Fish Oil Supplementation in Overweight/Obese Patients with Uncontrolled Asthma

A Randomized Trial

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Abstract

Rationale: Omega-3 fatty acid (n3PUFA) supplementation has been proposed as a promising antiasthma strategy. The rs59439148 *ALOX5* polymorphism affects leukotriene production and possibly inflammatory responses to n3PUFA.

Objectives: Assess the effects of n3PUFA supplementation and *ALOX5* genotype on asthma control in patients with obesity and uncontrolled asthma.

Methods: This multicenter trial among 12- to 25-year-olds with overweight/obesity and uncontrolled asthma randomized subjects in a 3:1 allotment to n3PUFA (4 g/d) or soy oil control for 24 weeks. Asthma Control Questionnaire was the primary outcome; secondary outcomes included blood leukocyte n3PUFA levels, urinary leukotriene-E4, spirometry, and asthma-related events. The number of SP1 tandem repeats in rs59439148 determined *ALOX5* genotype status. Simple and multivariable generalized linear models assessed effects on outcomes.

Results: Ninety-eight participants were randomized (77 to PUFA, 21 to control), and more than 86% completed all visits. Asthma and demographic characteristics were similar among treatment groups. n3PUFA treatment increased the n3-to-n6 PUFA ratio in circulating granulocytes ($P = 0.029$) and monocytes ($P = 0.004$) but did not affect mean Asthma Control Questionnaire change at 6 months (n3PUFA: mean, −0.09; 95% confidence interval [CI], 0.09 to 0.10; vs. control: mean, −0.18; 95% CI, −0.42 to 0.06; $P = 0.58$). Changes in urinary leukotriene-E4 ($P = 0.24$), forced expiratory volume in 1 second % predicted ($P = 0.88$), and exacerbations (relative risk [RR], 0.92; 95% CI, 0.30–2.89) at 6 months were similar in both groups. n3PUFA treatment was associated with reduced asthma-related phone contacts (RR, 0.34; 95% CI, 0.13–0.86; $P = 0.02$). *ALOX5* genotype did not affect n3PUFA treatment responses.

Conclusions: We did not find evidence that n3PUFA use improves most asthma-related outcomes and cannot recommend it as a prevention strategy for overweight/obese patients with asthma.

Clinical trial registered with www.clinicaltrials.gov (NCT01027143).

Keywords: asthma; obesity; fish oil; omega-3 fatty acids; EPA

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Asthma is a common, complex disease of the bronchial airways that involves diverse underlying inflammatory mechanisms and clinical phenotypes (1, 2). Uncontrolled asthma symptoms continue to cause impaired quality of life and urgent healthcare utilization. Obesity (3, 4) and adolescent age (5) are both risk factors for poor asthma symptom control. New therapeutic interventions to reduce airway inflammation and facilitate improved asthma control are greatly needed.

External factors such as diet and obesity status may alter the risk for incident asthma (6, 7) and also appear to worsen asthma severity (4, 8). Obesity is associated with reduced response to inhaled corticosteroids (9, 10), the most consistently effective antiasthma controller medication currently available. A diet low in fresh vegetables and fish and high in saturated fats and n-6 polyunsaturated fatty acid (PUFA) has been associated with both obesity (11) and greater risk for asthma (12). Obesity may promote greater arachidonic acid/5-lipoxygenase pathway activity and leukotriene production, leading to worsening of symptoms (13). Populations consuming high amounts of cold-water fish rich in long-chain polyunsaturated fatty acids, such as the omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), traditionally have a low incidence of asthma (14–17). In preclinical studies, omega-3 polyunsaturated fatty acid (n3PUFA) supplementation can increase plasma and inflammatory cell phospholipid membrane concentrations of EPA and DHA (18–26) and inhibit production of leukotrienes (27) via competitive inhibition of cytosolic phospholipase A2 (28).

Few large trials of n3PUFA have been conducted in asthma, and to our knowledge no trials have supplemented the at-risk obese asthma population. Results from small asthma trials have been inconsistent (18, 29–31), but encouraging (18, 21, 24, 28, 32–36). Inconsistent findings may stem from differences in daily dosing and trial duration. Dwyer and colleagues showed that the rs59439148 *ALOX5* promoter SP1 tandem repeat polymorphism influenced the response to n3PUFA in a study of adults with atherosclerosis (37). Previous supplementation trials in asthma have not conducted nutrigenetic analyses on asthma responses to n3PUFA.

The NOOA (Nutrigenetic Response to Omega-3 Fatty Acids in Obese Asthmatics) trial was designed as a randomized, double-blind, placebo-controlled 24-week intervention study to determine if supplemental omega-3 fatty acids improve symptoms among adolescents and young adults with overweight/obesity and uncontrolled asthma. NOOA measured change in Asthma Control Questionnaire (ACQ) score as its primary outcome, while evaluating secondary asthma outcomes, nutrigenetics, tolerability, and safety.

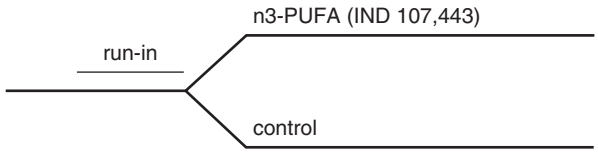
Methods

Study Design

A detailed description of the design of the NOOA trial, including screening and recruitment procedures and statistical analysis, has been reported elsewhere (38). Further description is provided in the Methods section of the online supplement. The NOOA study was a multicenter, double-blinded, randomized, placebo-controlled, 24-week parallel group intervention trial of omega-3 PUFA supplementation or placebo (3:1 allotment) in overweight/obese adolescents and young adults with poorly controlled asthma (Figure 1). Randomization was stratified by study site and body mass index (BMI) strata (I: BMI-percentile 85–94, II: BMI-percentile $\geq$ 95) using a randomization scheme generated using the SAS procedure PROC PLAN (SAS Institute Inc.). The NOOA study protocol was approved by the institutional review board at each participating site (Nemours Foundation IRB); all participants or legal caregivers provided written informed consent (and assent as appropriate), and a data and safety monitoring board monitored the study.

Participants

Adolescents and young adults aged 12 to 25 years were eligible provided they had a physician diagnosis of persistent asthma, evidence of poor asthma control despite taking a daily inhaled corticosteroid controller, and evidence of central overweight/obesity. Poor asthma control was defined as including one of the following: use of β-agonist more than twice per week on average over the past month; one or more nocturnal awakenings per week on average over the past month; two or more emergency room visits, unscheduled physician visits, prednisone courses, or hospitalizations for asthma (in the past 12 mo); or an asthma control questionnaire score greater than or equal to 1.25 at screening. Overweight and obesity status were defined using age-appropriate Centers for Disease Control and Prevention definitions based on BMI (39). In addition, participants had to have a waist circumference above the 90th percentile for age and sex. Asthma diagnosis was



NCT01027143	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Time	Minus 10–28 days	Minus 10–14 days	0	12 wks	24 wks
Screening	+	+			
Eligibility	+	+			
Start run-in		+			
Randomization			+		
ACQ	+		+	+	+
Spirometry	+		+	+	+
Biomarkers*			+	+	+

Figure 1. Study diagram and procedures. Screening included informed consent and medical history collection; eligibility and inclusion/exclusion criteria were assessed for run-in and randomization. Phone visits occurred 2, 6, 10, 16, and 20 weeks after visit 3. + indicates procedure was performed. *Urinary leukotriene E4, exhaled nitric oxide, blood for n3/n6 ratio. ACQ = Asthma Control Questionnaire; IND = Investigational New Drug number; PUFA = polyunsaturated fatty acid.

confirmed by evidence of either bronchodilator reversibility (forced expiratory volume in the first second of expiration [FEV_1] ⩾ 12% after 360 μg [four puffs] of albuterol) or airway hyperresponsiveness (provocative concentration of methacholine at which FEV_1 decreased by 20% ⩽ 16 mg/ml).

Treatment

Participants were randomized to either oral n3PUFA supplementation (3.18 g EPA, 822 mg DHA, 101 mg other omega-3 fatty acids), or similar weight ultrapurified (protein-free) soy oil control. The daily doses for both treatments were delivered in the form of six softgel capsules (Nordic Naturals, Inc.) and was similar to n3PUFA doses found to lead to reduced inflammation and airway responsiveness in two past studies (23, 24). The content purity was established by a certified and accredited reference laboratory (Nutrasource Diagnostics, Inc.). n3PUFA and soy oil placebo had identical look, taste, and texture.

Outcome Measures

The primary outcome was change in the ACQ at 6 months (40, 41). The ACQ ranges from 0 to 6 (higher values indicate worse asthma control) and considers a broad set of control indicators, including use of bronchodilators, cough, nocturnal symptoms, typical level of daily activity, and pulmonary function. A score greater than 1.25 in children is considered poor asthma control, and a change of 0.4 or greater is considered clinically meaningful (42). Asthma symptoms were also evaluated using the Asthma Control Test (43). Adherence to study drug was encouraged and monitored using daily diary cards, pill counts, and phone and clinic visits. Asthma exacerbations were defined by the need for urgent medical care (emergency room or urgent care clinic) or systemic corticosteroids to avoid severe worsening of asthma determined by study physician or local provider (44). Lung function measures (forced vital capacity and FEV_1) were measured using the Koko spirometric system per American Thoracic Society standards (45).

All biochemical parameters were measured using established and validated techniques in our laboratory (Nemours Biomedical Analysis Laboratory). Urinary leukotriene-E4 (LTE4) was measured by liquid chromatography tandem mass spectrometry (46, 47). Omega-3 and omega-6 PUFA content in histopaque isolated peripheral blood monocytes and granulocytes (48–50) was measured using gas chromatography mass spectrometry after derivation to respective fatty acid methyl esters as previously described (51). Alanine aminotransferase, platelet count, and the international normalized ratio were checked at baseline and 12 weeks. Participants were questioned about potential adverse effects of treatment at each visit.

Nutrigenetic Analysis

The *ALOX5* promoter SP1 tandem repeat polymorphism (marker rs59439148) was genotyped as previously described (37). Participant genomic DNA was prepared from mononuclear cells in whole blood samples. Hardy-Weinberg equilibrium between expected and observed genotype distributions was calculated using χ^2 goodness-of-fit tests. Participants with two copies of the wild-type SP1 tandem repeat (five repeats) were considered homozygous consensus (5/5), and participants with one or two copies of a non-5 SP1 tandem repeat were considered heterozygous variant (5/X) and homozygous variant (X/X), respectively (52).

Data Analysis

The primary analysis involved an intention-to-treat approach using all available data. All participants with baseline or follow-up data were included in the models to estimate treatment effects. Data were assumed to be missing at random. We used two sample *t* tests and analysis of covariance to determine whether the mean change in ACQ from the randomization to termination visit differed between treatment groups ($\alpha = 0.05$). Secondary outcomes that were continuous variables were analyzed similarly. In addition, we used an aligned rank test (nonparametric) to account for the multiple strata (two BMI strata × two clinic sites). For asthma exacerbations, we used negative binomial regression models. The statistical packages SAS 9.4 (SAS Institute Inc.) and STATA 11 (StataCorp, 2005) were used. Adjustments for multiple tests were made

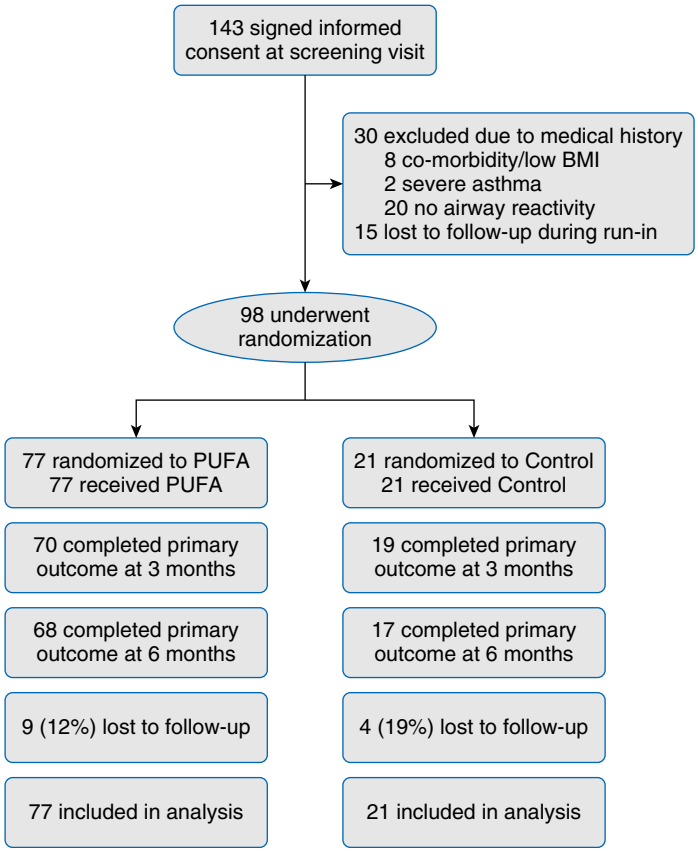


Figure 2. CONSORT Diagram of the study screening, randomization, and follow-up for overweight and obese adolescents with poorly controlled asthma. BMI = body mass index; PUFA = polyunsaturated fatty acid.

for exploratory outcomes but not for prescribed primary and secondary outcomes. No data were imputed. We assumed that 90 participants randomized in a 3:1 ratio and providing follow-up data would provide greater than 90% power to detect a 0.5-point difference in treatment group means and greater than 80% power to detect a nutrigenetic effect, with $\alpha = 0.05$ and assuming an ACQ standard deviation of 0.45. All tests were two-tailed at a level of significance of 0.05 (*see* online supplement for additional details).

Results

Characteristics of Study Participants

A total of 143 children were screened for eligibility and had caregivers sign informed consent. Ninety-eight children were randomized; 77 were assigned to n3PUFA and 21 to control soy oil (Figure 2). The baseline characteristics of study participants randomized to the two interventions were generally similar, with the following exceptions: n3PUFA-treated participants had greater baseline abdominal circumference and a higher prevalence of reported food allergies (Table 1). Among all participants, the mean age was 14.6 years, there were slightly more girls than boys, and roughly 50% of participants were African American. The mean and standard deviation for the BMI and BMI-percentile for all participants were 33.5 (7.8) and 96.8 (3.5), respectively. Participants had poor asthma control, with mean ACQ and Asthma Control Test values of 1.6 and 16.8, respectively. Nearly 70% of participants were taking National Asthma Education and Prevention Program (NAEPP) step-3 level treatment or higher to manage asthma, and roughly 80% reported allergies as a common asthma trigger (Table 1).

Recruitment and Follow-up

More than 86% of participants completed all study visits, and 88% of follow-up visits were completed. Self-reported missed days taking study drug over 24 weeks in n3PUFA and control-treated participants were similar (22 vs. 20 d, $P = 0.80$). Baseline characteristics of participants completing the study ($n = 85$) were similar to those not completing the study, with the following exceptions: noncompleters were more likely to be female (92% female vs. 46% in completers, $P = 0.002$), enrolled from the Orlando clinic (58% vs. 28%, $P = 0.049$), and have a history of panic disorder (25% vs. 5%, $P = 0.039$). Noncompleters also had significantly reduced baseline systolic (114.2 vs. 122.9, $P = 0.02$) and diastolic (66.4 vs. 73.2, $P = 0.02$) blood pressures.

Effects of n3PUFA on Circulating Leukocyte Fatty Acid Composition

The total n3 and n6PUFA concentration and n3/n6 ratios were determined in peripheral blood monocytes and granulocytes by treatment group at baseline and 3 and 6 months during the intervention period (Figure 3; *see* Table E2 in the online supplement) Participants randomized to active n3PUFA treatment showed significant increases in n3PUFA composition and n3/n6PUFA ratios in both granulocytes and monocytes compared with baseline. The changes in n3/n6PUFA ratio in both granulocytes and monocytes were significantly greater in the n3PUFA treated group than in the n6 soy oil treatment group at 3 and 6 months.

Effects of n3PUFA on Asthma Outcomes

Table 2 shows the asthma control and lung function measures and changes at 3 and 6 months during the intervention period.

Table 1. Baseline characteristics

Variables	*n*	n3PUFA	Soy Control
n		77	21
Age, yr, mean (SD)	98	14.6 (2.2)	14.6 (2.2)
Male, *n* (%)	98	37 (48)	10 (48)
Race, *n* (%)	98		
White		30 (39)	9 (43)
Black		40 (52)	10 (48)
Asian		1 (1)	0 (0)
Native American		1 (1)	0 (0)
Other		5 (6)	2 (10)
Hispanic/Latino, *n* (%)	97	20 (26)	7 (33)
Clinical center, *n* (%)	97		
Jacksonville, Florida		53 (70)	13 (62)
Orlando, Florida		23 (30)	8 (38)
Birth weight < 2.5 kg, *n* (%)	98	8 (10)	3 (14)
Birth weight, kg, mean (SD)	91	3.3 (0.7)	3.4 (0.6)
Gestational age at birth, wk, mean (SD)	96	38.2 (3.4)	38.4 (3.0)
Age of menarche, yr, mean (SD)	43	11.9 (1.3)	11.7 (1.4)
Anthropometrics, mean (SD)			
Weight in kilograms	98	91.2 (27.2)	81.6 (18.7)
Height in centimeters	98	163.2 (8.9)	162.2 (8.9)
BMI, kg/m^2	98	33.9 (8.4)	32.3 (4.9)
BMI percentile	98	96.8 (3.5)	96.9 (3.5)
Waist circumference, cm	90	102.7 (18.1)	99.2 (9.5)
Waist-to-height ratio	88	0.629 (0.104)	0.616 (0.067)
Hip circumference, cm	85	113.9 (17.6)	111.6 (8.0)
Neck circumference, cm	88	37.7 (3.9)	36.9 (3.1)
Abdominal circumference, cm	86	105.3 (17.8)	98.0 (9.7)
Activity level score, mean (SD)*	94	3.5 (1.7)	3.4 (2.0)
Blood pressure, mm Hg, mean (SD)			
Systolic	97	122.1 (12.1)	120.7 (12.9)
Diastolic	97	72.5 (9.6)	71.8 (9.2)
Age of asthma diagnosis, yr, mean (SD)	93	4.2 (4.3)	4.5 (4.9)
Baseline asthma control, mean (SD)			
Asthma Control Questionnaire	96	1.6 (0.9)	1.5 (1.1)
Asthma Control Test	95	16.7 (3.9)	17.2 (4.5)
Spirometry, mean (SD)			
FVC percent predicted	97	100.2 (13.7)	102.0 (17.3)
FEV_1% predicted	97	86.1 (20.3)	88.3 (19.4)
FEV_1/FVC	97	0.770 (0.093)	0.764 (0.091)
FEV_1 improvement post BD	88	12.1 (14.5)	13.4 (17.5)
FVC improvement post BD	88	4.1 (9.8)	6.6 (17.4)

Definition of abbreviations: BD = bronchodilator; BMI = body mass index; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PUFA = polyunsaturated fatty acid; SD = standard deviation.
*Ranges from 1 to 8 with higher score representing greater activity.

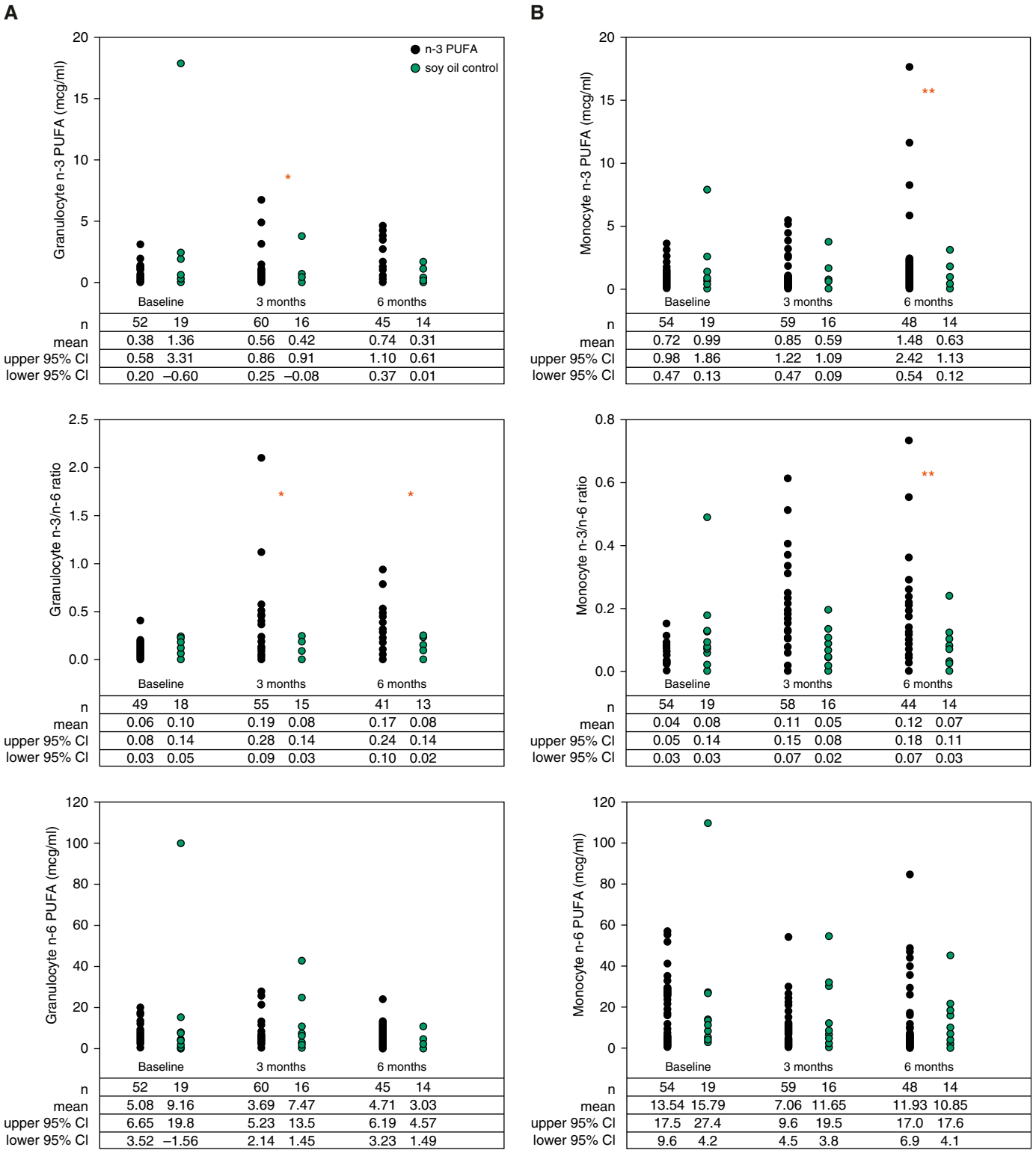


Figure 3. Total n3 and n6 polyunsaturated fatty acid (PUFA) concentration and n3/n6 ratios within peripheral blood (*A*) granulocytes and (*B*) monocytes and by treatment group at baseline and 3 and 6 months of the intervention period. *$P < 0.05$ and **$P < 0.01$ for the comparisons of 3-month and 6-month values adjusting for baseline values. CI = confidence interval.

Table 2. Asthma outcomes by treatment group

	PUFA	Control	*P* Value
ACQ			
Randomization	1.13 (0.95 to 1.31)	1.08 (0.78 to 1.39)	
Δ at 3 mo	−0.08 (−0.25 to 0.08)	−0.09 (−0.52 to 0.33)	0.95
Δ at 6 mo	−0.09 (−0.29 to 0.10)	−0.18 (−0.42 to 0.07	0.58
ACT			
Randomization	19.4 (18.5 to 20.2)	19.9 (18.2 to 21.6)	
Δ at 3 mo	0.10 (−0.95 to 1.15)	0.0 (−2.4 to 2.4)	0.93
Δ at 6 mo	0.62 (−0.35 to 1.60)	0.24 (−2.5 to 3.0)	0.74
FEV_1% predicted			
Randomization	90.2 (86.7 to 93.8)	90.3 (84.4 to 96.2)	
Δ at 3 mo	0.94 (−2.3 to 4.2)	−0.76 (−5.6 to 4.1)	0.63
Δ at 6 mo	0.55 (−2.9 to 4.0)	1.13 (−5.4 to 7.7)	0.88
FEV_1/FVC			
Randomization	0.781 (0.762 to 0.801)	0.763 (0.708 to 0.818)	
Δ at 3 mo	−0.01 (−0.02 to 0.01)	0.03 (−0.04 to 0.09)	0.37
Δ at 6 mo	0.001 (−0.01 to 0.02)	−0.01 (−0.04 to 0.03)	0.61

Definition of abbreviations: ACQ = Asthma Control Questionnaire; ACT = Asthma Control Test; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PUFA = polyunsaturated fatty acid. Data presented as mean (95% confidence interval). *P* values denote *t* test comparing change from baseline.

There were no significant differences in asthma control or lung function between treatment groups at 3 or 6 months and no significant differences in changes from baseline at either time point. Table 3 shows asthma-related events during the intervention period by treatment group. There were no significant differences in the prevalence or rate of severe exacerbations, urgent care visits for asthma, or change in controller medication. Urgent phone calls for asthma were significantly reduced among participants randomized to n3PUFA. No differences were noted for the need to step up or step down baseline asthma controller therapy.

ALOX5 sp1 Promoter Status and Nutrigenetic Treatment Response

ALOX5 promoter genotype was determined in 93 participants (Table E2). At baseline, genotypes with variant alleles were associated with significantly higher urinary LTE4 values (adjusted for creatinine) but not with other clinically meaningful inflammatory, oxidative, or asthma outcomes (Table 4). *ALOX5* genotype did not affect treatment responses to n3PUFA on the primary outcome (ACQ) or to any secondary outcomes, including lung function, urinary LTE4, or the asthma control test at 3 and 6 months.

Adverse Events

n3PUFA was well tolerated in the vast majority of participants. Nonasthma adverse events did not differ significantly between the treatment groups (Table 5). No cases of anemia, thrombocytopenia, or elevated liver enzymes were noted in either treatment group. The group treated with n3PUFA experienced a small drop in mean platelet count versus soy control (−6.0 vs. 14.2, $P = 0.052$) that did not reach statistical significance. Treatment did not affect changes from baseline in alanine aminotransferase ($P = 0.49$) or international normalized ratio ($P = 0.89$).

Discussion

This randomized, controlled clinical trial found little evidence for the efficacy of daily fish oil supplementation (4 g/d) for 24 weeks on clinically important asthma outcomes as compared with a soy oil control. The dose of n3PUFA used in the study significantly increased total n3PUFA concentration and the ratio of n3PUFA to n6PUFA in circulating leukocytes compared with soy oil control. The secondary outcomes of spirometry, Asthma Control Test, asthma exacerbations, and urgent care visits for asthma were unaffected by the n3PUFA intervention. We did see a significant reduction in urgent asthma-related phone visits in the active treatment group, but this was of uncertain clinical significance, considering that 10 of the 11 primary and secondary asthma-related outcomes tested were not affected by treatment allocation. Because of the lack of adjustment for multiple comparisons of secondary outcomes, this finding needs to be considered exploratory until confirmed in further study.

The NOOA trial was also powered to detect an *ALOX5* nutrigenetic effect of n3PUFA. Prior clinical studies have shown that the rs59439148 *ALOX5* promoter SP1 tandem repeat polymorphism affects the production of circulating cysteinyl leukotrienes and influenced response to leukotriene antagonists in patients with asthma (52–55) and thus may affect the

Table 3. Asthma-related events by treatment group

	n3 PUFA (*n* = 77)		Control (*n* = 21)		Event Rate Ratio	95% CI		*P* Value
	Total	*n* (%)	Total	*n* (%)		LL	UL	
Steroid bursts for asthma	17	13 (17)	5	4 (19)	0.92	0.30	2.89	0.89
Asthma-related episodes (*n* = 89), median (IQR)	0 (0, 0)	17 (24)	1 (0, 2)	11 (58)	0.78	0.25	2.44	0.67
Urgent clinic visit for asthma	16	13 (17)	6	5 (24)	0.72	0.26	2.00	0.53
Urgent phone call for asthma	10	9 (12)	6	5 (24)	0.34	0.13	0.86	0.02
Controller step up	—	5 (6)	—	3 (14)				0.35
Controller step down	—	5 (6)	—	0 (0)				0.58

Definition of abbreviations: CI = confidence interval; IQR = interquartile range; LL = lower limit; PUFA = polyunsaturated fatty acid; UL = upper limit. Counts reflect the number of a particular asthma-related event, unless noted. *n* (%) denotes the number and percentage of individual participants within the intervention group with at least one episode. Reference group is the group receiving soy-oil control.

Table 4. Baseline asthma characteristics by *ALOX5* promoter genotype

	Genotype				*ALOX5* × Treatment Interaction	
	5/5	5/X	X/X	*P* Value T	*P* Value (3 mo)	*P* Value (6 mo)
n	37	43	13			
ACQ	1.04 (0.76)	1.18 (0.77)	1.22 (0.76)	0.4717	0.2303	0.2565
FEV_1% predicted	87.6 (16.2)	91.4 (14.1)	89.5 (10.8)	0.6914	0.8543	0.7604
FEV_1/FVC	0.770	0.787	0.737	0.2686	0.1203	0.2463
LTE4/Cr	61.1 (46.3)	77.1 (40.8)	108.2 (25.7)	0.0012	0.8955	0.6022
C-reactive protein	2.49 (3.13)	3.70 (5.90)	3.32 (3.92)	0.5969	0.0209	0.9427

Definition of abbreviations: ACQ = Asthma Control Questionnaire; Cr = creatinine; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; LTE4 = leukotriene E4. T = *P* values represent ANOVA test for trend.
Genotype describes the number of tandem Sp1 binding motifs. Common alleles/wildtype = 5, X = non-5 number of tandem Sp1 binding motifs, 5/5 = homozygous wildtype, 5/X = heterozygous, X/X = homozygous mutant.

treatment response to n3PUFA supplementation (37). Dwyer and colleagues found that diets higher in n3PUFA associated with a significantly greater reduction in carotid intimal-medial thickness in participants with X/X homozygous variant genotype than in participants with 5/5 or 5/X, and higher n6PUFA intake associated with increased intimal-medial thickness among participants with X/X genotype and not among those with 5/5 or 5/X genotype (37). Interestingly, our results did not find a similar treatment × genotype interaction. At baseline, *ALOX5* genotype did affect circulating cysteinyl LTE4 levels, which confirmed our previous findings that variant allele carriage leads to increased cysteinyl leukotriene production (54). Many authors have posited that the mechanism by which n3PUFA supplementation may improve asthma and allergy conditions is by reducing available n6 substrate for the arachidonic acid pathway, leading to reduced proinflammatory mediators including the proasthma cysteinyl leukotrienes (56, 57). In our study, n3PUFA supplementation did lead to n3-to-n6PUFA ratio changes in both circulating monocytes and granulocytes but did not affect systemic cysteinyl leukotriene production, measured by urinary LTE4. It is possible that neither the n3PUFA concentration nor the n3/n6 ratio within leukocytes reached the required threshold to reduce leukotriene production and asthma symptoms.

A novel feature of the current study is the assessment of specific biomarkers of inflammation along with lung function and asthma control measures. Strengths of the NOOA study also included its randomized multicenter design with an ultrapurified soy oil control intervention and its focus on a high-morbidity asthma phenotype. A strength also was its "nutrigenetic" analysis of *ALOX5* and responses to circulating cysteinyl LTE4 and asthma control to assess the proposed mechanism of n3PUFA supplementation. Despite the requirement to ingest six softgel caps per day, our reported adherence was generally good, which demonstrated tangible increases in n3PUFA leukocyte levels.

The results from this study must be interpreted in the context of a number of potential limitations. It is possible that the failure to observe a significant treatment effect is attributable to inadequate statistical power or inadequate dose of n3PUFA or both. Our study was well powered ($\beta < 0.05$) to detect a 0.5-point change in ACQ (a relatively moderate effect size) at the current dose. Thus, it is possible that studies using a higher dose or longer duration are needed to fully resolve the question of n3PUFA efficacy on obese adolescents with asthma. Second, although we demonstrated significant increases in n3-to-n6PUFA ratios at 3 and 6 months, there was a wide range of observed n3 plasma membrane levels, suggesting that variable response may occur depending on baseline level of PUFA (58). In addition, the dose of n3PUFA selected in our study had previously shown improvements in airway inflammation and exercise-related lung function over shorter treatment periods (23, 24). It is possible that the dose used in the current study was not large enough to reduce leukotriene production and improve asthma control in the right population. Our population was generally sedentary. Future studies at this dose may yield better efficacy focusing on

Table 5. Adverse events reported during intervention period

	PUFA (*n* = 77)		Control (*n* = 21)		*P* Value, W	*P* Value, F
	Total	*n* (%)	Total	*n* (%)		
Headaches	91	39 (51)	19	8 (38)	0.52	0.34
Dry mouth	27	14 (18)	9	5 (24)	0.41	0.55
Nausea	40	21 (27)	8	4 (19)	0.60	0.58
Bloating	14	11 (14)	4	2 (10)	0.69	0.73
Diarrhea	13	11 (14)	7	5 (24)	0.20	0.32
Constipation	10	8 (10)	6	2 (10)	0.91	0.99
Flatulence	27	14 (18)	8	5 (24)	0.51	0.55
Rash	27	15 (19)	3	2 (10)	0.33	0.35
URI	37	31 (40)	9	8 (38)	0.96	0.99
Sore throat	25	20 (26)	10	8 (38)	0.19	0.29
Sinusitis	12	9 (12)	1	1 (5)	0.38	0.69

Definition of abbreviations: F = Fisher exact test; PUFA = polyunsaturated fatty acid; URI = upper respiratory infection; W = Wilcoxon test.
Total counts reflect total number of a particular adverse event type. *N* (%) denote the number and percent of individual participants with at least one episode.

patients with exercise-induced symptoms or those who primarily report activity limitation. Other hypothesized mechanisms for n3PUFA treatment include their precursor status as proresolving autacoids, resolvins, and protectins, which are believed to reduce inflammatory cytokine production and leukocyte chemotaxis (59). By design, the soy control group is relatively small, compared with the n3PUFA group, but the two groups did not differ in most demographic and other baseline risk factors for asthma. However, n3PUFA-treated participants had a higher mean abdominal circumference and prevalence of food allergy, although adjustments for these factors did not affect the main results. Furthermore, given that the body composition parameters such as the lean body mass and fat mass may vary in adolescents during growth and development, reliance on BMI percentiles instead of body fat percentage may have biased our interpretation of the n3PUFA intervention.

In adolescents and young adults with overweight/obesity and uncontrolled asthma, fish oil supplementation at 4 g/d increased n3PUFA concentration in peripheral blood monocytes and granulocytes. However, these enhancements did not translate to a measurable reduction in LTE4 production, asthma control, or most secondary outcomes. These findings do not support a strategy of therapeutic n3PUFA supplementation in these patients with symptomatic asthma. ■

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