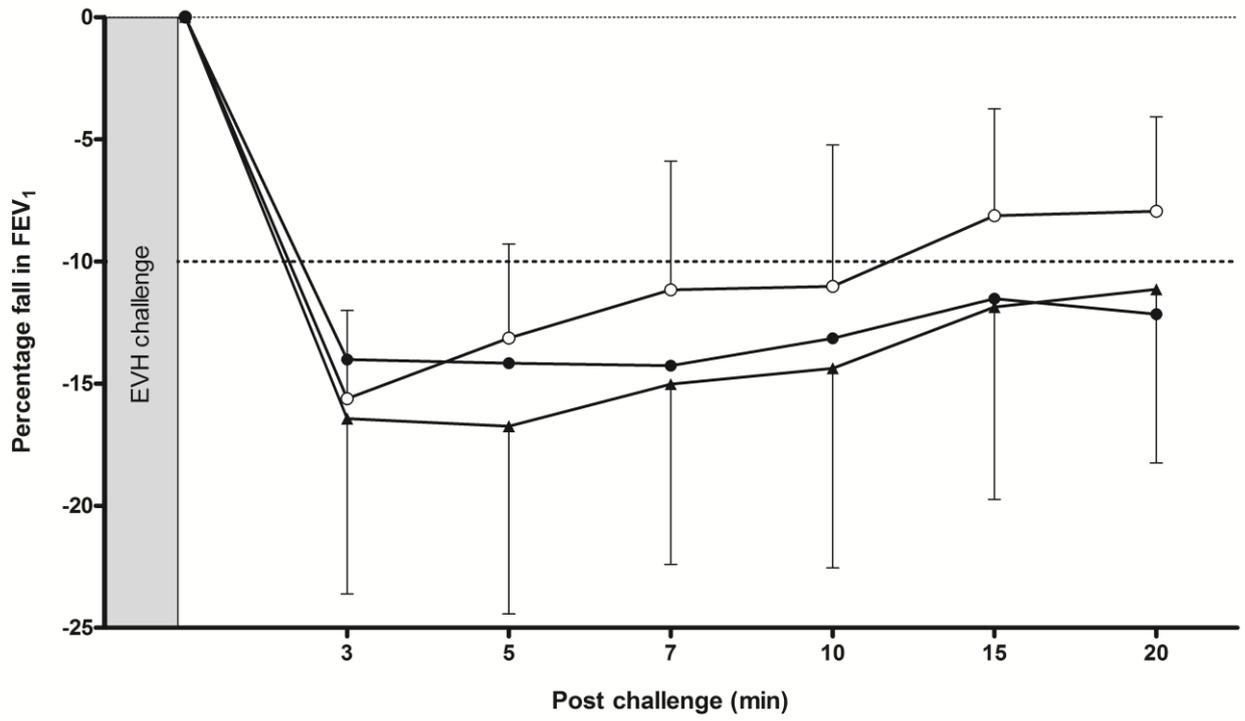
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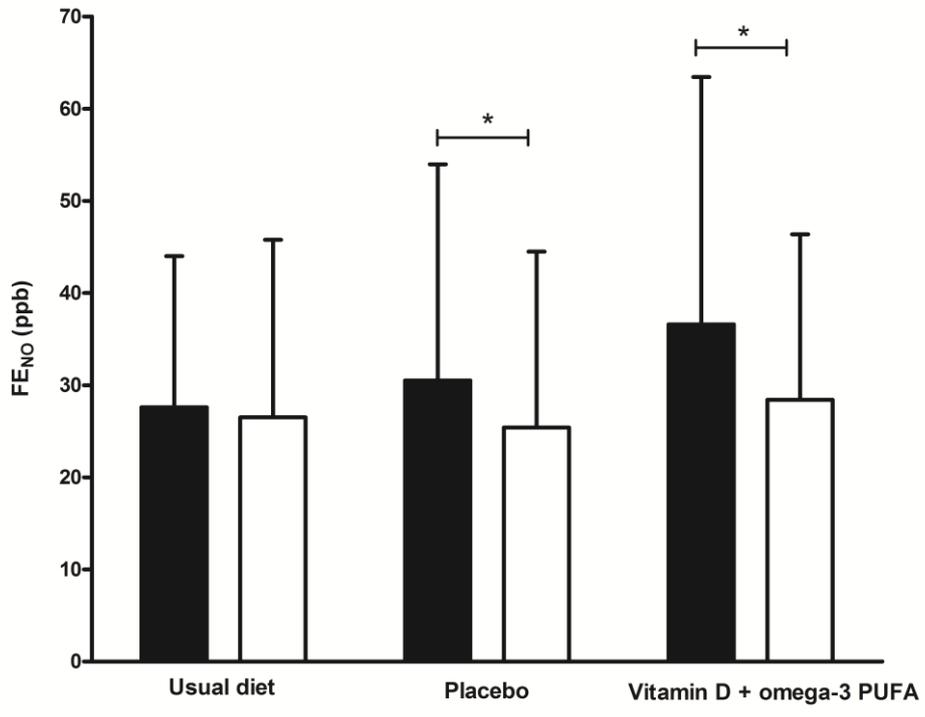
415 **Figure 1.**



416

417 **Figure 2.**

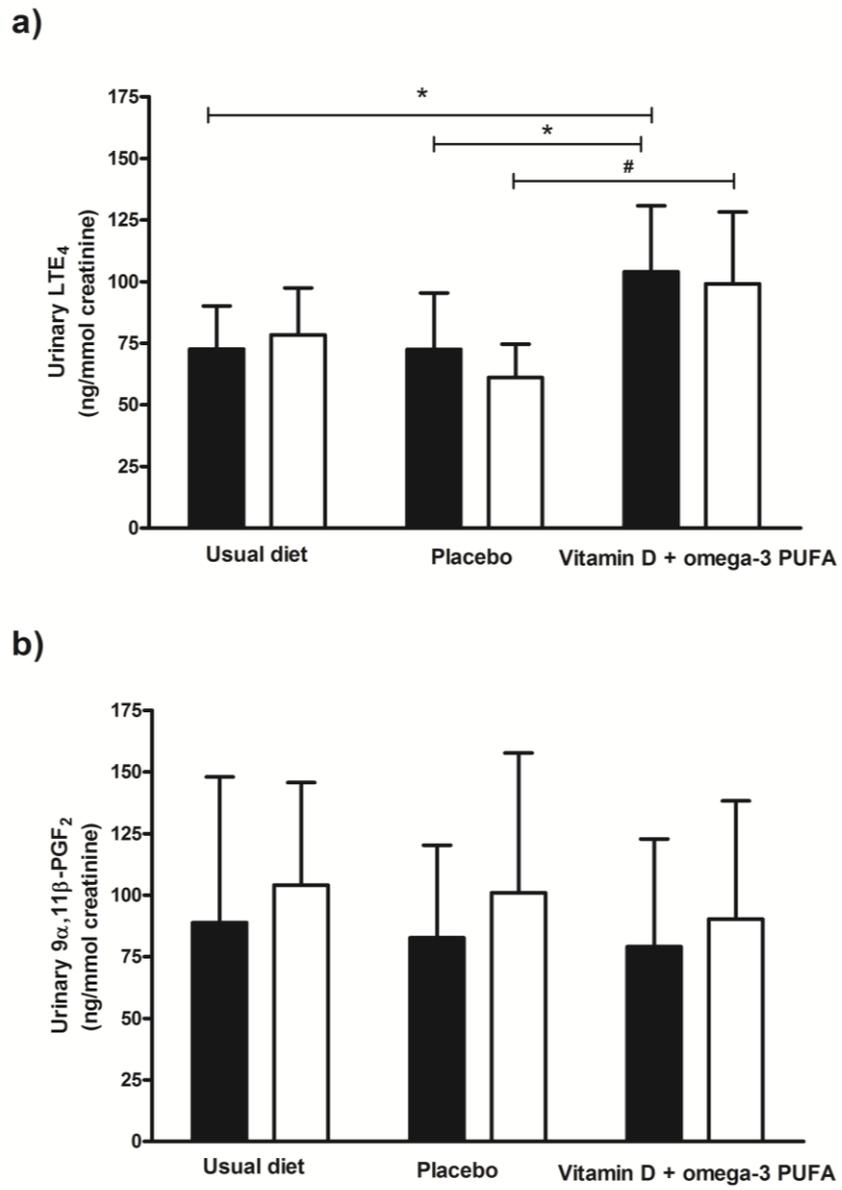
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420

421 **Figure 3.**

422



424

425 **Figure 4.**

426

427 **REFERENCES**

- 428 1. Parsons JP, Hallstrand TS, Mastrorade JG *et al.* An official American Thoracic  
429 Society clinical practice guideline: exercise-induced bronchoconstriction. *Am J Respir*  
430 *Crit Care Med*, 187(9), 1016-1027 (2013).  
431
- 432 2. Price OJ, Ansley L, Menzies-Gow A, Cullinan P, Hull JH. Airway dysfunction in elite  
433 athletes—an occupational lung disease? *Allergy*, 68(11), 1343-1352 (2013).  
434
- 435 3. Molphy J, Dickinson J, Hu J, Chester N, Whyte G. Prevalence of bronchoconstriction  
436 induced by eucapnic voluntary hyperpnoea in recreationally active individuals.  
437 *Journal of Asthma*, 51(1), 45-50 (2014).  
438
- 439 4. Kippelen P, Anderson SD. Pathogenesis of Exercise-Induced Bronchoconstriction.  
440 *Immunology and allergy clinics of North America*, 33(3), 299-312 (2013).  
441
- 442 5. Hallstrand TS, Altemeier WA, Aitken ML, Henderson WR. Role of Cells and  
443 Mediators in Exercise-Induced Bronchoconstriction. *Immunology And Allergy Clinics*  
444 *of North America*, 33(3), 313-328 (2013).  
445
- 446 6. Karjalainen E, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, Laitinen LA. Evidence  
447 of airway inflammation and remodeling in ski athletes with and without bronchial  
448 hyperresponsiveness to methacholine. *Am J Respir Crit Care Med*, 161(6), 2086  
449 (2000).  
450
- 451 7. Davis MS, Schofield B, Freed AN. Repeated peripheral airway hyperpnea causes  
452 inflammation and remodeling in dogs. *Medicine & Science in Sports & Exercise*,  
453 35(4), 608-616 (2003).  
454
- 455 8. Mickleborough TD. A nutritional approach to managing exercise-induced asthma.  
456 *Exercise and sport sciences reviews*, 36(3), 135-144 (2008).  
457
- 458 9. Wraight JM, Smith AD, Cowan JO, Flannery EM, Herbison GP, Taylor DR. Adverse  
459 effects of short-acting beta-agonists: Potential impact when anti-inflammatory therapy  
460 is inadequate. *Respirology*, 9(2), 215-221 (2004).  
461
- 462 10. Mickleborough TD, Lindley MR, Ionescu AA, Fly AD. Protective effect of fish oil  
463 supplementation on exercise-induced bronchoconstriction in asthma. *CHEST Journal*,  
464 129(1), 39-49 (2006).  
465
- 466 11. Mickleborough TD, Murray RL, Ionescu AA, Lindley MR. Fish oil supplementation  
467 reduces severity of exercise-induced bronchoconstriction in elite athletes. *Am J Respir*  
468 *Crit Care Med*, 168(10), 1181-1189 (2003).  
469
- 470 12. Mickleborough TD, Vaughn CL, Shei R-J, Davis EM, Wilhite DP. Marine lipid  
471 fraction PCSO-524™(lyprinol<sup>®</sup>/omega XL<sup>®</sup>) of the New  
472 Zealand green lipped mussel attenuates hyperpnea-induced bronchoconstriction in  
473 asthma. *Respir Med*, 107(8), 1152-1163 (2013).  
474

- 475 13. Head S, Mickleborough T. Randomized Cross-Over Controlled Pilot Study of  
476 Docosahexaenoic Acid Supplementation on Airway Inflammation and Hyperpnea-  
477 Induced Bronchoconstriction in Adults with Asthma. *The Internet Journal of Asthma,*  
478 *Allergy and Immunology*, 9(1) (2013).  
479
- 480 14. Williams N, Hunter K, Johnson M, Sharpe G. A randomised placebo controlled trial  
481 to compare the effects of two dosages of omega-3 PUFA on exercise-induced  
482 bronchoconstriction (EIB). *Br J Sports Med*, 47(17), e4-e4 (2013).  
483
- 484 15. Brannan JD, Bood J, Alkhabaz A *et al.* The effect of omega-3 fatty acids on bronchial  
485 hyperresponsiveness, sputum eosinophilia and mast cell mediators in asthma. *CHEST*  
486 *Journal*, (2014).  
487
- 488 16. Arm J, Horton C, Mencia-Huerta J *et al.* Effect of dietary supplementation with fish  
489 oil lipids on mild asthma. *Thorax*, 43(2), 84-92 (1988).  
490
- 491 17. Mickleborough T, Rundell K. Dietary polyunsaturated fatty acids in asthma-and  
492 exercise-induced bronchoconstriction. *European journal of clinical nutrition*, 59(12),  
493 1335-1346 (2005).  
494
- 495 18. Foong RE, Zosky GR. Vitamin D deficiency and the lung: disease initiator or disease  
496 modifier? *Nutrients*, 5(8), 2880-2900 (2013).  
497
- 498 19. Chinellato I, Piazza M, Sandri M *et al.* Serum vitamin D levels and exercise-induced  
499 bronchoconstriction in children with asthma. *Eur Respir J*, 37(6), 1366-1370 (2011).  
500
- 501 20. Toyota N, Sakai H, Takahashi H, Hashimoto Y, Iizuka H. Inhibitory effect of 1 $\alpha$ , 25-  
502 dihydroxyvitamin D3 on mast cell proliferation and A23187-induced histamine  
503 release, also accompanied by a decreased c-kit receptor. *Archives of dermatological*  
504 *research*, 288(11), 709-715 (1996).  
505
- 506 21. Baroni E, Biffi M, Benigni F *et al.* VDR-dependent regulation of mast cell maturation  
507 mediated by 1, 25-dihydroxyvitamin D3. *Journal of leukocyte biology*, 81(1), 250-262  
508 (2007).  
509
- 510 22. Benigni F, Baroni E, Zecevic M *et al.* Oral treatment with a vitamin D3 analogue  
511 (BXL628) has anti-inflammatory effects in rodent model of interstitial cystitis. *BJU*  
512 *international*, 97(3), 617-624 (2006).  
513
- 514 23. Bosse Y, Maghni K, Hudson TJ. 1 $\alpha$ ,25-dihydroxy-vitamin D3 stimulation of  
515 bronchial smooth muscle cells induces autocrine, contractility, and remodeling  
516 processes. *Physiol Genomics*, 29(2), 161-168 (2007).  
517
- 518 24. Berraies A, Hamzaoui K, Hamzaoui A. Link between vitamin D and airway  
519 remodeling. *Journal of asthma and allergy*, 7, 23-30 (2014).  
520
- 521 25. International Olympic Committee - Medical Commission. Beta2 adrenoceptor agonists  
522 and the Olympic Games in Beijing. Available at:  
523 [http://www.olympic.org/Documents/Reports/EN/en\\_report\\_1302.pdf](http://www.olympic.org/Documents/Reports/EN/en_report_1302.pdf) (accessed 14  
524 Aug 2014) (2008).

- 525 26. Jones G. Pharmacokinetics of vitamin D toxicity. *The American journal of clinical*  
526 *nutrition*, 88(2), 582S-586S (2008).  
527
- 528 27. Choi IS, Ki W-J, Kim T-O, Han E-R, Seo I-K. Seasonal factors influencing exercise-  
529 induced asthma. *Allergy, asthma & immunology research*, 4(4), 192-198 (2012).  
530
- 531 28. Bousquet J, Heinzerling L, Bachert C *et al.* Practical guide to skin prick tests in  
532 allergy to aeroallergens. *Allergy*, 67(1), 18-24 (2012).  
533
- 534 29. Bonini M, Braido F, Baiardini I *et al.* AQUA: allergy questionnaire for athletes.  
535 Development and validation. *Med Sci Sports Exerc*, 41(5), 1034-1041 (2009).  
536
- 537 30. Miller MR, Hankinson J, Brusasco V *et al.* Standardisation of spirometry. *Eur Respir*  
538 *J*, 26(2), 319-338 (2005).  
539
- 540 31. Ansley L, Kippelen P, Dickinson J, Hull J. Misdiagnosis of exercise-induced  
541 bronchoconstriction in professional soccer players. *Allergy*, 67(3), 390-395 (2012).  
542
- 543 32. Argyros GJ, Roach JM, Hurwitz KM, Eliasson AH, Phillips YY. Eucapnic voluntary  
544 hyperventilation as a bronchoprovocation technique. *Chest*, 109(6), 1520-1524  
545 (1996).  
546
- 547 33. American Thoracic Society/European Respiratory Society recommendations for  
548 standardized procedures for the online and offline measurement of exhaled lower  
549 respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*,  
550 171(8), 912-930 (2005).  
551
- 552 34. Emmen J, Wielders JP, Boer A-K, van den Ouweland JM, Vader HL. The new Roche  
553 Vitamin D Total assay: fit for its purpose? *Clinical Chemistry and Laboratory*  
554 *Medicine*, 50(11), 1969-1972 (2012).  
555
- 556 35. Holick MF. Vitamin D deficiency. *New England Journal of Medicine*, 357(3), 266-  
557 281 (2007).  
558
- 559 36. Tecklenburg-Lund S, Mickleborough TD, Turner LA, Fly AD, Stager JM,  
560 Montgomery GS. Randomized controlled trial of fish oil and montelukast and their  
561 combination on airway inflammation and hyperpnea-induced bronchoconstriction.  
562 *PloS one*, 5(10), e13487 (2010).  
563
- 564 37. Kippelen P, Larsson J, Anderson SD, Brannan JD, Dahlén B, Dahlén SE. Effect of  
565 sodium cromoglycate on mast cell mediators during hyperpnea in athletes. *Med Sci*  
566 *Sports Exerc*, 42(10), 1853-1860 (2010).  
567
- 568 38. Bartels H, Böhmer M, Heierli C. Serum creatinine determination without protein  
569 precipitation. *Clinica chimica acta; international journal of clinical chemistry*, 37,  
570 193-197 (1972).  
571
- 572 39. Anderson S, Argyros G, Magnussen H, Holzer K. Provocation by eucapnic voluntary  
573 hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med*, 35(5),  
574 344-347 (2001).

575 40. Scragg R, Camargo CA. Frequency of leisure-time physical activity and serum 25-  
576 hydroxyvitamin D levels in the US population: results from the Third National Health  
577 and Nutrition Examination Survey. *American journal of epidemiology*, 168(6), 577-  
578 586 (2008).  
579

580 41. Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin d and  
581 pulmonary function in the third national health and nutrition examination survey.  
582 *CHEST Journal*, 128(6), 3792-3798 (2005).  
583

584 42. Devereux G, Wilson A, Avenell A, McNeill G, Fraser W. A case-control study of  
585 vitamin D status and asthma in adults. *Allergy*, 65(5), 666-667 (2010).  
586

587 43. Bar Yoseph R, Livnat G, Schnapp Z *et al.* The effect of vitamin D on airway  
588 reactivity and inflammation in asthmatic children: A double-blind placebo-controlled  
589 trial. *Pediatric pulmonology*, (2014).  
590

591 44. Owens DJ, Fraser WD, Close GL. Vitamin D and the athlete: Emerging insights. *Eur*  
592 *J Sport Sci*, 15(1), 73-84 (2015).  
593

594 45. Moreira A, Moreira P, Delgado L *et al.* Pilot study of the effects of n-3  
595 polyunsaturated fatty acids on exhaled nitric oxide in patients with stable asthma.  
596 *Journal of investigative allergology and clinical immunology*, 17(5), 309-313 (2007).  
597

598 46. Rundell KW, Anderson SD, Spiering BA, Judelson DA. Field exercise vs laboratory  
599 eucapnic voluntary hyperventilation to identify airway hyperresponsiveness in elite  
600 cold weather athletes. *CHEST Journal*, 125(3), 909-915 (2004).  
601

602 47. Dickinson J, Whyte G, McConnell A, Harries M. Screening elite winter athletes for  
603 exercise induced asthma: a comparison of three challenge methods. *Br J Sports Med*,  
604 40(2), 179-182 (2006).  
605

606 48. Schuster GU, Bratt JM, Jiang X *et al.* Dietary Long-Chain Omega-3 Fatty Acids Do  
607 Not Diminish Eosinophilic Pulmonary Inflammation in Mice. *American journal of*  
608 *respiratory cell and molecular biology*, 50(3), 626-636 (2014).  
609

610 49. Anderson SD, Pearlman DS, Rundell KW *et al.* Reproducibility of the airway  
611 response to an exercise protocol standardized for intensity, duration, and inspired air  
612 conditions, in subjects with symptoms suggestive of asthma. *Respir Res*, 11(120)  
613 (2010).  
614

615 50. Price OJ, Ansley L, Hull JH. Diagnosing Exercise-Induced Bronchoconstriction With  
616 Eucapnic Voluntary Hyperpnea: Is One Test Enough? *The Journal of Allergy and*  
617 *Clinical Immunology: In Practice*, (2014).  
618

619 51. Gupta A, Sjoukes A, Richards D *et al.* Relationship between serum vitamin D, disease  
620 severity, and airway remodeling in children with asthma. *Am J Respir Crit Care Med*,  
621 184(12), 1342-1349 (2011).  
622

623

624 **REFERENCE ANNOTATIONS**

625 *References of considerable interest\*\**

626 \*\*Chinellato I, Piazza M, Sandri M *et al.* Serum vitamin D levels and exercise-  
627 induced bronchoconstriction in children with asthma. *Eur Respir J*, 37(6), 1366-1370 (2011).

628

629 *First study to show a relationship between low serum vitamin D levels and severity of EIB in*  
630 *asthmatic children.*

631

632

633 \*\*Arm J, Horton C, Mencia-Huerta J *et al.* Effect of dietary supplementation with fish oil  
634 lipids on mild asthma. *Thorax*, 43(2), 84-92 (1988).

635

636 *Early work indicating no beneficial effect of omega-3 PUFA supplementation in patients with*  
637 *mild asthma.*

638

639

640 \*\*Mickleborough TD, Murray RL, Ionescu AA, Lindley MR. Fish oil supplementatio reduces  
641 severity of exercise-induced bronchoconstriction in elite athletes. *Am J Respir Crit Care*  
642 *Med*, 168(10), 1181-1189 (2003).

643

644 *Fish oil supplementation (i.e. omega-3 PUFA) provides a protective effect in suppressing EIB*  
645 *in elite athletes due to their anti-inflammatory properties.*

646

647

648 \*\*Mickleborough TD, Lindley MR, Ionescu AA, Fly AD. Protective effect of fish oil  
649 supplementation on exercise-induced bronchoconstriction in asthma. *CHEST Journal*, 129(1),  
650 39-49 (2006).

651

652 *Fish oil supplementation (i.e. omega-3 PUFA) provides a protective effect in suppressing*  
653 *EIB in elite athletes with asthma.*

654

655

656 \*\*Brannan JD, Bood J, Alkhabaz A *et al.* The effect of omega-3 fatty acids on bronchial  
657 hyperresponsiveness, sputum eosinophilia and mast cell mediators in asthma. *CHEST*  
658 *Journal*, (2014).

659

660 *Omega-3 supplementation does not improve bronchial hyper-responsiveness to mannitol or*  
661 *inhibit urinary inflammatory mediator excretion in adults with mild-moderate asthma.*

662

663

664

665 **References of interest\***

666 \*Parsons JP, Hallstrand TS, Mastrorade JG *et al.* An Official American Thoracic Society  
667 Clinical Practice Guideline: Exercise-induced Bronchoconstriction. *Am J Respir Crit Care*  
668 *Med*, 187(9), 1016-1027 (2013).

669 *The recent American Thoracic Society guidelines concluded that whilst it is reasonable to*  
670 *employ omega-3 PUFA supplementation in receptive patients with EIB, the evidence is not*  
671 *currently strong enough to suggest that they are effective in a large majority cases.*  
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674 \*Tecklenburg-Lund S, Mickleborough TD, Turner LA, Fly AD, Stager JM, Montgomery GS.  
675 Randomized controlled trial of fish oil and montelukast and their combination on airway  
676 inflammation and hyperpnea-induced bronchoconstriction. *PloS one*, 5(10), e13487 (2010).

677

678 *Bronchoconstrictor response to EVH attenuated following fish oil supplementation (i.e.*  
679 *omega-3 PUFA) in asthmatic patients with EIB.*

680

681

682 \*Mickleborough TD, Vaughn CL, Shei R-J, Davis EM, Wilhite DP. Marine lipid fraction  
683 PCSO-524™(lyprinol<sup>®</sup>/omega XL<sup>®</sup>) of the New Zealand green  
684 lipped mussel attenuates hyperpnea-induced bronchoconstriction in asthma. *Respir Med*,  
685 107(8), 1152-1163 (2013).

686

687 *Bronchoconstrictor response to EVH attenuated following omega-3 PUFA supplementation*  
688 *derived from New Zealand green lipped mussel (Perna canaliculus)in asthmatic patients with*  
689 *EIB.*

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The authors have no real or perceived conflict of interest in respect of this manuscript.

## **GUARANTOR STATEMENT**

OP confirms full responsibility for the content of the manuscript, including data and analysis.

## **CONTRIBUTION STATEMENT**

OP was involved in the conception and design of the study, acquisition, interpretation of data, drafting and critical revision of manuscript and final approval of the version to be published.

JH was involved in the conception and design of the study, interpretation of data, drafting and critical revision of manuscript and final approval of the version to be published

GH was involved in the conception and design of the study, drafting and critical revision of manuscript and final approval of the version to be published.

PA was involved in the conception and design of the study, drafting and critical revision of manuscript and final approval of the version to be published.

LA was involved in the conception and design of the study, interpretation of data, drafting and critical revision of manuscript and final approval of the version to be published