Fish Oil supplementation in Overweight/Obese Patients with Uncontrolled Asthma: a Randomized Trial

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Abstract

Introduction. Omega-3 fatty acid (n3PUFA) supplementation has been proposed as a promising anti-asthma strategy. The rs59439148 *ALOX5* polymorphism affects leukotriene production and possibly inflammatory responses to n3PUFA. No studies have assessed n3PUFA supplementation and *ALOX5* genotype on asthma control in patients with obesity and uncontrolled asthma.

Methods. This multi-center trial among 12-25 year olds with overweight/obesity and uncontrolled asthma randomized subjects in a 3:1 allotment to n3PUFA (4g/day) or soy oil control for 24 weeks. Asthma Control Questionnaire (ACQ) was the primary outcome; secondary outcomes included blood leukocyte n3PUFA levels, urinary leukotriene-E4 (uLTE4), 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 > 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 (95% CI) ACQ change at 6 months (N3PUFA: -0.09 (-.09, .10) vs. control: -0.18 (-.42, .06), p=0.58). Changes in uLTE (p=0.24), FEV1 percent predicted (p=0.88) and exacerbations (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.

Conclusion. 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.

Introduction

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 anti-asthma controller medication currently available. A diet low in fresh vegetables and fish and high in saturated fats and n-6 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 pre-clinical 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 Alayee 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 *Nutrigenetic response to Omega-3 Fatty acids in Obese Asthmatics* (NOOA) trial was designed as a randomized, double-blind, placebo controlled 24 week intervention study to determine if supplemental omega-3 fatty acids improves symptoms among adolescents and young adults with overweight/obesity and uncontrolled asthma. NOOA measured change in asthma control questionnaire 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 Supplementary Appendix (available with the full text of this article online). The NOOA study was a multi-center, 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 (see Fig 1). Randomization was stratified by study site and BMI strata (I: BMI-percentile 85-94, II: BMI-percentile≥95) using a randomization scheme generated using the SAS procedure PROC PLAN. 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 of age 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 beta-agonist >twice/week on average over the past month; ≥1 nocturnal awakenings/week on average over the past month; ≥ 2 emergency room (ER) visits, unscheduled physician visits, prednisone courses, or hospitalizations for asthma (in the past 12 months); or an asthma control questionnaire score ≥1.25 at screening. Overweight and obesity status were defined using age-appropriate CDC definitions based on body mass index (BMI)(39). In addition, participants had to have a waist-circumference above the 90th percentile for age and sex. Asthma diagnosis was confirmed by evidence of either bronchodilator reversibility (forced expiratory volume in the first second of expiration [FEV1] ≥ 12% following 360 μg [4 puffs] of albuterol) or airway hyperresponsiveness

(provocative concentration of methacholine at which FEV1 decreased by 20% [PC20] ≤16mg/mL).

Treatment. Participants were randomized to either oral n3PUFA supplementation (3.18g EPA, 822mg DHA, 101mg 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., Watsonville, CA USA), 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, Guelph, Canada). Both n-3PUFA and soy oil placebo had identical look, taste and texture.

Outcome measures. The primary outcome was change in the Asthma Control Questionnaire (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 > 1.25 in children is considered poor asthma control and a change of 0.4 or greater is considered a clinically meaningful (42). Asthma symptoms were also evaluated using the Asthma Control Test (ACT)(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 (ER 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 (FVC) and forced expiratory volume in 1 second (FEV1)) 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, Jacksonville, FL). Urinary LTE4 was measured by liquid chromatography tandem mass spectrometry (LC MS-MS)(46, 47). Omega-3

and omega-6 PUFA content in histiopaque isolated peripheral blood monocytes and granulocytes (48-50) was measured using gas chromatography mass spectrometry (GCMS) after derivation to respective fatty acid methyl esters as previously described (51). Alanine aminotransferase (ALT), platelet count, and the international normalized ratio (INR) werechecked 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 (HWE) between expected and observed genotype distributions was calculated using χ^2 goodness-of-fit tests. Participants with two copies of the wildtype SP1 tandem repeat (5 repeats) were considered homozygous consensus (5/5), while participants with 1 or 2 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 utilizing 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 utilized two sample t-tests and ANCOVA to determine whether the mean change in ACQ from the randomization to termination visit differed between treatment groups (α=.05). Secondary outcomes that were continuous variables were analyzed similarly. Additionally, we used an aligned rank test (non-parametric) test to account for the multiple strata (two BMI strata X 2 clinic sites). For asthma exacerbations, we used negative binomial regression models. The statistical packages SAS 9.4 (SAS Institute Inc, Cary NC, USA) and STATA 11 (College Station, TX: StataCorp, 2005) were used. Adjustments for multiple tests were made 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 >90% power to detect a 0.5-point difference in treatment group means and >80% power to detect a nutrigenetic effect, with α=0.05 and assuming an ACQ standard deviation=0.45. All tests were two-tailed at a level of significance of 0.05 (see online supplement for additional details).

Trial oversight

The trial was funded by the National Heart, Lung, and Blood Institute and the Office of Dietary Supplements and approved by the NOOA data and safety monitoring committee. Nordic Naturals, Inc. did not play a role in the trial design or the collection or interpretation of the data. The authors are responsible for the trial design, data collection, data interpretation and analysis, manuscript preparation, and decision to submit the manuscript for publication. The authors vouch for the accuracy and completeness of the data, for the accuracy of the analyses, and for the fidelity of the trial to the protocol.

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 (Fig 2). The baseline characteristics of study participants randomized to the two interventions were generally similar, with the exceptions: n3PUFA treated participants had greater baseline abdominal circumference and a higher prevalence of reported food allergies (see 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 body mass index (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 ACT values of 1.6 and 16.8, respectively. Nearly 70% of participants were taking NAEPP step-3 level treatment or higher to manage asthma, and roughly 80% reported allergies as a common asthma trigger. Co-morbid conditions were frequent in the study population including sleep apnea, nightly snoring, gastroesophageal reflux (GERD), and anxiety/depression (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 n3-PUFA and control-treated were similar (22 vs. 20 days, p=.80). Baseline characteristics of participants completing the study (n=85) were similar to those not completing the study, with the following exceptions: non-completers were more likely to be female (92% female vs. 46% in completers, p=.002), enrolled from the Orlando clinic (58% vs. 28%, p=.049), and have a history of panic disorder (25% vs. 5%, p=.039). Non-completers also had significantly reduced baseline systolic (114.2 vs. 122.9, p=.02) and diastolic (66.4 vs. 73.2, p=.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, Table e2) Participants randomized to active n3PUFA treatment showed significant increases in n3PUFA composition and n-3/n6PUFA ratios in both granulocytes and monocytes compared to baseline. The changes in n-3/n6PUFA ratio in both granulocytes and monocytes were significantly greater in the n3PUFA treated group compared to the n-6 soy oil treatment group at 3 and 6 months.

Effects of n3PUFA on Asthma Outcomes

Table 3 shows the asthma control and lung function measures and changes at 3 and 6 months during the intervention period. 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 4 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 was significantly reduced among participants randomized to n-PUFA. 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 (eTable 2). 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 5). *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. Non-asthma adverse events did not differ significantly between the treatment groups (Table 6). 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 ALT (p=0.49) or INR (p=0.89).

Discussion

This randomized, controlled clinical trial found little evidence for the efficacy of daily fish oil supplementation (4g/day) 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 n3 PUFA to n6 PUFA in circulating leukocytes compared to 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 asthmatics (52-55) and thus may affect the treatment response to n3PUFA supplementation(37). Dwyer et al 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 compared to participants with 5/5 or 5/X; while higher n6PUFA intake associated with increased IMT 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 n-6 substrate for the arachidonic acid pathway, leading to reduced pro-inflammatory mediators including the pro-asthma cysteinyl leukotrienes(56, 57). In our study, n3-PUFA supplementation did lead to n-3 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 multi-center design with an ultra-purified 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 n-3 plasma membranes 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 patients with exercise-induced symptoms or those who report primarily report activity limitation. Other hypothesized mechanisms for n3PUFA treatment include their precursor status as pro-resolving autacoids, resolvins and protectins which are thought to reduce inflammatory cytokine production and leukocyte chemotaxis(59). By design, the soy-control group is relatively small, compared to the n3-PUFA 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, though adjustments for these factors did not affect the main results. Further, 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 4g/day increased n3PUFA concentration in peripheral blood monocytes and granulocytes. However, these enhancements did not translate to a measurable reduction in LTE4 production, asthma control, nor most secondary outcomes. These findings do not support a strategy of therapeutic n3PUFA supplementation in these patients with symptomatic asthma.

Figure Legends

Figure 1. Study diagram and procedures. Screening included informed consent and medical history collection, Eligibility, inclusion/exclusion criteria assessed for run-in and randomization, ACQ – asthma control questionnaire, Phone visits occurred 2, 6, 10, 16 and 20 weeks after visit 3, +, procedure was performed. * - urinary LTE4, exhaled nitric oxide, blood for n3/n6 ratio.

Figure 2. CONSORT Diagram of the Study Screening, Randomization and Follow-up for Overweight and Obese adolescents with poorly controlled asthma. PUFA – polyunsaturated fatty acid.

Figure 3. Total n3 and n6PUFA concentration and n3/n6 ratios within peripheral blood granulocytes (A) and monocytes (B) and by treatment group at baseline and 3 and 6 months of the intervention period. ** - represent $p<0.01$ * - represent $p<0.05$ for the comparisons of 3 month and 6 month values adjusting for baseline values.

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References

1. Stream AR, Sutherland ER. Obesity and asthma disease phenotypes. Curr Opin Allergy Clin Immunol. 2012;12(1):76-81.
2. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med. 2012;18(5):716-25.
3. Lang JE, Fitzpatrick AM, Mauger DT, Guilbert TW, Jackson DJ, Lemanske RF, Jr., et al. Overweight/obesity status in preschool children associates with worse asthma but robust improvement on inhaled corticosteroids. J Allergy Clin Immunol. 2017.
4. Lang JE, Hossain MJ, Lima JJ. Overweight children report qualitatively distinct asthma symptoms: analysis of validated symptom measures. J Allergy Clin Immunol. 2015;135(4):886-93 e3.
5. Guilbert TW, Bacharier LB, Fitzpatrick AM. Severe asthma in children. J Allergy Clin Immunol Pract. 2014;2(5):489-500.
6. Camargo CA, Jr., Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. Arch Intern Med. 1999;159(21):2582-8.
7. Gold DR, Damokosh AI, Dockery DW, Berkey CS. Body-mass index as a predictor of incident asthma in a prospective cohort of children. Pediatr Pulmonol. 2003;36(6):514-21.
8. Quinto KB, Zuraw BL, Poon KY, Chen W, Schatz M, Christiansen SC. The association of obesity and asthma severity and control in children. J Allergy Clin Immunol. 2011;128(5):964-9.
9. Forno E, Lescher R, Strunk R, Weiss S, Fuhlbrigge A, Celedon JC. Decreased response to inhaled steroids in overweight and obese asthmatic children. J Allergy Clin Immunol. 2011;127(3):741-9.
10. Sutherland ER, Goleva E, Strand M, Beuther DA, Leung DY. Body mass and glucocorticoid response in asthma. Am J Respir Crit Care Med. 2008;178(7):682-7.
11. Pereira MA, Kartashov AI, Ebbeling CB, Van Horn L, Slattery ML, Jacobs DR, Jr., et al. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet. 2005;365(9453):36-42.
12. Nagel G, Weinmayr G, Kleiner A, Garcia-Marcos L, Strachan DP. Effect of diet on asthma and allergic sensitisation in the International Study on Allergies and Asthma in Childhood (ISAAC) Phase Two. Thorax. 2010;65(6):516-22.
13. Yadav UC, Srivastava SK. Cysteinyl Leukotrienes (CysLTs): Role in Obesity-Induced Asthma. Curr Mol Med. 2015;15(7):598-605.
14. Herxheimer H, Schaefer O. Letter: Asthma in Canadian Eskimos. N Engl J Med. 1974;291(26):1419.
15. Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950-1974. Acta Med Scand. 1980;208(5):401-6.
16. Bang HO, Dyerberg J, Hjoorne N. The composition of food consumed by Greenland Eskimos. Acta Med Scand. 1976;200(1-2):69-73.
17. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med. 1985;312(19):1205-9.
18. Nagakura T, Matsuda S, Shichijyo K, Sugimoto H, Hata K. Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. Eur Respir J. 2000;16(5):861-5.
19. Hodge L, Salome CM, Hughes JM, Liu-Brennan D, Rimmer J, Allman M, et al. Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children. Eur Respir J. 1998;11(2):361-5.
20. Surette ME, Koumenis IL, Edens MB, Tramposch KM, Clayton B, Bowton D, et al. Inhibition of leukotriene biosynthesis by a novel dietary fatty acid formulation in patients with atopic asthma: a randomized, placebo-controlled, parallel-group, prospective trial. Clin Ther. 2003;25(3):972-9.

21. Mihrshahi S, Peat JK, Webb K, Oddy W, Marks GB, Mellis CM. Effect of omega-3 fatty acid concentrations in plasma on symptoms of asthma at 18 months of age. Pediatr Allergy Immunol. 2004;15(6):517-22.
22. Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr. 1996;63(1):116-22.
23. Arm JP, Horton CE, Mencia-Huerta JM, House F, Eiser NM, Clark TJ, et al. Effect of dietary supplementation with fish oil lipids on mild asthma. Thorax. 1988;43(2):84-92.
24. Mickleborough TD, Lindley MR, Ionescu AA, Fly AD. Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. Chest. 2006;129(1):39-49.
25. Mickleborough TD, Murray RL, Ionescu AA, Lindley MR. Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. Am J Respir Crit Care Med. 2003;168(10):1181-9.
26. Romieu I, Tellez-Rojo MM, Lazo M, Manzano-Patino A, Cortez-Lugo M, Julien P, et al. Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. Am J Respir Crit Care Med. 2005;172(12):1534-40.
27. Surette ME, Koumenis IL, Edens MB, Tramposch KM, Chilton FH. Inhibition of leukotriene synthesis, pharmacokinetics, and tolerability of a novel dietary fatty acid formulation in healthy adult subjects. Clin Ther. 2003;25(3):948-71.
28. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr. 1997;65(4):1011-7.
29. Reisman J, Schachter HM, Dales RE, Tran K, Kourad K, Barnes D, et al. Treating asthma with omega-3 fatty acids: where is the evidence? A systematic review. BMC Complement Altern Med. 2006;6:26.
30. Hodge AM, Dowse GK, Gareeboo H, Tuomilehto J, Alberti KG, Zimmet PZ. Incidence, increasing prevalence, and predictors of change in obesity and fat distribution over 5 years in the rapidly developing population of Mauritius. Int J Obes Relat Metab Disord. 1996;20(2):137-46.
31. Marks GB, Mihrshahi S, Kemp AS, Tovey ER, Webb K, Almqvist C, et al. Prevention of asthma during the first 5 years of life: a randomized controlled trial. J Allergy Clin Immunol. 2006;118(1):53-61.
32. Emelyanov A, Fedoseev G, Krasnoschekova O, Abulimity A, Trendeleva T, Barnes PJ. Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomised clinical trial. Eur Respir J. 2002;20(3):596-600.
33. Okamoto M, Mitsunobu F, Ashida K, Mifune T, Hosaki Y, Tsugeno H, et al. Effects of perilla seed oil supplementation on leukotriene generation by leucocytes in patients with asthma associated with lipometabolism. Int Arch Allergy Immunol. 2000;122(2):137-42.
34. Villani F, Comazzi R, De Maria P, Galimberti M. Effect of dietary supplementation with polyunsaturated fatty acids on bronchial hyperreactivity in subjects with seasonal asthma. Respiration. 1998;65(4):265-9.
35. Dry J, Vincent D. Effect of a fish oil diet on asthma: results of a 1-year double-blind study. Int Arch Allergy Appl Immunol. 1991;95(2-3):156-7.
36. Arm JP, Horton CE, Spur BW, Mencia-Huerta JM, Lee TH. The effects of dietary supplementation with fish oil lipids on the airways response to inhaled allergen in bronchial asthma. Am Rev Respir Dis. 1989;139(6):1395-400.
37. Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. N Engl J Med. 2004;350(1):29-37.
38. Lang JE, Mougey EB, Allayee H, Blake KV, Lockey R, Gong Y, et al. Nutrigenetic response to omega-3 fatty acids in obese asthmatics (NOOA): rationale and methods. Contemp Clin Trials. 2013;34(2):326-35.
39. Barlow SE. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics. 2007;120 Suppl 4:S164-92.

40. Juniper EF, Gruffydd-Jones K, Ward S, Svensson K. Asthma Control Questionnaire in children: validation, measurement properties, interpretation. Eur Respir J. 2010;36(6):1410-6.
41. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J. 1999;14(4):902-7.
42. Nguyen JM, Holbrook JT, Wei CY, Gerald LB, Teague WG, Wise RA, et al. Validation and psychometric properties of the Asthma Control Questionnaire among children. J Allergy Clin Immunol. 2014;133(1):91-7 e1-6.
43. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol. 2006;117(3):549-56.
44. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med. 2009;180(1):59-99.
45. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36.
46. Severien C, Artlich A, Jonas S, Becher G. Urinary excretion of leukotriene E4 and eosinophil protein X in children with atopic asthma. Eur Respir J. 2000;16(4):588-92.
47. Mai XM, Bottcher MF, Bruhammar M, Nilsson L, Zetterstrom O. Urinary inflammatory mediators and inhalation of hypertonic saline in children. Allergy. 2005;60(1):60-4.
48. Slifkin M, Cumbie R. Comparison of the Histopaque-1119 method with the Plasmagel method for separation of blood leukocytes for cytomegalovirus isolation. J Clin Microbiol. 1992;30(10):2722-4.
49. Boyum A. Separation of leukocytes from blood and bone marrow. Introduction. Scand J Clin Lab Invest Suppl. 1968;97:7.
50. English D, Andersen BR. Single-step separation of red blood cells. Granulocytes and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. J Immunol Methods. 1974;5(3):249-52.
51. Guijas C, Perez-Chacon G, Astudillo AM, Rubio JM, Gil-de-Gomez L, Balboa MA, et al. Simultaneous activation of p38 and JNK by arachidonic acid stimulates the cytosolic phospholipase A2-dependent synthesis of lipid droplets in human monocytes. J Lipid Res. 2012;53(11):2343-54.
52. Lima JJ, Zhang S, Grant A, Shao L, Tantisira KG, Allayee H, et al. Influence of leukotriene pathway polymorphisms on response to montelukast in asthma. Am J Respir Crit Care Med. 2006;173(4):379-85.
53. Tantisira KG, Drazen JM. Genetics and pharmacogenetics of the leukotriene pathway. J Allergy Clin Immunol. 2009;124(3):422-7.
54. Mougey E, Lang JE, Allayee H, Teague WG, Dozor AJ, Wise RA, et al. ALOX5 polymorphism associates with increased leukotriene production and reduced lung function and asthma control in children with poorly controlled asthma. Clin Exp Allergy. 2013;43(5):512-20.
55. Telleria JJ, Blanco-Quiros A, Varillas D, Armentia A, Fernandez-Carvajal I, Jesus Alonso M, et al. ALOX5 promoter genotype and response to montelukast in moderate persistent asthma. Respir Med. 2008;102(6):857-61.
56. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans. 2017;45(5):1105-15.
57. Kumar A, Mastana SS, Lindley MR. n-3 Fatty acids and asthma. Nutr Res Rev. 2016;29(1):1-16.
58. Tremblay BL, Cormier H, Rudkowska I, Lemieux S, Couture P, Vohl MC. Association between polymorphisms in phospholipase A2 genes and the plasma triglyceride response to an n-3 PUFA supplementation: a clinical trial. Lipids Health Dis. 2015;14:12.
59. Mickleborough TD, Lindley MR. Omega-3 fatty acids: a potential future treatment for asthma? Expert Rev Respir Med. 2013;7(6):577-80.

Table 1: Baseline Characteristics

Variables	n	n3 PUFA	Soy Control
n		77	21
Age in years, 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)
Birthweight<2.5kg, n (%)	98	8 (10)	3 (14)
Birth weight in kilograms, mean (SD)	91	3.3 (0.7)	3.4 (0.6)
Gestational age at birth in weeks, mean (SD)	96	38.2 (3.4)	38.4 (3.0)
Age of menarche in years, 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/m2	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	.629 (.104)	.616 (.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)[1]	94	3.5 (1.7)	3.4 (2.0)
Blood Pressure in mmHg, 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 in years, mean (SD)	93	4.2 (4.3)	4.5 (4.9)
Baseline Asthma Control, mean (SD)			
Asthma Control Questionnaire	96	1.6 (.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)
FEV1 percent predicted	97	86.1 (20.3)	88.3 (19.4)
FEV1/FVC	97	.770 (.093)	.764 (.091)
FEV1 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)

Table 2: Asthma Outcomes by Treatment Group

	PUFA	Control	p-value[1]
ACQ, mean (95% CI)			
Randomization	1.13 (0.95, 1.31)	1.08 (0.78, 1.39)	
Δ at 3 months	-0.08 (-0.25, 0.08)	-0.09 (-0.52, 0.33)	.95
Δ at 6 months	-0.09 (-0.29, 0.10)	-0.18 (-0.42, 0.07	.58
ACT, mean (95% CI)			
Randomization	19.4 (18.5, 20.2)	19.9 (18.2, 21.6)	
Δ at 3 months	0.10 (-0.95, 1.15)	0.0 (-2.4, 2.4)	.93
Δ at 6 months	0.62 (-0.35, 1.60)	0.24 (-2.5, 3.0)	.74
FEV1 percent predicted, mean (95% CI)			
Randomization	90.2 (86.7, 93.8)	90.3 (84.4, 96.2)	
Δ at 3 months	0.94 (-2.3, 4.2)	-0.76 (-5.6, 4.1)	.63
Δ at 6 months	0.55 (-2.9, 4.0)	1.13 (-5.4, 7.7)	.88
FEV1/FVC, mean (95% CI)			
Randomization	0.781 (0.762, 0.801)	0.763 (0.708, 0.818)	
Δ at 3 months	-0.01 (-0.02, 0.01)	0.03 (-0.04, 0.09)	.37
Δ at 6 months	.001 (-0.01, 0.02)	-0.01 (-0.04, 0.03)	.61
ACQ – Asthma Control Questionnaire, ACT – Asthma Control Test, PUFA – polyunsaturated Fatty Acid, FVC – forced vital capacity, FEV1 – forced expiratory volume in 1 seconds. P-values denote t-test comparing change from baseline.			

Table 3. Asthma-related Events by Treatment Group

	n3 PUFA (n=77)		Control (n=21)		Event Rate Ratio	95% CI		
	total	N (%)	total	n (%)		LL	UL	p-value
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

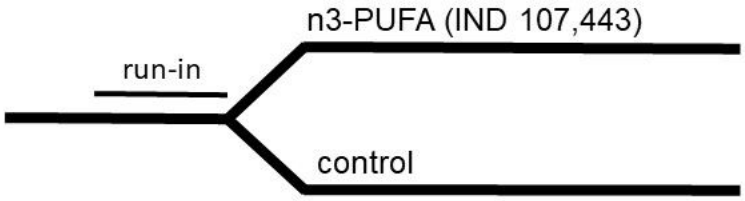
Counts reflect the number of a particular asthma-related event, unless noted. N (%) denote the number and percent of individual participants within the intervention group with at least one episode. Reference group is the group receiving soy-oil control. IQR – intra-quartile range, PUFA – polyunsaturated fatty acid, CI – confidence interval, LL – lower limit, UL – upper limit.

Table 4. Baseline Asthma Characteristics by ALOX5 promoter Genotype

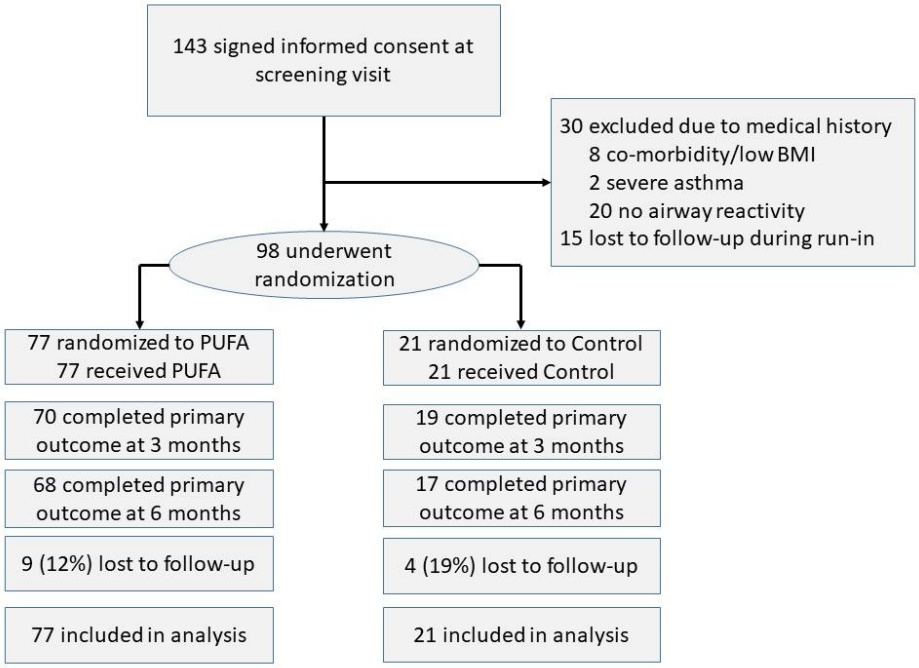
	Genotype				ALOX5*Treatment Interaction	
	5/5	5/x	x/x	p-valueT	p-value (3 months)	p-value (6 months)
n	37	43	13			
ACQ	1.04 (0.76)	1.18 (0.77)	1.22 (0.76)	0.4717	0.2303	0.2565
FEV1 percent predicted	87.6 (16.2)	91.4 (14.1)	89.5 (10.8)	0.6914	0.8543	0.7604
FEV1/FVC	0.770	0.787	0.737	0.2686	0.1203	0.2463
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
Genotype describes the number of tandem Sp1 binding motifs. Common allele=5 ACQ – asthma control questionnaire, FEV1 – forced expiratory volume in 1 second, FVC – forced vital capacity, LTE – leukotriene E4, EBC – exhaled breath condensate						

Table 5. Adverse Events Reported During Intervention Period

	PUFA (n=77)		Control (n=21)			
	total	N (%)	total	N (%)	p-valueW	p-valueF
Headaches	91	39 (51)	19	8 (38)	.52	.34
Dry mouth	27	14 (18)	9	5 (24)	.41	.55
Nausea	40	21 (27)	8	4 (19)	.60	.58
Bloating	14	11 (14)	4	2 (10)	.69	.73
Diarrhea	13	11 (14)	7	5 (24)	.20	.32
Constipation	10	8 (10)	6	2 (10)	.91	.99
Flatulence	27	14 (18)	8	5 (24)	.51	.55
Rash	27	15 (19)	3	2 (10)	.33	.35
URI	37	31 (40)	9	8 (38)	.96	.99
Sore throat	25	20 (26)	10	8 (38)	.19	.29
Sinusitis	12	9 (12)	1	1 (5)	.38	.69
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. W – Wilcoxon test, F – Fisher exact test.						



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


143 signed informed consent at screening visit
30 excluded due to medical history
8 co-morbidity/low BMI
2 severe asthma
20 no airway reactivity
15 lost to follow-up during run-in
98 underwent randomization
77 randomized to PUFA
77 received PUFA
21 randomized to Control
21 received Control
70 completed primary outcome at 3 months
19 completed primary outcome at 3 months
68 completed primary outcome at 6 months
17 completed primary outcome at 6 months
9 (12%) lost to follow-up
4 (19%) lost to follow-up
77 included in analysis
21 included in analysis

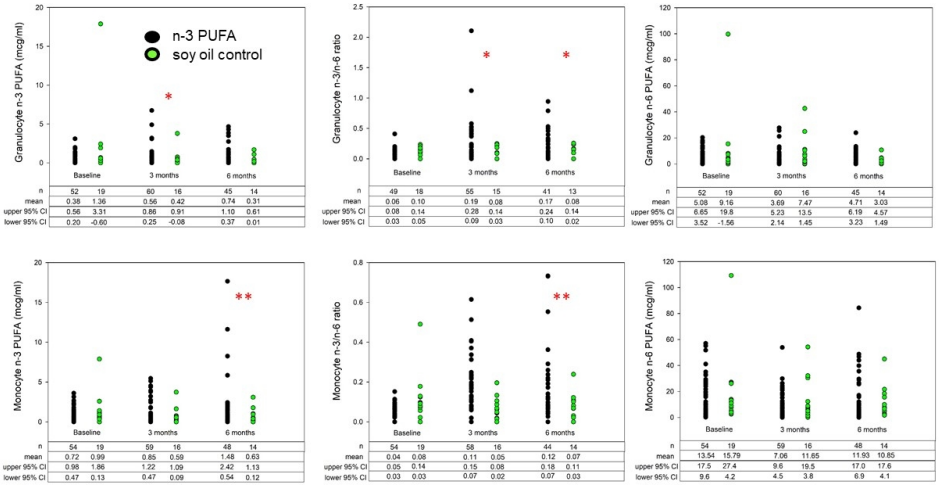


Figure 3. Total n3 and n6PUFA concentration and n3/n6 ratios within peripheral blood granulocytes (A) and monocytes (B) and by treatment group at baseline and 3 and 6 months of the intervention period. ** - represent p<0.01 * - represent p<0.05 for the comparisons of 3 month and 6 month values adjusting for baseline values.

338x190mm (96 x 96 DPI)

Table e1: Characteristics of Participants lost to follow-up			
Variables	Completers	Lost to follow-up	p-value[F]
n	N=85	N=13	
Age in years, mean (SD)	14.6 (2.1)	14.2 (1.7)	.533
Male, n (%)	46 (54)	1 (8)	.002
Race, n (%)			
White	35 (41)	4 (31)	.520[F]
Black	43 (51)	7 (54)	
Asian	1 (1)	0 (0)	
Native American	1 (1)	0 (0)	
Other	5 (6)	2 (15)	
Hispanic/Latino, n (%)	22 (26)	5 (38)	.338[F]
Clinical Center, n (%)			.049[F]
Jacksonville	61 (72)	5 (42)	
Orlando	24 (28)	7 (58)	
Birthweight<2.5kg, n (%)	10 (12)	1 (9)	>.999[F]
Birth weight in kilograms, mean (SD)	3.4 (.60)	3.3 (.80)	.661
Gestational age at birth in weeks, mean (SD)	38.2 (3.3)	39.0 (3.4)	.431
Age of menarche in years, mean (SD)	11.9 (1.4)	11.9 (1.2)	.944
Anthropometrics, mean (SD)			
Weight in kilograms	88.9 (26.3)	90.1 (23.1)	.890
Height in centimeters	163.2 (9.0)	162.3 (8.6)	.741
BMI, kg/m2	33.5 (8.0)	33.9 (6.5)	.883
BMI-percentile	96.7 (3.6)	97.2 (3.3)	.642
Waist circumference, cm	102.0 (17.0)	101.6 (13.8)	.952
Waist-to-height ratio	.63 (.10)	.63 (.10)	.913
Hip circumference, cm	113.6 (16.0)	111.4 (16.0)	.704
Neck circumference, cm	37.5 (3.8)	37.2 (3.3)	.807
Abdominal circumference, cm	104.1 (16.8)	100.5 (14.4)	.567
Activity level score, mean (SD)[1]	3.5 (1.7)	3.1 (2.0)	.476
BP in mmHg, mean (SD)			
Systolic	122.9 (11.9)	114.2 (12.5)	.020
Diastolic	73.2 (9.6)	66.4 (6.5)	.020
Age of asthma diagnosis in years, mean (SD)	4.2 (4.3)	4.5 (5.6)	.812
Baseline Asthma Control, mean (SD)			
Asthma Control Questionnaire	1.50 (.82)	2.31 (1.3)	.070
Asthma Control Test	17.0 (3.8)	14.9 (5.5)	.235
Spirometry, mean (SD)			
FVC percent predicted	100.8 (13.0)	99.3 (23.2)	.823
FEV1 percent predicted	87.6 (17.1)	79.2 (34.3)	.419
FEV1/FVC	.773 (.087)	.741 (.126)	.401
FEV1 improvement post bronchodilator	11.2 (13.1)	27.5 (28.9)	.230
FVC improvement post bronchodilator	4.1 (10.7)	12.3 (20.7)	.380

Table I Continued

Variables - continued	Completers	Lost follow-up	p-value[F]
	N=85	N=13	
Controller medication, n (%)			.027[F]
Step 2	21 (25)	4 (36)	
Step 3	27 (33)	4 (36)	
Step 4	28 (34)	0 (0)	
Step 5	7 (8)	3 (27)	
Family history of asthma, n (%)	61 (72)	10 (77)	>.999
Allergies worsen asthma, n (%)	70 (82)	10 (77)	.702
Co-morbid conditions, n (%)			
Hay Fever	26 (31)	1 (8)	.105
Food Allergy	24 (28)	5 (38)	.518
Snoring	65 (76)	11 (85)	.727
Sleep Apnea	27 (32)	2 (15)	.334
CPAP/BiPAP	5 (6)	0 (0)	>.999
GERD	17 (20)	3 (23)	.725
Depression	20 (24)	6 (46)	.100
Anxiety	21 (25)	4 (31)	.734
Panic	4 (5)	3 (25)	.039
Hyperventilation	3 (4)	2 (15)	.129
Vocal cord dysfunction	2 (2)	0 (0)	>.999
Diabetes			
Type 1	1 (1)	0 (0)	>.999
Type 2	2 (2)	1 (8)	.351
Former smoker, n (%)	2 (2)	0 (0)	>.999
Smoke exposure, n (%)			
Home	16 (19)	1(8)	.456
Other	19 (22)	1 (8)	.294

Values are means (SD) or counts (%) as noted. n-3 PUFA – omega 3 polyunsaturated fatty acid, SD – standard deviation, BMI – body mass index, kg – kilograms, cm – centimeters, GERD – gastroesophageal reflux disease, CPAP – continuous positive airway pressure, BiPAP – bi-level positive airway pressure, NAEPP – National Asthma Education & Prevention Program, F – Fisher exact test was used to compare ratios. 1 – activity level score ranges from 1 to 6 with a higher score denoting greater daily activity.

Table e2: Leukocyte composition of n3 and n6 fatty acids at baseline and 3 and 6 months												
	Baseline				3 months				6 months			
Granulocytes	n	PUFA	Control	p-value	n	PUFA	Control	p-value	n	PUFA	Control	p-value
Total n6 (ug/ml)	52/19	5.08 (5.64)	9.16 (22.23)	0.223	60/16	3.69 (5.98)	7.47 (11.30)	0.517	45/14	4.71 (4.93)	3.03 (2.67)	0.073
Total n3 (ug/ml)	52/19	0.38 (0.65)	1.36 (4.06)	0.094	60/16	0.56 (1.19)	0.42 (0.93)	0.031	45/14	0.74 (1.22)	0.31 (0.51)	**0.011**
n3/n6 ratio	49/18	0.06 (0.08)	0.10 (0.09)	0.087	55/15	0.19 (0.34)	0.08 (0.10)	**0.088**	41/13	0.17 (0.23)	0.08 (0.10)	**0.029**
Monocytes												
Total n6 (ug/ml)	54/19	13.54 (14.60)	15.79 (24.02)	0.630	59/16	7.06 (9.90)	11.65 (14.75)	0.397	48/14	11.93 (17.46)	10.86 (11.63)	0.699
Total n3 (ug/ml)	54/19	0.73 (0.93)	0.99 (1.79)	0.412	59/16	0.85 (1.45)	0.59 (0.94)	0.036	48/14	1.48 (3.23)	0.63 (0.87)	0.300
n3/n6 ratio	54/19	0.04 (0.04)	0.08 (0.11)	0.014	58/16	0.11 (0.15)	0.05 (0.06)	**0.003**	44/14	0.12 (0.18)	0.07 (0.07)	**0.004**
Values are means (SD), p-values represent differences in change from baseline, n3 – omega 3, n6 – omega 6												

Table e3. Genotype Frequencies of Sp1-binding motifs in the ALOX5 promoter by Treatment Group				
	All	N3-PUFA	Soy Oil	p-value
Genotype, n (%)				.0546
3/3	5 (5.38)	5 (6.9)	0 (0)	
4/3	6 (6.45)	2 (2.8)	4 (19.1)	
5/3	21 (22.58)	17 (23.6)	4 (19.1)	
5/4	20 (21.51)	18 (25.0)	2 (9.5)	
5/5	37 (39.78)	28 (38.9)	9 (42.9)	
6/4	2 (2.15)	1 (1.4)	1 (4.8)	
6/5	2 (2.15)	1 (1.4)	1 (4.8)	
totals	93 (100)	72 (77.4)	21 (22.6)	
Totals combined, n (%)	All	N3-PUFA	Soy Oil	.2344
5/5	37 (39.8)	28 (38.9)	9 (42.9)	
5/X	43 (46.2)	36 (50.0)	7 (33.3)	
X/X	13 (14.0)	8 (11.1)	5 (23.8)	
totals	93 (100)	72 (77.4)	21 (22.6)	
Genotype describes the number of tandem Sp1 binding motifs. Common allele=5				

Table e4: Additional Baseline Characteristics

Variables - continued	n3 PUFA	Soy Control	p-value[F]
Controller medication, n (%)			0.7561
Step 2	21 (28.8)	4 (19.1)	
Step 3	24 (32.9)	7 (33.3)	
Step 4	20 (27.4)	8 (38.1)	
Step 5	8 (11.0)	2 (9.5)	
Family history of asthma, n (%)	56 (72.7)	15 (71.4)	>0.999
Allergies worsen asthma, n (%)	63 (81.8)	17 (81.0)	>0.999
Co-morbid conditions, n (%)			
Hay Fever	22 (28.6)	5 (23.8)	0.7869
Snoring	60 (77.9)	16 (76.2)	>0.999
Sleep Apnea	23 (29.9)	6 (28.6)	>0.999
CPAP/BiPAP	4 (5.2)	1 (4.8)	>0.999
GERD	15 (19.5)	5 (23.8)	0.7609
Depression	21 (27.3)	5 (23.8)	>0.999
Anxiety	20 (26.0)	5 (23.8)	>0.999
Panic	3 (4.0)	4 (19.1)	0.0376
Hyperventilation	4 (5.2)	1 (4.8)	>0.999
Vocal cord dysfunction	2 (2.6)	0 (0)	>0.999
Diabetes			
Type 1	1 (1.3)	0 (0)	>0.999
Type 2	2 (2.6)	1 (4.8)	0.5191
Former smoker, n (%)	2 (2.6)	0 (0)	>0.999
Smoke exposure, n (%)			
Home	13 (16.9)	4 (19.1)	0.7556
Other	17 (22.1)	3 (14.3)	0.5511

Values are means (SD) or counts (%) as noted. n-3 PUFA – omega 3 polyunsaturated fatty acid, SD – standard deviation, BMI – body mass index, kg – kilograms, cm – centimeters, GERD – gastroesophageal reflux disease, CPAP – continuous positive airway pressure, BiPAP – bi-level positive airway pressure, NAEPP – National Asthma Education & Prevention Program, F – Fisher exact test was used to compare ratios. 1 – activity level score ranges from 1 to 6 with a higher score denoting greater daily activity.