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Nutrigenetic Response to Omega-3 Fatty Acids in Obese Asthmatics (NOOA): Rationale and Methods

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Abstract

Uncontrolled asthma is a major cause of hospitalizations and emergency room visits. Factors including obesity, African ancestry and childhood are associated with increased asthma severity. Considering the high morbidity caused by asthma, relatively few classes of drugs exist to control this common disease. Therefore, new therapeutic strategies may be needed to reduce asthma's impact on public health. Data suggest that a high fat diet that is deficient in omega-3 fatty acids could promote both obesity and excessive inflammation, resulting in greater asthma severity. Small trials with supplemental omega-3 fatty acids have been conducted with encouraging but

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COMPETING INTERESTS

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(A complete listing of the Nemours Network for Asthma Research (NNAR) and collaborating partners can be found at the end of this article.)

inconsistent results. The variability in response seen in past trials may be due to the past subjects' genetics (specifically *ALOX5* rs59439148) or their particular asthma phenotypes. Therefore, the "Nutrigenetic response to Omega-3 Fatty acids in Obese Asthmatics (NOOA)" trial is currently underway and was designed as a randomized, double-blind, placebo controlled intervention study to determine if supplemental omega-3 fatty acids improves symptoms among obese adolescents and young adults with uncontrolled asthma. Here we report the design and rationale for the NOOA trial. Participants were given either 3.18g daily of eicosapentaenoic acid and 822mg daily docosahexaenoic acid, or matched control soy oil, for 24 weeks. Change in the asthma control questionnaire score was the primary outcome. Secondary outcomes included spirometry, impulse oscillometry, exacerbation rate, airway biomarkers, systemic inflammation, leukotriene biosynthesis and T-lymphocyte function. NOOA may lead to a new therapeutic treatment strategy and greater understanding of the mechanistic role of diet in the pathogenesis of asthma.

Keywords

Asthma Control; Obesity; Children; Nutrigenetics; Omega-3 Fatty Acids

1. INTRODUCTION

Asthma is a common, complex disease of the bronchial airways that involves diverse underlying mechanisms and clinical phenotypes [1, 2]. Uncontrolled asthma symptoms continue to cause impaired quality of life and urgent healthcare utilization. Factors such as obesity and younger age are risk factors for poor symptom control. Relatively few classes of pharmacologic medications exist to control this common disease. Therefore, new therapeutic interventions to facilitate improved asthma control are greatly needed, particularly for subgroups with severe disease.

External factors such as diet and obesity-status may alter the risk for incident asthma and may worsen existing disease. Obesity is associated with incident asthma [3, 4], greater asthma-related symptoms, and altered treatment response [5, 6]. In addition, a poor diet (low in vegetables and fish, and high in saturated fats and omega-6 fatty acids) has been associated with obesity[7] and onset of asthma symptoms[8–10]. Observational studies note a lower rate of asthma among populations consuming high amounts of cold-water fish [11–15]. Cold-water fish are rich in the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Plasma and intracellular concentrations of EPA and DHA increase following ingestion of omega-3 fatty acid [16–19] and increase within inflammatory cell phosholipid membranes. Supplementation also increases the ratio of omega-3 versus omega-6 polyunsaturated fatty acids (PUFA) within cell phosholipid membranes[20–24]. There exist several plausible mechanisms by which EPA and DHA may reduce inflammation important in asthma. First, omega-3 PUFAs serve as a competitive inhibitor of the arachidonic acid cascade enzymes phospholipase A2, 5-lipoxygenase and cyclooxygenase. Greater omega-3 PUFA availability diverts from inflammatory pathways that lead to eicosanoids (leukotrienes, thromboxanes and prostaglandins) which have asthma-promoting features[25]. Next, EPA and DHA inhibit production of TNF-alpha [17, 20, 26] and IL-1[20, 26–28] through altered NF-kappaB activity and reduced gene transcription. EPA/DHA may affect T-regulatory cell (Treg) production and activity [29] , FoxP3 expression[30–32], and effector T-lymphocyte proliferation[33–35]. Finally, omega-3 PUFAs are precursor molecules to the anti-inflammatory mediators, resolvins and protectins[36–40], which function to curtail neutrophil chemotaxis, and reduce chemokine and cytokine production[38, 39]. All of these molecular mechanisms are relevant to asthma.

Supplementation of the diet of asthmatics with omega-3 PUFA has been evaluated in several small trials (see table 1), the results of which are encouraging but inconsistent[16, 41–43]. Several studies report significant asthma-related improvements [16, 19, 22, 25, 44–48]. The inconsistent past results could be explained by inadequate dose or duration of EPA/DHA, or by reduced response among participants with polymorphisms in arachidonic acid pathway genes. The ALOX5 promoter polymorphism rs59439148 has been shown to be an important modifier of treatments acting on the leukotriene pathway[49] including fish oil[50]. Further studies involving a longer duration of treatment and involving consideration of possible nutrigenetic effects are needed.

Therefore, 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 obese adolescents and young adults with uncontrolled asthma. NOOA will measure change in asthma control questionnaire score as its primary outcome, while evaluating nutrigenetics, safety and tolerability.

2. STUDY DESIGN AND RATIONALE

The NOOA study is a controlled, 24-week parallel group intervention trial involving 100 obese children and young adults with asthma randomized to either omega-3 PUFA treatment (3.18g EPA, 822mg DHA per day) or similar soy oil control (fig 1). The study was reviewed by the National Institutes of Health (NIH), the Food and Drug Administration (FDA) and by the Institutional Review Boards (IRB) at all participating sites.

2.1 Study Cohort and Rationale

NOOA selection criteria (Table 2) were established to study obese asthmatic adolescents and young adults with inadequately controlled asthma. Trained nurse coordinators measured height, weight and waist circumference at visit 1 and subsequent visits using standardized procedures[51, 52]. We utilized body mass index (BMI)-based CDC guidelines for the definition of obesity[53, 54]. We included only participants with a waist-circumference above the 90^{th} percentile to target participants with central obesity[53]. We enrolled only asthma patients with recent inadequate control (Table 3) similar to recent large asthma trials[55].

2.2 Study Medication and Dosing

Participants were randomized to either oral omega-3 PUFA supplementation (3.18g EPA, 822mg DHA, 101mg other omega-3 fatty acids), or similar weight control soy oil. The daily dose for both treatments comes in the form of six softgel capsules and was justified based on past studies (Table 1) and recent recommendations[56]. Nordic Naturals, Inc (NNI) (Watsonville, CA USA) was the supplier of active treatment (EPA/DHA) and control treatment (soy oil) and reports third-party testing results that show precision of dosing and purity from contaminants[57]. Testing of content purity was performed by Nutrasource Diagnostics, Inc (Guelph, Candada) which is a certified and accredited reference laboratory. Both EPA/DHA and soy oil treatments were similar in look, taste and feel..

2.3 Randomization Procedure

Eligible participants were monitored for 14–28 days prior to randomization to assess asthma control and asthma diary card adherence. Participants were randomized in a 3:1 ratio, with 3 participants receiving EPA/DHA for each participant receiving control soy oil. This unbalanced randomization scheme was devised to increase the power to detect a nutrigenetic effect of EPA/DHA. Participants were also stratified into one of two groups according to

their BMI in order to ensure that there was a balanced distribution of participants with an extreme BMI between treatments assignments. The randomization scheme was generated using the SAS procedure PROC PLAN. A block randomization was used (instead of a completed randomized design) by dividing subjects into 2 BMI blocks or strata (details of BMI strata exist in the online supplement). Neither participants nor the research staff were informed of the treatment group assignment to maintain the double-mask. The randomization scheme was devised by our statistical consultant (YG), and was controlled and implemented by an unblinded on-site collaborator (EM).

2.4 Safety Monitoring

EP A and DHA in the form of nutritional oils have been found to be safe and well-tolerated in several post-marking studies [16, 18, 19, 21–23, 25, 41, 44, 58]. There have been fewer safety studies in children. Therefore, all patients had blood work for alanine aminotransferase (ALT), hemagram and prothrombin time (PT)/partial thromboplastin time (PTT) drawn at baseline and 12 weeks. We assessed for side-effects at visits 3, 4 and 5, and via phone contacts at weeks 2, 4, 6, 10, 16, and 20.

All subjects continued on their previously prescribed asthma control regimen that included an inhaled corticosteroid (either from previous asthma plan or conversion from leukotriene modifier (LTM) monotherapy (see table 2 and online supplement for details). The participant's asthma during the intervention period was managed by the participant's designated asthma physician.

2.5 Data collection and study visits

Certified research staff determined eligibility using a baseline medical history, anthropometrics, pulmonary function/lung responsiveness testing, asthma symptom questionnaires, and pregnancy testing (females) at visit 1. Participants or guardians (if needed) signed informed consent documents. Patients who met all requirements for inclusion with the sole exception of the lung responsiveness criteria were scheduled for methacholine challenge at visit 2. Once lung responsiveness criterion was met, participants started on a monitored 2–4 week run-in period. If participants were adherent to peak flow and current asthma controller on 5 out of 7 days (on average), they met eligibility for randomization at visit 3.

Willing participants previously on LTM were taken off LTM according to the step-down protocol. Because a possible mechanism of EPA/DHA may be reduced leukotriene production, participants were taken off all leukotriene modifying agents at visits 2. The timing and details of the baseline data collection are in table 4. The screening visit (V1) included informed consent, instructions regarding study format and asthma action plan, and testing per table 4. Participants with documented lung responsiveness criteria at visit 1 or within the past 24 months had visits 1 and 2 combined in the same day. Visit 2 signified the start of the run-in period. At visit 2, staff reviewed the participant's asthma action plan and the participant and accompanying parent were told to continue with their normal diet and level of activity. Participants were given a peak flow device (and trained on its use) and daily diary cards to document peak flow values and controller medicine use.

At the randomization visit (V3), participants returned diary cards and were assessed for randomization. Testing occurred per table 5 and fig 1. If subjects did not meet inclusion criteria for randomization due to adherence and the study personnel agreed, they were permitted to repeat visit 3 after continuing on their routine daily asthma medications and run-in specifications for an additional 2 weeks. Participants who had one or more of the following: 1) took daily asthma treatment < 10 of the preceding 14 days as documented by

diary cards. 2) FEV1 ≤ 60% predicted pre-bronchodilator; or 3) febrile illness (>38.0ºC or 100.4ºF) within last 24 hours, had the opportunity to be re-evaluated and randomized after an additional 2 week monitoring period.

After 12 weeks of intervention, participants returned their daily diary cards at visit 4 (V4) and had testing performed per table 5. At the termination visit (V5), participants returned their diary cards. They received an exit letter unmasking their treatment assignment and counseling information regarding a healthy lifestyle and diet. Testing proceeded per table 5.

2.6 Outcome Measures

2.6.1 Asthma Symptom Scoring—Change in the 'Juniper' Asthma Control Questionnaire (ACQ) was the primary outcome. The ACQ instrument has been validated in children[59] and adults, and used in several multi-center asthma trials[55, 60]. The ACQ ranges from 0 to 6 (higher values indicate worse asthma control) and considered a broad set of control indicators including use of bronchodilators, cough, nocturnal symptoms, level of activity, and pulmonary function. At all visits we also performed the Asthma Control Test (ACT), Asthma Symptom Utility Index (ASUI) and several tools measuring quality of life. We computed rate and prevalence of asthma symptom exacerbations for both treatment groups (see online supplement for details).

2.6.2 Lung Function—The Koko spirometric system was used per American Thoracic Society standards[61]. Outcomes included forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and forced expiratory flows at 25–75% of vital capacity ($FEF_{25–75}$). Raw data and percent predicted values were recorded before and after bronchodilator. Forced oscillation testing was also performed (Jaeger MasterScreen Impulse Oscillometry system, Jaeger Co, Germany)[62–68] at all clinic visits (at two sites, Jacksonville and Orlando). Impedance, resistance and reactance values were calculated at discrete frequencies from 5 to 35 Hz. Lastly, expiratory peak flow measurements were measured at home daily during the intervention period in order to calculate peak expiratory flow variability (see on-line repository).

2.6.3 Inflammatory mechanisms—Exhaled nitric oxide, exhaled breath condensate (pH, 8-isoprostane), plasma C-reactive protein and urinary LTE4 were collected at visits 3, 4 and 5. (See online web repository for details on methodology.) Omega-3 to omega-6 PUFA ratio within leukocyte plasma membranes was assessed at visits 3–5. Phospholipids from cell lysates were purified and transmethylated. The fatty acid methyl esters were analyzed by gas-liquid chromatography at Nemours Bioanalytic Lab and the amounts of individual fatty acids will be expressed as relative percentages, together totaling 100 percent.

2.6.4 Regulatory T-lymphocyte (CD4+-CD25+-CD127--FoxP3+) number and function—We collected blood and sputum on a subset of participants to measure regulatory T-lymphocytes. CD4+-CD25hi-CD127lo/- regulatory T cells were magnetically isolated from whole blood, and counted by flow cytometry[69]. The level of FoxP3 expression will be measured by RT-qPCR and by flow cytometry, and Treg activity will be assessed by chemotaxis and T cell proliferation-inhibition assays[70] (See online repository for more detail on methodology).

2.7 Assessment of Dietary Intake

We assessed each participant's diet using a Block validated food frequency questionnaire (at randomization and again at week 24) (Block Kids FFQ 2004, Block Adult FFQ 2005) [71–74]. Two 72 hour dietary recalls were performed at the beginning and the end of the study period, along with three additional 24-hour dietary recalls periodically throughout the

intervention period. We had access to the Food Processor 9.3 (Standard Version, Esha Research, Salem, OR USA) software package that analyzed intake in order to describe dietary patterns among obese asthmatics, and to report the comparability in diet (vitamin D, antioxidant vitamins, soy, saturated fats) between intervention groups. Our research nutritionist (KK) assisted in quantifying particular dietary components including omega-3 and omega-6 PUFA, antioxidant vitamins (such as vitamin A, vitamin E, Vitamin C and Selenium), and total fat intake.

2.8 Genetic analysis

The *ALOX5* promoter SP1 tandem repeat polymorphism genotype has been shown to affect responses to drugs acting on the 5-lipoxygenase/leukotriene pathway[49, 75]. Therefore, the *ALOX5* promoter SP1 tandem repeat polymorphism (marker rs59439148) will be genotyped at the completion of the study as previously described [50] (see online repository for details). 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. We planned to test both additive (5/5 vs. 5/X vs. X/X) and dominant (5/5 vs. 5/X + X/X) general linear models. The final model will depend on how accurately each model describes the distribution of the data, and will be consistent with past analyses[49, 76, 77]. Accounting for attrition and a racially heterogenous sample, we estimate that we should have at minimum > 70% power to detect a nutrigenetic effect if one exists.

2.9 Organization of the Study

The NOOA study utilized the Nemours Network for Asthma Research. The study was initiated at the Nemours Clinic in Jacksonville, Florida and was expanded to the Nemours Children's Hospital, Orlando and three other sites in Florida and Delaware. A data and safety monitoring board was created to monitor for safety and data integrity and met yearly. An investigational new drug application was submitted under the section 505(i) of the Federal Food, Drug, and Cosmetic Act for the omega-3 polyunsaturated fatty acid intervention (ProEPA Xtra) and granted in March 2010 (IND107443).

2.10 Data management

Study data were collected and managed using the Research Electronic Data Capture (REDCap) electronic data capture system and tools, hosted by collaborators within Nemours Bioinformatics/Nemours Foundation[78]. REDCap was chosen because it is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

2.11 Analysis

2.11.1 Sample size calculation—We determined that 100 participants will need to be randomized according to a 3:1 allocation ratio, with a goal of 75 receiving EPA/DHA and 25 receiving control. We assumed a 10% dropout rate with a final goal of 90 participants completing intervention. Power estimation for EPA/DHA effect on asthma control was performed depending on a two-sided t-test of a hypothesis about two population means (EPA/DHA-treated and control-treated). We let n be the minimum sample size per group to detect a deviation of magnitude Δ from the null value in the direction of the research hypothesis. The appropriate sample size per group to reach a defined power would be calculated as:

$$n \geq 2(t(v,\alpha/2)+t(v,\beta))^2(\frac{\sigma}{\Delta})^2$$ [[79]]

(Where σ was the population standard deviation; v denoted the degree of freedom, and $v = 2(n-1)$ for a two-sample t test; α is type I error set as 0.05; β is type II error and the power can be estimated as $1-\beta$). With large sample size (e.g. $n \geq 30$), the t-distribution can be approximated by a standard normal distribution. In this case, the above inequality could be simplified as the following equation:

$$n=2[z(\alpha/2)+z(\beta)]^2(\frac{\sigma}{\Delta})^2$$ [[79]]

Sample size was determined based on the ACQ (primary outcome measure). Power to detect a treatment effect using alternate clinical outcomes (assuming 90 participants with type I error $\alpha = .05$)are shown in table 6.

The trial had at least 98% power to detect a significant effect size in clinical outcomes of ACQ, ASUI and 8-isoprostane. We sought a power >70% to improve the likelihood of detecting a *nutrigenetic* effect (see on-line supplement).

2.11.2 Data analysis—We assumed an intention-to-treat approach. When the trial ends, we will utilize a two sample t-test to determine whether the change in ACQ from the randomization to termination visit differs between treatment groups, (α=.05). Other secondary outcomes that are continuous numeric variables will be analyzed similarly. We will also compare the proportion of diary card asthma-free days between the two treatment arms using the Mann-Whitney-Wilcoxon test. Additionally, we will use an aligned rank test (non-parametric) test to account for the multiple strata (two BMI strata X 4 clinic sites). For asthma exacerbations, we will likely employ Poisson regression analysis because of its relatively low event frequency. The statistical packages SAS 9.2 (SAS Institute Inc, Cary NC, USA) and STATA 11 (College Station, TX: StataCorp, 2005) were used. All tests were two-tailed at a level of significance of 0.05 (see online supplement for additional details).

3. Discussion

The NOOA study aimed to determine if the addition of oral EPA/DHA to corticosteroid therapy in obese children and young adults with inadequately controlled asthma leads to improved asthma control. Though there have been several small interventional trials involving EPA/DHA for asthma, past results have been inconsistent (Table 1). Considering the large problem that uncontrolled asthma symptoms pose to public health, the relative paucity of effective therapies for asthma control, and the general safety of EPA/DHA, further exploration of the efficacy of EPA/DHA for the treatment of asthma is warranted. In addition, increased omega-3 fatty acid intake leading to altered leukotriene production may prove to be particularly effective in asthmatics with obesity. Obesity has been associated with greater leukotriene production, while evidence also suggests that the montelukast, a leukotriene receptor antagonist, may have a modestly increased effect with higher body mass index[80].

We chose to use softgel capsules to deliver 4g of daily omega-3 fatty acids primarily in the form of EPA and DHA, or matching control soy oil, over a 24 week duration. A 24 week intervention was chosen to ensure adequate time to: 1) alter omega-3/omega-6 fatty acid ratios in leukocyte plasma membranes, and 2) establish effectiveness (that fish oil could be

tolerated and adhered to among patients with persistent asthma). The primary outcome measure was change in Asthma Control Questionnaire on therapy, which included assessment of lung function and several clinically relevant symptom measures. The NOOA study also evaluated several likely molecular mechanisms before, during and after intervention to try to determine causal effects of omega-3 fatty acid therapy on inflammation. Data collected as a part of NOOA will be analyzed to determine is omega-3 fatty acid treatment is modified secondary factors such as level of obesity, dietary omega-3 fatty acid intake, urinary leukotriene levels or previous response to LTRAs. Our current hypothesis is that omega-3 fatty acids will improve asthma particularly in those with obesity and leukotriene-driven disease. However, because omega-3 fatty acids affect other mechanisms, it is possible that larger studies will be needed testing the effect of omega-3 fatty acids in both obese and non-obese asthmatics.

As depicted in table 1, smaller studies have been completed that included a range of ages, dosing regimens and clinical phenotypes. Ages have ranged from 8–65 years, with the majority of studies involving young and middle-aged adults. Very few randomized controlled studies have used an intervention more than 12 weeks, and the largest 6 month trial in asthmatics to our knowledge involved 39 participants[17]. A systematic review by Reisman in 2006 attempted to perform a meta-analysis of omega-3 fatty acid supplementation for asthma, however the authors were not able due to widely varying reporting of allocation concealment, baseline characteristics, asthma severity, disease phenotype, primary outcome and missing data[41]. Additionally, there may be a dose-response effect, with Reisman et al speculating that a daily dose greater 3 grams may be required to see a significant and clinically meaningful effect[41]. Several studies with encouraging results have shown that doses in the range of greater than 5 grams/day succeed in reducing bronchial reactivity among persistent asthmatics or asthmatics with exercise-induced bronchospasm[22, 23, 48]. One of the largest and most controlled studies used dietary manipulation to increase omega-3 fatty acid intake but failed to show clinical improvement[17]. Inconsistent past results could reflect a nutrigenetic or phenotype-specific effect with EPA/DHA. The current NOOA study focused on the important problem of poorly controlled asthma symptoms despite inhaled corticosteroids, and used a 24 week intervention period at a high dose of greater than 4g per day. In addition, we focus on obese asthmatics, a subset of asthmatics who have been shown to be more resistant to inhaled steroids. Importantly, obese asthmatics may be more responsive to omega-3 fatty acid supplementation if their past diets are excessively high in n-6 fatty acids. Dietary assessment and intervention (‘medical nutrition therapy’) has even been advocated for asthmatic children[81]. With our detailed dietary assessments, we will report the nature of the dietary intake among obese asthmatics and evaluate how diet affects asthma control and response to omega-3 fatty acids.

Nausea and abdominal discomfort were consistently reported, and could limit the future effectiveness of EPA/DHA supplementation as a treatment strategy. Concerns about taste and tolerability led us to use a pharmaceutical-grade product that was lemon-flavored and masked the commonly reported fishy taste and dyspepsia. So far, gastrointestinal complaints have been rarely reported and have not led to problems of withdrawal.

The choice of specifically which omega-3 PUFAs to use, the daily dose and treatment duration, and the choice of optimal ‘placebo’ control agent to use were important design questions. A fixed daily dose of 4g using a discrete number of softgels has optimize adherence and maintain blinding. We used a similar number of softgels as our control treatment which were matched for taste, appearance and consistency. Various products have been used in past trials as a ‘placebo’. The choice of soy oil seemed to be the best option after considering the factors of safety, need for blinding, and need for a true inert placebo.

The soy oil control is isovolumetric and similar in caloric content to EPA/DHA treatment. The control intervention constitutes a negligible fraction of the daily omega-6 PUFA consumption in a typical Western diet and therefore should not significantly alter omega-3/omega-6 ratios within inflammatory cells[24]. Soy oil contains a small fraction of (non-EPA, non-DHA) α-linolenic acid, however conversion of α-linolenic acid into EPA is limited. Soybeans contain isoflavone glycoproteins that theoretically could be anti-inflammatory, however our soy oil product has been filtered and highly purified to remove proteins and glycoproteins, and any likely anti-inflammatory effect.

Several drugs show inter-individual variability of response due to nutrigenetic effects which can limit their efficacy within populations. If responses to EPA/DHA were dependent on genetics (as we are postulating), results of EPA/DHA intervention trials would vary depending on the genetic make-up of the treated group. Past results from asthma trials cited above have been consistent with a nutrigenetic effect. Because EPA/DHA appears to reduce inflammation through action on the arachidonic acid pathway, we chose to evaluate associations between common gene variants in the arachidonic acid and leukotriene pathways and treatment response. The *ALOX5* gene is located on Chromosome 10q11.21 and has been associated with altered response to asthma therapy[49, 76] and risk for obesity in both asthmatics and non-asthmatics. Dwyer and Alayee showed that omega-3 PUFA displays a protective effect on carotid intimal thickening[50] that is also dependent on ALOX5 rs59439148 promoter polymorphism. This promoter polymorphism in the gene encoding 5-lipoxygenase leads to increased production of inflammatory leukotrienes, and to a gene-environment interaction[50]. Compared to heterozygotes and wild type homozygotes, individuals that are mutant homozygotes (carrying 2 mutant alleles) have increased carotid intima-media thickness, an atherogeneic effect that was exacerbated by increased intake of dietary arachidonic acid (n-6 PUFA). Increased dietary intake of n-3 PUFA blunted the atherogenic effect in carriers of the mutant variant. These data suggest that the mutant allele of the addition/deletion promoter polymorphism for the *ALOX5* gene up-regulates production of proinflammatory leukotrienes, and that n-3 PUFA supplementation is effective in one genotype but not the other at ameliorating the inflammatory and atherogenic effect.

In addition, Tantisira recently concluded that pharmacogenetic variability exists in the response to zileuton therapy (a 5-lipoxygenase inhibitor). The results of this study are important because it is reasonable to hypothesize that these same genetic variants may also influence response to EPA/DHA[82].In a clinical trial of montelukast (selective cysLTR1 antagonist), Lima et al reported that asthmatics carrying one or 2 mutant alleles responded better to montelukast treatment compared to individuals carrying 2 wild type alleles[83], suggesting that mutant variants in asthmatics up-regulated 5-LO activity. Since obesity is accompanied by up-regulation of pro-inflammatory mediators, it could be hypothesized that the mutant allele for the addition/deletion promoter polymorphism for the *ALOX5* gene might be more prevalent in obese compared to non-obese individuals. Consistent with this, other authors have shown improved clinical response to montelukast with increasing BMI[80]. Taken together these data make a compelling argument that n-3 PUFA supplementation will reduce leukotriene-mediated inflammation and improve asthma control particularly among obese asthmatics.

In summary, we have presented a review of the largest clinical trial to assess the efficacy of omega-3 fatty acids for the treatment of asthma. Furthermore, this is the first trial to our knowledge that targets inadequately controlled obese asthmatics with a novel nutrient with potential pharmacologic effects. We are assessing for a nutrigenetic effect by interrogating a select arachidonic acid pathway polymorphism. If this trial discovers that omega-3 fatty acids improve asthma control, a new class of therapy will potentially become available and could significantly improve the way asthma is managed in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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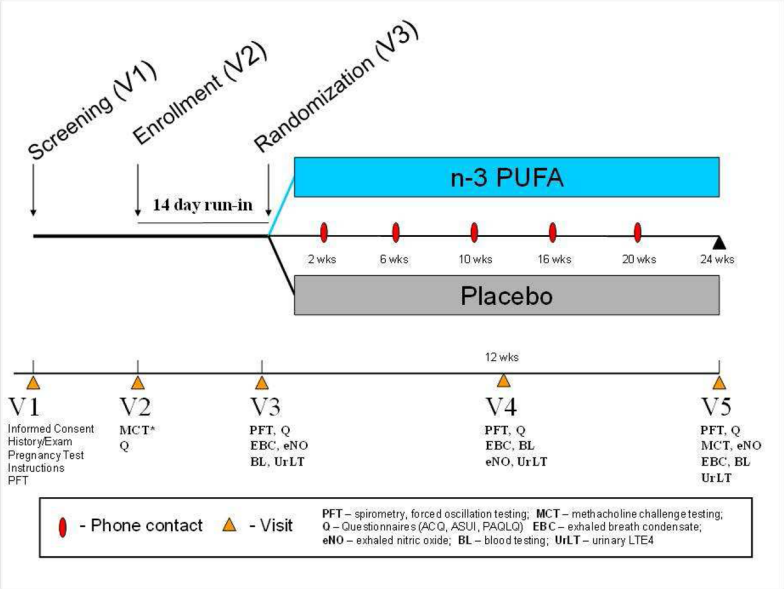

Figure 1.

Schema for the Nutrigenetic response to Omega-3 fatty acids in Obese Asthmatics (NOOA) trial. NOOA is a 24-week randomized, controlled, double-blinded parallel intervention trial. Acronyms not depicted above: ACQ – asthma control questionnaire, ASUI – asthma symptom utility index, PAQLQ – paediatric asthma quality of life questionnaire.

Table 1

Asthma Trials involving omega-3 fatty acids

Study	age	Size (n)	Daily EPA/DHA dose (g)[1]	Control agent	duration	Effect seen
Hodge, 1998	8–12	39	.72/.036	Safflower/Sunflower	6 months	No
Broughton, 1997	19–25	19	--	--	1 month	Reduced AHR
Arm, 1988	15–42	25	3.2/2.2	Olive oil	10 weeks	Reduced inflammation
Arm, 1989	15–42	17	3.2/2.2	Olive oil	10 weeks	Lung function
Thien, 1993	15–65	25	3.2/2.2	Olive oil	6 months	No
Surette, 2003	18–56	43	.5–.75/--	--	4 weeks	Reduced LT production
Emelyanov, 2002	18–56	23	Combined	Olive oil	8 weeks	Reduced symptoms
Mickleborough, 2006	Mean = 23	16	3.2/2.0	Olive oil	3 weeks	Lung function

AHR – airway hyperresponsiveness, LT – leukotriene.

[1] Institute of Medicine's adequate intake for omega-3 polyunsaturated fatty acids is 1.1 and 1.6 grams/day for females and males, respectively (IOM report, 2002). A typical serving of Salmon contains just less than 1 gram of EPA + DHA.

Table 2

Participant Selection

Criteria for Enrollment - Inclusion

1. M ales or females between and including the ages 12 through 25 years.
2. Participant must have physician-diagnosed persistent asthma (cannot have only exercise-induced or intermittent asthma).
3. Lung Function Responsiveness Criteria (needs at least one of the following within the past 24 months):
   - >12% β-agonist reversibility, using up to 4 puffs albuterol or levalbuterol or,
   - PC_{20} FEV_1 methacholine < 16 mg/ml or,
   - Exercise bronchoprovacation test with a 20% or greater decrease in FEV_1.
4. FEV_1 > 60% predicted pre-bronchodilator;
5. Waist-circumference > 90^{th} percentile based on age- and sex-based normative data.
6. Participant must have a local asthma care provider to assist with clinical management.
7. Evidence of Inadequate Recent Asthma Control: Participant must meet at least 1 of the following 4 criteria:
   - Use of beta-agonist for asthma symptoms twice/week or more on average over the past month or,
   - Nocturnal awakenings at least once per week on average due to asthma symptoms over the past month or,
   - Two or more emergency room visits, unscheduled physician visits for asthma, prednisone courses, or hospitalizations for asthma in the past 12 months or, a Score of 1.25 or greater on the Juniper Scale of Asthma Control (ACS) at the screening visit.
8. Participant must have been on some form of daily asthma controller therapy, can include ICS-LABA or LTRA. Patients on LTRA will proceed to step-down/conversion to inhaled corticosteroids for controller therapy (see Leukotriene Modifier (LM) Stepdown/ ICS Converion Procedure).
9. Participants must be adhering to daily therapy at least 5 or more days per week on average;
10. For adults, asthma symptoms must have had onset during childhood (before age 18);

Criteria for Enrollment – Exclusion (participant cannot have any of the following):

- Currently taking nutritional oil supplements;
- Daily LTRA therapy currently or within last 8 weeks;
- Daily oral corticosteroid use;
- Hospitalization or urgent medical care visit for asthma within past 4 weeks;
- Allergy to fish or fish oil supplements or omega-3-acid ethyl esters or soy in the past;
- Febrile illness within past 24 hours (>38.0C) or upper respiratory infections within the past 2 weeks;
- Currently planning any changes in diet during the run-in or treatment period;
- Any smoking history within the past 2 years;
- Any patient with a history of prolonged bleeding time, coagulopathy, or severe clinical bleeding (prolonged nose bleeds or menorrhagia);
- Any patient currently taking daily therapeutic anti-coagulants or blood thinners (such as aspirin, warfarin, heparin or NSAIDS);
- For girls: Current pregnancy, or recent pregnancy and currently lactating; females enrolled must agree to practice an adequate birth control method (abstinence, combination, barrier/spermicide, OCP) for the duration of the study;
- Concurrent diseases that, in the investigator's opinion, would interfere with participation in the study or might put the participant at risk by participating;
- Inability to give informed consent or comply with study procedures.

Table 3

Definition of Inadequately Controlled Asthma

Participants were considered to have evidence of inadequately controlled asthma if one or more of the following criteria were met.

1. Use of beta-agonist for asthma symptoms twice/week or more on average over the past month or,
2. Nocturnal awakenings at least once per week on average due to asthma symptoms over the past month or,
3. Two or more emergency room visits, unscheduled physician visits for asthma, prednisone courses, or hospitalizations for asthma in the past 12 months or,
4. Score of 1.25 or greater on the Juniper Scale of Asthma Control (ACS) at the screening visit
5. Score of 19 or less on the Asthma Control Test (ACT) at the screening visit.

Table 4

Schedule for Collection of Screening and Baseline Data

	Visit 1 Screening	Visit 2 Enrollment	Visit 3 Randomization
Timeline	**Minus 2–8 weeks**	**Minus 2–4 weeks**	**0**
Consent/Assents and eligibility evaluation	•	•	•
Medical History, participant instructions	•		
Anthropometrics	•		
Review inhaler technique	•		•
Distribute Asthma Action Plan	•		
Issue Asthma Diary Cards		•	•
Review Asthma Diary		•	•
Asthma Questionnaire Scoring		•	•
Pregnancy test (females)	•	•	•
Methacholine Challenge (if needed for inclusion)		•	
Block Food Frequency Questionnaire			•
72 hour Dietary Recall			•
Spirometry, Forced oscillation	•		•
Exhaled nitric oxide and breath condensate			•
Urinary LTE4			•
Blood for CRP, ALT, F2-isoprostane			•
Blood for T-regulatory Cell analysis			•
Granulocyte membrane PUFA analysis			•

LTE4 – leukotriene E4, CRP – C-reactive protein, ALT – alanine aminotransferase, CBC – complete blood count, PUFA – polyunsaturated fatty acid

Table 5

Schedule for Collection of Response Data during Intervention

	Visit 3 Randomization	Visit 4 Midpoint	Visit 5 Termination
Timeline	**0**	**+12 (10–14)**	**+24 (22–26)**
Anthropometrics	•	•	•
Review inhaler technique	•	•	•
Issue Asthma Diary Cards	•	•	
Asthma Questionnaire Scoring (ACQ, ACT)	•	•	•
Asthma-related Quality of Life questionnaires[1]	•	•	•
Pregnancy test (females)	•	•	•
Adverse Event screening	•	•	•
Interval Health/Asthma assessment	•	•	•
Block Food Frequency Questionnaire	•		•
24 hour Dietary Recall		•	
72 hour Dietary Recall	•		•
Spirometry and Forced oscillation testing	•	•	•
Exhaled nitric oxide and breath condensate	•	•	•
Urinary LTE4	•	•	•
Blood for CRP, ALT, CBC, F2-isoprostane	•	•	•
Blood for T-regulatory Cell analysis	•	•	•
Granulocyte membrane PUFA analysis	•	•	•

ACQ – asthma control questionnaire, ACT – asthma control test; LTE4 – leukotriene E4, CRP – C-reactive protein, ALT – alanine aminotransferase, CBC – complete blood count, PUFA – polyunsaturated fatty acid,

[1]consists of the asthma quality of life questionnaire, the pediatric asthma quality of life questionnaire, and the pediatric caregiver asthma quality of life questionnaire;

Table 6

Power Analysis for EPA/DHA Treatment

Outcome	Standardized effect size (change/standard deviation)	Estimated Power[1]
ACQ	0.5/0.45 = 1.1	98
ASUI	0.1/0.1 = 1.0	98
8-isoprostane	1.0/0.7 = 1.43	99
FEV1/FVC	0.05/0.07 = 0.71	83
FEV1	0.07/0.11 = 0.64	75

[1] Based on 90 completing intervention, 67 treated, 23 controls;

ACQ – asthma control questionnaire; ASUI – asthma symptom utility index, FEV – forced expiratory volume, FVC – forced vital capacity