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The Effect of Omega-3 Fatty Acids on Bronchial Hyperresponsiveness, Sputum Eosinophilia, and Mast Cell Mediators in Asthma

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BACKGROUND: Omega-3 fatty acid supplements have been reported to inhibit exercise-induced bronchoconstriction (EIB). It has not been determined whether omega-3 supplements inhibit airway sensitivity to inhaled mannitol, a test for bronchial hyperresponsiveness (BHR) and model for EIB in people with mild to moderate asthma.

METHODS: In a double-blind, crossover trial, subjects with asthma who had BHR to inhaled mannitol (n = 23; 14 men; mean age, 28 years; one-half taking regular inhaled corticosteroids) were randomized to omega-3 supplements (4.0 g/d eicosapentaenoic acid and 2.0 g/d docosahexaenoic acid) or matching placebo for 3 weeks separated by a 3-week washout. The primary outcome was the provoking dose of mannitol (mg) to cause a 15% fall in FEV_1 (PD_{15}). Secondary outcomes were sputum eosinophil count, spirometry, Asthma Control Questionnaire (ACQ) score, serum triacylglyceride level, and lipid mediator profile in urine and serum.

RESULTS: PD_{15} (geometric mean, 95% CI) to mannitol following supplementation with omega-3s (78 mg, 51-119 mg) was not different from placebo (88 mg, 56-139 mg, $P = .5$). There were no changes in sputum eosinophils (mean ± SD) in a subgroup of 11 subjects (omega-3, 8.4% ± 8.2%; placebo, 7.8% ± 11.8%; $P = .9$). At the end of each treatment period, there were no differences in FEV_1 % predicted (omega-3, 85% ± 13%; placebo, 84% ± 11%; $P = .9$) or ACQ score (omega-3, 1.1% ± 0.5%; placebo, 1.1% ± 0.5%; $P = .9$) (n = 23). Omega-3s caused significant lowering of blood triglyceride levels and expected shifts in serum fatty acids and eicosanoid metabolites, confirming adherence to the supplements; however, no changes were observed in urinary mast cell mediators.

CONCLUSIONS: Three weeks of omega-3 supplements does not improve BHR to mannitol, decrease sputum eosinophil counts, or inhibit urinary excretion of mast cell mediators in people with mild to moderate asthma, indicating that dietary omega-3 supplementation is not useful in the short-term treatment of asthma.

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ABBREVIATIONS: 11β-$PGF_{2\alpha}$ = 11β-prostaglandin F2α; BHR = bronchial hyperresponsiveness; DHA = docosahexaenoic acid; EIB = exercise-induced bronchoconstriction; EPA = eicosapentaenoic acid; EVH = eucapnic voluntary hyperventilation; HEPE = hydroxyeicosapentaenoic acid; ICS = inhaled corticosteroid; LTE_4 = leukotriene E4; PD_{15} = provoking dose of mannitol (mg) to cause a 15% fall in FEV_1; PGD_2 = prostaglandin D2; PGE_2 = prostaglandin E2

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Dietary supplementation using omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) plays an uncertain role in the management of asthma, which has led to calls for more prospective studies to investigate whether there are any benefits from an omega-3-enriched diet in asthma.[1] Results have not been consistent when assessing high doses of dietary omega-3s on bronchial hyperresponsiveness (BHR), a key feature of asthma. Early studies administering omega-3s daily over 10 weeks demonstrated no attenuation of exercise-induced bronchoconstriction (EIB)[2] while causing a small decrease of the late airway response to inhaled allergen.[3] More recently, 3 weeks of omega-3s were reported to inhibit mild airway responses to exercise and eucapnic voluntary hyperventilation (EVH) in both elite athletes and symptomatic subjects with asthma.[4-6] This was observed in association with reductions in markers of airway inflammation, such as reduced cytokine levels, reduced excretion of the urinary eicosanoids 11β-prostaglandin F2α (11β-$PGF_{2\alpha}$) and leukotriene E4 (LTE_4), and decreased sputum eosinophil counts, suggesting that the benefits of an omega-3-enriched diet on EIB was through antiinflammatory mechanisms.

Inhaled mannitol as a test for BHR has been demonstrated as a useful model for EIB,[7] and all pharmacotherapies effective at inhibiting EIB inhibit BHR to mannitol.[8-11] Mannitol causes bronchoconstriction through an increase in the osmolarity of the airway surface fluid, leading to mediator release from mast cells similar to the proposed mechanism for EIB.[12] Evidence for mediator release to mannitol is an increase in urinary eicosanoids 11β-$PGF_{2\alpha}$ and LTE_4 following a mannitol challenge[11] similar to what has been observed with exercise and EVH.[13,14] BHR to mannitol identifies EIB in elite athletes[15] and predicts the severity of EIB in people with asthma.[7]

Considering these similarities, we evaluated whether dietary supplementation with omega-3 fatty acids could inhibit BHR to inhaled mannitol to assess the efficacy of omega-3s in people with mild to moderate BHR. We assessed the effect of 3 weeks of dietary omega-3s on BHR to mannitol, sputum eosinophil counts, spirometry, and asthma symptoms in subjects with clinically defined steroid-naive asthma as well as in those using regular inhaled corticosteroids (ICSs). Urine and serum were collected during the study to determine the biochemical effects of the omega-3 supplementation on mast cell mediators derived from fatty acids.

Materials and Methods

Study Design

The study used a randomized, double-blind, placebo-controlled, and crossover trial design. Subjects attended the laboratory in the morning on four occasions occurring before and after two 3-week treatment periods. People taking ICSs were required to attend an extra initial visit 2 weeks prior to the commencement of the trial in order to perform a mannitol challenge to document that their airway sensitivity in the presence of ICS was reproducible over the run-in period and that their asthma was clinically stable.

Each visit comprised self-administered questionnaires (Asthma Control Questionnaire and Asthma Quality of Life Questionnaire); taking blood to assess fasting blood triglyceride, omega-3, and omega-6 fatty acid levels; obtaining a urine sample to assess urinary eicosanoid counts; assessing baseline lung function using spirometry; and performing a mannitol challenge followed by sputum induction. More details are provided in e-Appendix 1. Upon meeting entry criteria, subjects were randomized to a 3-week supply of a daily dose of 10 capsules of either a matched placebo containing a blend of omega-6 and omega-9 or a ratio of omega-3 fatty acids comprising 400 mg EPA and 200 mg DHA (40/20EE capsules; Ocean Nutrition Canada). This equated to a daily dose of 4.0 g EPA and 2.0 g DHA. Each treatment period was separated by a 3-week washout. More details are provided in e-Figure 1.

The protocol was approved by the ethics review board of St. Joseph's Healthcare, Hamilton, Ontario, Canada (R.P.#06-2750), and Health Canada (Approval No. 120532). All subjects gave written informed consent.

Subjects

Nonsmoking subjects with clinically diagnosed asthma and current asthma symptoms with an $FEV_1 > 70\%$ predicted were entered into the trial (Fig 1, Table 1). Short-acting β_2-agonists were withheld for 8 h and long-acting β_2-agonists for 48 h, and no ICSs were taken on the morning of the study. Antihistamines and leukotriene antagonists were not permitted throughout the whole study period. No caffeine-containing food or drink and no vigorous exercise were permitted on the study day prior to the study visit. Subjects were asked to abstain from fish meals or other significant sources of omega-3s for at least 2 weeks prior to the study and throughout the study.

Part of this article has been presented in abstract form (Brannan JD, Alkhabaz A, Otis J, et al. Effect of omega-3 fatty acid [O3FA] from fish oils on mannitol-induced airway hyperresponsiveness [AHR] and sputum eosinophils in patients with asthma. *Eur Respir J*. 2009;34[suppl 33]:333s).

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Biochemical Measurements

Serum triacylglyceride levels and the fatty acid composition of the serum phospholipids were analyzed for triglycerides as well as omega-3 and omega-6 fatty acids. The effects of the dietary intervention on eicosanoid levels of 11β-$PGF_{2\alpha}$ and LTE_4 in urine were assessed by enzyme immunoassay,[11,14] and an ultraperformance liquid chromatography-tandem mass spectrometry platform[16] that measures > 90 different lipid mediators. More details are provided in e-Appendix 1.

Statistics

The primary outcome was the airway sensitivity to a provoking dose of mannitol (mg) to cause a 15% fall in FEV_1 (PD_{15}). Secondary outcomes were sputum eosinophil counts, baseline FEV_1, and asthma symptoms scores. Spearman rank correlation was performed on nonnormally distributed data. For comparisons between groups, the Student *t* test was used for paired measurements, with $P < .05$ considered significant. A sample size for each treatment group for PD_{15} was based on a prior study demonstrating a clinically significant improvement in association with decreased airway sensitivity to inhaled mannitol using ICS.[17] When raw data for PD_{15} values were log2 converted, this study demonstrated a 1.9 doubling dose shift in the presence of ICS and a within-subject SD of 0.7, yielding a sample size of four subjects at 80% power and a significance of .05. When calculating a minimal important difference of 1 doubling dose, assuming the same within-subject SD, this yielded a sample size of seven at 80% power and a significance of .05. The rate of spontaneous recovery of lung function following the challenge was calculated as an area under the % fall in FEV_1 vs time curve.

Results

Omega-3 fatty acid supplements were not effective at attenuating BHR to mannitol. The geometric mean PD_{15} was 88 mg (95% CI, 56-139 mg) after placebo treatment and 78 mg (95% CI, 51-119 mg) after omega-3 fatty acid supplements ($P = .5$). This represented a doubling dose shift in favor of placebo of −0.25 (SD, 1.1) and less than the minimal important difference of 1 doubling dose. Although the airway sensitivity to mannitol was in the moderate range for most subjects ($PD_{15} < 155$ mg), those taking β_2-agonists as needed were more sensitive to mannitol than those taking ICSs ($P = .03$) (Table 2). However, no differences were observed based on subjects' daily treatment (Fig 2, Table 2, e-Fig 2). In addition, there were no differences in the spontaneous recovery from mannitol between the omega-3 supplement and placebo groups. However, a subgroup analysis found those taking ICS had a slower recovery while on omega-3 supplements compared with placebo, and recovery time for the whole group was longer in the presence of omega-3 supplements compared with baseline (Table 2, e-Fig 3).

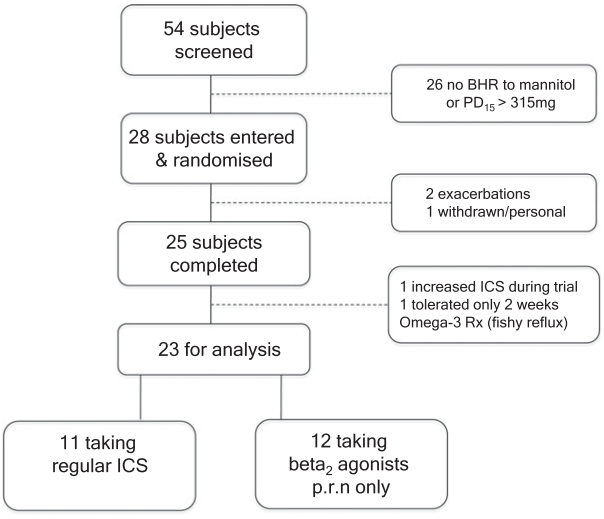

Figure 1 – *Participant flow diagram. BHR = bronchial hyperresponsiveness; ICS = inhaled corticosteroid; PD_{15} = provoking dose of mannitol (mg) to cause a 15% fall in FEV_1; p.r.n = as needed; Rx = supplement.*

The majority of subjects who could produce an adequate sputum sample had evidence of eosinophils (> 2%) (Table 1), and there was a significant relationship between the percentage of eosinophils and mannitol PD_{15} ($rs = 0.53$, $P = .047$, n = 14). However, in subjects who provided suitable sputum samples at the end of each treatment period (n = 11), there were no differences in eosinophil counts following omega-3 supplementation (8.4% ± 8.2%) compared with placebo (7.8% ± 11.8%) (Fig 3).

There were no significant improvements in either lung function (baseline FEV_1) or asthma symptoms with the omega-3 supplements, with no changes in asthma control or asthma quality of life scores (Table 2). However, there was a 27% reduction in fasting blood triglyceride levels in subjects taking the omega-3 supplements compared with those taking placebo ($P < .001$) as well as evidence of raised EPA and DHA serum levels in the presence of the omega-3 supplements compared with placebo ($P < .001$) (Figs 4, 5). This finding indicated biologic activity of the omega-3 supplements and that subjects were adherent to the dietary supplementation.

As further evidence of the effectiveness of the intervention, serum levels (mean ± SEM) of the omega-3 fatty acid EPA-derived metabolites 12-hydroxyeicosapentaenoic acid (HEPE) and 15-HEPE increased significantly following omega-3 supplementation (0.6 ± 0.1 pM vs 6.9 ± 2.6 pM, $P = .022$) compared with placebo (0.1 ± 0.02 pM vs 0.6 ± 0.1 pM, $P = .000024$). Measurement of the analogous omega-6 fatty acid arachidonic acid-derived metabolites 12- and 15-hydroxyeicosatetraenoic acid showed no changes (11 ± 2 pM vs 17 ± 5 pM and 1.3 ± 0.1 pM vs 1.3 ± 0.1 pM, respectively).

TABLE 1] Anthropometric Data, Medication, FEV_1, Response to Mannitol, and % Eosinophils in Sputum at Baseline

Subject No.	Age, y	Sex	Height, cm	Weight, kg	FEV_1 % Predicted	Asthma Medications	ICS Dose, μg/d	Mannitol PD_{15}, mg	% Eosinophils in Sputum
1	31	M	168	84	75.6	BEC, S	200	71.1	0.5
2	38	M	183	78	88.6	BUD/Ef, S	800	50.6	…
3	43	F	172	76	84.7	BUD/Ef	100	181.6	0.0
4	26	F	156	74	80.3	BUD/Ef, S	200	88.4	8.0
5	23	M	179	85	102.6	FL/Sm, S	250	126.6	13.5
6	30	M	172	110	76.4	BUD/Ef, S	400	136.4	0.2
7	33	F	167	99	88.1	BUD/Ef, S	200	65.5	…
8	24	M	176	87	98.0	FL/Sm, S	500	81.5	…
9	28	F	165	61	72.1	FL/Sm, S	500	293.4	4.3
10	21	F	169	107	71.1	FL/Sm, S	500	45.4	18.6
11	20	M	175	101	84.7	FL, S	125	282.0	7.0
12	28	M	172	102	89.6	S	prn	85.8	…
13	26	M	186	93	110.0	S	prn	20.9	8.0
14	33	F	167	70	81.8	S	prn	20.6	33.5
15	29	M	183	63	82.5	S	prn	34.9	…
16	22	F	171	65	99.5	S	prn	70.9	…
17	24	F	166	55	81.1	S	prn	73.7	…
18	54	M	174	82	76.8	S	prn	122.6	…
19	47	M	173	79	76.2	S	prn	33.4	5.3
20	19	M	179	126	70.2	S	prn	116.0	…
21	39	F	177	73	103.2	S	prn	9.9	21.3
22	26	M	170	87	79.8	S	prn	37.4	46.0
23	22	M	169	72	74.5	S	prn	220.2	4.7
Mean	30	…	…	…	84.7	…	…	72[a]	11.5
Range	19-54	…	…	…	70.2-110	…	…	49.7-104[b]	0-33.5

/ = in combination; BEC = beclomethasone; BUD = budesonide; Ef = eformoterol; F = female; FL = fluticasone; ICS = inhaled corticosteroid; M = male; PD_{15} = provoking dose of mannitol (mg) to cause a 15% fall in FEV_1; prn = as needed; S = salbutamol; Sm = salmeterol.
[a]Geometric mean.
[b]95% CI.

The measurements of urinary levels (ng/mmol creatinine) of LTE_4 and the prostaglandin D2 (PGD_2) metabolite 11β-$PGF_{2\alpha}$ revealed no differences between the two treatments (Table 2); thus, further characterization of the effect of omega-3 supplements on lipid mediator profiles using ultra-performance lipid chromatography-tandem mass spectrometry was performed in a subset of subjects (n = 5). No changes were found in the urinary excretion of any of the key metabolites of the primary prostaglandins or LTE_4. However, there was a decrease in urinary prostaglandin E2 (PGE_2) by omega-3 supplements compared with placebo (29 ± 19 units vs 141 ± 52 units, respectively; $P = .048$). A decrease was also observed for 8,12-iso-i$PF_{2\alpha}$-VI after omega-3 supplementation compared with placebo (581 ± 103 units vs 730 ± 129 units, respectively; $P = .0117$). The levels of all lipid mediators measured in the urine are provided in e-Table 1.

A post hoc power calculation of sample size using the data for PD_{15} obtained in this study showed that the total sample size of 23 was of sufficient power for assessing a minimal important difference of one doubling dose. The within-subject SD for a comparison of PD_{15} values at the beginning of each treatment period before any treatment was administered and following washout (each separated by 6 weeks), was 1.2. The SD for the PD_{15} values in the presence of the placebo and omega-3 fatty acid treatments (each also separated by 6 weeks) was 1.1. This yielded a sample size of 13 and 12 subjects,

TABLE 2] Total Group or Individual Treatment Groups of Symptoms, FEV_1 % Predicted, PD_{15}, and Spontaneous Recovery in Lung Function and Baseline Urinary Metabolites Either at Baseline or Following Omega-3 Supplementation or Placebo

Category	Total Group	Treatment Subgroup	
		ICS[a]	β_2-Agonist
No. subjects	23	11	12
Male (female) sex	14 (9)	6 (5)	8 (4)
Mean age (range), y	28 (19-54)	28 (20-43)	27 (19-54)
Atopy	20	8	12
AQLQ score			
Baseline	5.6 ± 0.8	5.5 ± 0.9	5.7 ± 0.7
ACQ score			
Baseline	1.1 ± 0.5	1.1 ± 0.4	1.0 ± 0.6
Omega-3	1.0 ± 0.5	1.1 ± 0.6	0.9 ± 0.4
Placebo	1.1 ± 0.5	1.2 ± 0.6	1.0 ± 0.5
FEV_1 % predicted			
Baseline	84.7 ± 11.2	83.8 ± 11.2	85.4 ± 13.2
Omega-3	84.5 ± 13.2	83.2 ± 11.5	85.7 ± 15.0
Placebo	84.2 ± 11.2	83.8 ± 9.8	84.6 ± 12.8
PD_{15}, mg			
Baseline	72.0 (49.7-104.3)	106.5 (69.1-164.1)	50.3 (28.2-89.4)
Omega-3	77.8 (50.9-119.1)	110.0 (57.2-211.3)	56.7 (31.4-102.5)
Placebo	88.1 (55.9-138.8)	118.2 (73.2-190.7)	67.2 (29.9-151.2)
Response dose ratio, % fall/mg			
Baseline	0.25 ± 0.22	0.15 ± 0.08	0.35 ± 0.27
Omega-3	0.25 ± 0.23	0.17 ± 0.17	0.32 ± 0.27
Placebo	0.24 ± 0.29	0.16 ± 0.16	0.31 ± 0.38
AUC FEV_1 recovery, % fall.min			
Baseline	403 ± 142	418 ± 183	390 ± 103
Omega-3	468 ± 145[b]	484 ± 172[c]	455 ± 124
Placebo	424 ± 166	397 ± 172	446 ± 164
Urinary mediators, No.	22[d]	11	11[d]
11β-$PGF_{2\alpha}$, ng/mmol creatinine			
Baseline	57 ± 27	62 ± 31	51 ± 23
Omega-3	51 ± 35	58 ± 47	44 ± 18
Placebo	64 ± 59	60 ± 33	69 ± 79
LTE_4, ng/mmol creatinine			
Baseline	46 ± 13	50 ± 14	43 ± 12
Omega-3	53 ± 17[b]	58 ± 18	49 ± 16
Placebo	54 ± 20	55 ± 17	53 ± 24

Data are presented as mean ± SD or geometric mean (95% CI) unless otherwise indicated. 11β-$PGF_{2\alpha}$ = 11β-prostaglandin F2α; ACQ = Asthma Control Questionnaire; AQLQ = Asthma Quality of Life Questionnaire; AUC = area under the curve; %fall.min = % fall in FEV_1 vs time; LTE_4 = leukotriene E4. See Table 1 legend for expansion of other abbreviations.

[a]ICS with or without long-acting β_2-agonist.

[b]$P < .05$ with baseline.

[c]$P < .05$ with placebo.

[d]For β_2-agonist group, urine data only in 11 subjects.

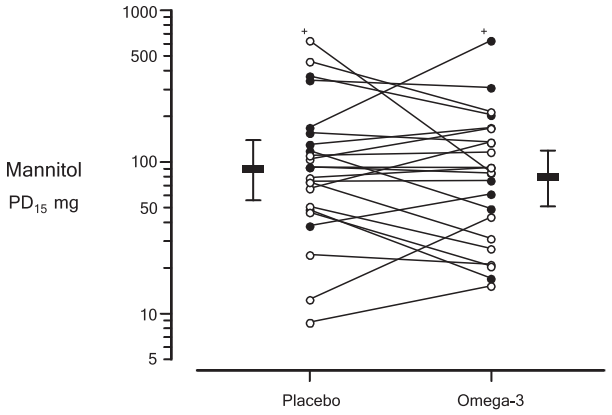

Figure 2 – *The individual data and geometric mean (95% CI) for PD_{15} in subjects with asthma following 3 wk of daily omega-3 fatty acid supplementation compared with placebo, demonstrating no change in PD_{15}. ($n = 23$, $P = .5$). ● = inhaled corticosteroid group; ○ = β_2-agonist group; + = two subjects who did not have a PD_{15} value. See Figure 1 legend for expansion of abbreviation.*

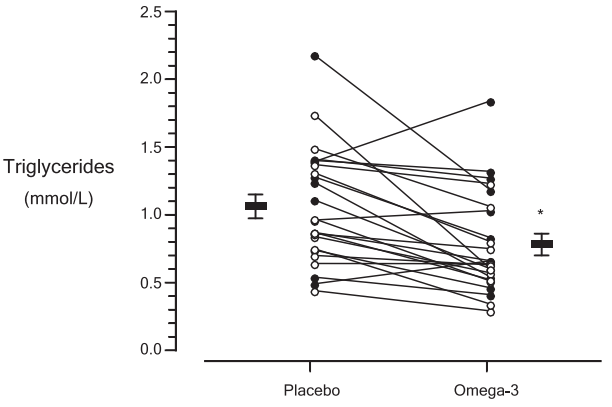

Figure 4 – *The individual data and mean (SE) for serum triglyceride levels in subjects with asthma following 3 wk of daily omega-3 fatty acid supplementation compared with placebo, demonstrating a significant decrease in serum triglyceride concentration following omega-3 fatty acid supplementation ($n = 23$, $P = .0011$). *$P < .0011$. ● = inhaled corticosteroid group; ○ = β_2-agonist group.*

respectively, at a power of 80% and a significance of 0.05. Individual data for PD_{15} can be found in e-Table 2. The doubling dose difference comparing the PD_{15} values at the commencement of each treatment period was 0.25.

Discussion

This study demonstrates that a high daily dose of an omega-3 fatty acid supplement has no effect on either BHR to mannitol or sputum eosinophil percentage in subjects with mild to moderate asthma in association with no changes in asthma symptom control. The lack of effect was seen irrespective of whether the subjects had baseline treatment with as-needed β_2-agonists or were maintained on ICS. Neither did the omega-3 fatty acid supplementation change the resting levels of key arachidonic acid-derived mediators of mast cell responses, such as the cysteinyl leukotrienes and prostaglandin D2. However, we did see an increase in the serum levels of the omega-3 fatty acid-derived products 12- and 15-HEPE after the active supplementation in association with the expected lowering of serum triglyceride levels, which together provide firm evidence that the subjects were adherent to the treatment.

The findings are consistent with the initial observation that omega-3 fatty acids had no effect on EIB[2] but contradictory to other studies where similar doses of omega-3 supplements over the same treatment duration of 3 weeks were found to inhibit EIB[4,5] and EVH.[6,18] The most obvious difference is that we used inhaled mannitol, a bronchial provocation challenge that has been derived from the understanding of the effects of dry

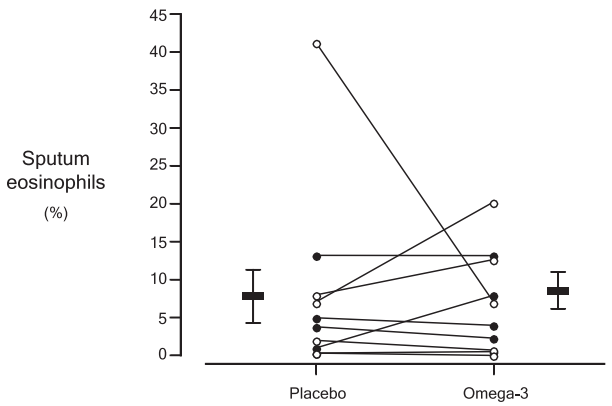

Figure 3 – *The individual data and mean (SE) for the percentage of eosinophils in sputum in a subgroup of subjects with asthma following 3 wk of daily omega-3 fatty acid supplementation compared with placebo, demonstrating no change in percent eosinophils ($n = 11$, $P = .9$). ● = inhaled corticosteroid group; ○ = β_2-agonist group.*

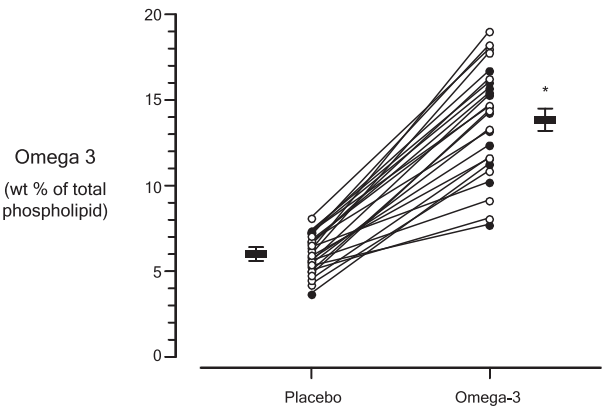

Figure 5 – *The individual data and mean (SE) for combined omega-3 fatty acids in serum (weight [wt] as a percentage of total phospholipid) in subjects with asthma following 3 weeks of daily omega-3 fatty acid supplementation compared with placebo, demonstrating a significant increase in omega-3 fatty acid levels in serum following omega-3 fatty acid supplementation ($n = 23$, $P < .001$). *$P < .001$. ● = inhaled corticosteroid group; ○ = β_2-agonist group.*

air hyperpnea-induced BHR, which is a well-established alternative challenge stimulus to study EIB. There is strong evidence that the airway response to mannitol closely mimics central components of EIB, such as the activation of mast cells and eosinophils, resulting in the release of bronchoconstrictive mediators.[7,9-13,19,20] However, the differences may lie in the intensity of the stimulus to the airways. The studies showing benefit performed the exercise challenge until volitional exhaustion, which is a test that has a known low sensitivity to identifying EIB and which may induce milder airway responses in those with EIB.[4,5] In both exercise and EVH studies by Mickleborough and colleagues,[4,5] no data were provided in relation to the intensity or reproducibility of the exercise and ventilatory stimulus administered or whether this achieved the minimum requirements for each protocol to induce EIB.

Moreover, the current findings are consistent with earlier studies demonstrating that a 10-week treatment period with omega-3 supplements does not attenuate EIB using the standardized 8-min exercise protocol.[2] The authors assessed airway resistance (specific airway conductance) and not FEV_1 as a measure of airflow limitation. The same investigators, however, did demonstrate a small effect of omega-3s on the late airway response to allergen.[3] Interestingly, in a treatment study, the same group also found that omega-3 supplements changed the profile of blood fatty acids and lipid mediator biosynthesis in circulating leukocytes but did not improve the clinical course of rhinitis or asthma.[21]

In contrast to Mickleborough and colleagues,[4,5] we did not find any significant reductions in resting levels of inflammatory markers, such as sputum eosinophils or the urinary metabolites of PGD_2 and LTE_4 with omega-3 fatty acids. The present findings are consistent with studies in mice showing no effect of a 6-week treatment with EPA and DHA (using a similar equivalent dose) on suppressing ovalbumin-augmented eosinophils in an asthma mouse model, with evidence that DHA alone augments eosinophilia.[22] However, it was also shown in mice that both EPA and DHA can suppress PGE_2 in BAL. We also observed significant reductions in PGE_2 excretion (its source likely from the kidney), which suggests that omega-3 fatty acids can modify prostaglandin metabolism. PGE_2 in the airway is believed to have a beneficial effect as an endogenous bronchodilator, providing bronchoprotection after exercise.[23] We did not notice any detrimental effect of the omega-3 supplements other than a small, but statistically significant prolonged recovery from bronchoconstriction to mannitol in the presence of the omega-3 treatment; however, the clinical relevance of this is unclear. Slower recovery from mannitol challenge does suggest a direct role of leukotrienes.[10,19] One study in humans has demonstrated worsening of daily peak flow and increased β_2-agonist use with longer treatment of a daily dose of 3 g omega-3 fatty acids over 6 weeks in subjects with asthma and aspirin sensitivity.[24]

The presumed mechanism for explaining the possible beneficial effects of omega-3 fatty acids on inflammatory pathways is through diversion of the production of bronchoconstricting prostaglandins and leukotrienes from the omega-6 substrate arachidonic acid to mediators biosynthesized from the alternative omega-3 substrates. This assumes that these alternative metabolites have weaker effects on the airway smooth muscle and inflammatory effector cells.[4,25] However, it has been shown that the 5-series leukotrienes derived from omega-3 fatty acids have the same bronchoconstrictive potency as the 4-series leukotrienes derived from arachidonic acid.[26] In addition, the generation of the 3-series prostaglandins with omega-3 supplements may not provide any benefits on airway inflammation and BHR in an animal model.[22] Considering this, it may be that omega-3 supplements, even in high doses, fail to substantially alter the substrate flow in the tissue-residing cells, whereas in the blood, significant concentrations exist to divert metabolism. It is well established that omega-3 fatty acids can effectively decrease serum triglyceride levels, as we observed in this study. Thus, omega-3 supplements may be effective in altering plasma fatty acid composition; function of blood cells; and metabolism in other cells exposed to high levels of circulating omega-3 fatty acids, such as vascular endothelium and renal tubular cells. This is supported by the current finding that the intervention caused a decrease in urinary PGE_2, which is known to be derived directly from the kidney.[27] We suggest that it may be more difficult to change the metabolic profile of cells within tissues, such as that of the airway mast cell, where there is most likely still sufficient arachidonic acid available as the preferred substrate for the activation-induced biosynthesis of cysteinyl leukotrienes and PGD_2. During stimulation, arachidonic acid is liberated mainly by group 4 phospholipase A2 (or cytosolic phospholipase A2). This enzyme has preference for phospholipids with arachidonic acid, which may explain why in acute processes, displacing arachidonic acid with omega-3s is not effective to shield the production of arachidonic acid-derived eicosanoids.[28]

The study was adequately powered for the primary outcome, and this was supported with the post hoc calculations. We powered the study expecting a clinically important difference based on the existing studies showing a 64% protection in the percent fall in FEV_1 after exercise in subjects with asthma taking omega-3 fatty acids[5] and considering this inhibition to be similar to what has been observed using ICS on both exercise and mannitol in subjects with asthma.[17,29] Independent of ICS therapy, a patient with asthma who has a PD_{15} will demonstrate considerable room for improvement in PD_{15} in the presence of improved ICS treatment (higher dose or improvement in adherence)[17,30] and acute therapy using cromolyn drugs and β_2-agonists.[11] A post hoc sample size analysis demonstrated that the individual treatment groups based on the presence of ICS therapy or β_2-agonists as needed were either adequately powered or slightly underpowered, suggesting that a minimum of 12 to 13 subjects are required when using inhaled mannitol in a study assessing an intervention and looking for a minimal important difference of 1 doubling dose. Considering the 0.25 doubling dose difference in favor of placebo, this possible slight underpowering for each treatment group is likely of no significance.

In conclusion, a diet enriched with omega-3 fatty acids over a 3-week treatment period had no clinical benefits in either steroid-treated or steroid-naive subjects with asthma, as confirmed by no observed improvements on the two key features of asthma: BHR and airway inflammation. Furthermore, there was no evidence of significant changes in resting eicosanoid metabolism caused by the omega-3 supplements, even though expected changes in serum omega-3 fatty acid metabolism and decreases in serum triglyceride levels were seen. These data suggest that in people with asthma, high daily doses of omega-3 fatty acids from fish oils are not an alternative or additive treatment strategy for asthma. Further studies need to elucidate whether the degree of the stimulus to elicit EIB is a determining factor on the protective effect of omega-3 fatty acids and whether the same effect is observed following longer-term therapy. Considering this, the findings have general ramifications, indicating that distinct manipulation of tightly controlled biologic responses in tissues by dietary intervention of omega-3 fatty acids is difficult.

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Additional information: The e-Appendix, e-Figures, and e-Tables can be found in the Supplemental Materials section of the online article.

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