

## ORIGINAL ARTICLE

# Impact of a novel nutritional formula on asthma control and biomarkers of allergic airway inflammation in children

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## Clinical & Experimental Allergy

### Summary

**Background** A novel nutritional formula (NNF) enriched in eicosapentaenoic (EPA) and  $\gamma$ -linolenic fatty acids and antioxidants reduces airway inflammation and improves clinical outcomes in critically ill patients, but NNF has not been evaluated in chronic inflammatory diseases such as persistent asthma.

**Objective** To evaluate the efficacy, compliance, and safety of NNF in asthmatic children.

**Methods** Children, 6–14 years of age, with mild to moderate persistent asthma, on as needed albuterol alone, were randomized to receive daily NNF ( $n = 23$ ) or control formula ( $n = 20$ ) for 12 weeks, with multiple assessments of asthma control, spirometry, measures of airway inflammation, formula tolerance, and adverse events.

**Results** Daily consumption of either NNF or a control formula showed improvement in asthma-free days over time ( $P = 0.04$ ) but there was no difference between groups. However, the NNF group had lower exhaled nitric oxide levels compared with the control group at weeks 4, 8, and 12 ( $P < 0.05$ ). An overall group difference in log FEV<sub>1</sub> PC<sub>20</sub> ( $P = 0.05$ ) was found in favour of the NNF group as well. Significantly higher levels of EPA in plasma ( $P < 0.01$ ) and peripheral blood mononuclear cell (PBMC) ( $P < 0.01$ ) phospholipids in the NNF group compared with control group within 2 weeks indicated good adherence with daily NNF intake. There were no differences in adverse events for NNF vs. control after 12 weeks.

**Conclusions** Both NNF and control groups demonstrated improvement in asthma-free days. NNF-treated group had reduced biomarkers of disease activity. Rapid PBMC fatty acid composition changes reflected an anti-inflammatory profile. Dietary supplementation with NNF was safe and well tolerated (ClinicalTrials.gov number NCT01087710).

**Keywords** airway inflammation, antioxidants, bronchial hyperresponsiveness, childhood asthma, eicosapentaenoic acid,  $\gamma$ -linolenic acid

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### Introduction

Although inhaled corticosteroids are the preferred long-term control therapy for persistent asthma, concerns remain regarding the potential for adverse effects particularly in children, limitation in treatment effect, and variable responses [1–4]. Recently, questions have been raised whether the increased consumption of a westernized diet deficient in antioxidants and rich in pro-inflammatory fatty acids ( $\omega$ -6 fatty acids) is a contributing factor for the increased prevalence of asthma and allergies [5–8]. The protective effects of diets rich in very

long chain  $\omega$ -3 PUFA [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)], antioxidants, and whole grains against asthma or wheezing illnesses in childhood have been shown in epidemiologic studies [6, 9–12]. EPA and DHA compete with arachidonic acid (AA) as substrates for the formation of leukotrienes (LTEs) and prostaglandins (PGs), but the EPA-derived metabolites have a much weaker pro-inflammatory activity compared with the 4-series cysteinyl LTEs and 2-series prostanoid products of AA metabolism [13]. In addition to the benefits provided by consumption of  $\omega$ -3 PUFA, diets rich in antioxidant vitamins (Vitamins C, E, and  $\beta$ -carotene),

phenolic, and phytic acids [14, 15] may reduce the susceptibility of the lung to oxidant damage and inflammation.

The results of clinical trials using dietary strategies on various asthma outcomes offer limited and conflicting information [16–29]. A specialized enteral formula combining anti-inflammatory fatty acids [EPA and  $\gamma$ -linolenic acid (GLA)] and antioxidants (Oxepa<sup>®</sup>, Abbott Nutrition, Abbott Laboratories, Columbus, OH, USA) is an adjunctive nutritional therapy in the clinical management of acute lung injury (ALI) and/or acute respiratory distress syndrome, disease states characterized by a marked inflammatory response. Improvement in clinical outcomes (e.g. improved oxygenation and reduction of ventilator requirement, reduced new organ failure, and reduced time on mechanical ventilation and ICU stay) have been demonstrated [30–34]. The components of this nutritional support decrease the production of pro-inflammatory eicosanoids and ameliorate the functional and physiologic changes associated with ALI [35–38]. EPA modulates pro-inflammatory eicosanoid production from AA, consequently reducing the level of AA in tissue and immune cell phospholipids. GLA is rapidly elongated to dihomo- $\gamma$ -linolenic acid (DGLA) and is incorporated into tissue and immune cell phospholipids. DGLA suppresses LTE biosynthesis and is further metabolized to PGE<sub>1</sub>, a potent vasodilator of pulmonary and systemic circulation [34]. Mechanistic studies showed that enteral nutrition for at least 4–7 days with Oxepa<sup>®</sup> as compared with the control diet reduced pulmonary protein permeability, suppressed intrapulmonary inflammatory mediators IL-8, LTB<sub>4</sub>, and TNF- $\alpha$ , and reduced pulmonary neutrophil recruitment [31]. Thus, this combination of fatty acids can favourably reduce an elevated inflammatory response while promoting vasodilatation and oxygen delivery [34].

The ability of Oxepa<sup>®</sup> to modulate an exuberant inflammatory response during acute illness has led us to investigate if a similar formula can serve as adjunctive therapy for a chronic inflammatory condition such as asthma. The purpose of this randomized, placebo-controlled, feasibility study was to evaluate whether a nutritional formula containing EPA, GLA, and selected antioxidants would improve asthma control and reduce allergic airway inflammation in children with mild persistent asthma. In addition, assessment of peripheral blood mononuclear cell (PBMC) incorporation of anti-inflammatory fatty acids along with safety of this strategy was evaluated in a 12-week study.

## Methods

This study was a randomized, double-blind, placebo-controlled, single centre trial that recruited patients from the National Jewish Health (NJH) outpatient clinics and the Denver metropolitan area community. Children, aged 6–14 years old, with mild to moderate persistent asthma

for at least 1 year who had not received inhaled, nasal, or systemic corticosteroids for at least 1 month before the initial study evaluation or other long-term controller for asthma were recruited. Eligibility criteria included the presence of diary-reported symptoms or inhaled bronchodilator use (not including pre-exercise) or peak flows <80% of personal best on average of at least three times per week during the run-in period. Additional inclusion criteria included ability to perform reliable spirometry, forced expiratory volume in 1 s (FEV<sub>1</sub>)  $\geq$  80% predicted (at a minimum 4 h post-bronchodilator) at screening and  $\geq$  70% predicted at randomization, and a methacholine FEV<sub>1</sub> PC<sub>20</sub>  $\leq$  12.5 mg/mL.

Exclusion criteria included asthma symptoms with night awakenings more than 2 days/week on average and/or albuterol use (more than eight inhalations per day, not including pre-exercise treatment); presence of other lung diseases or medical illness other than asthma; two or more asthma hospitalizations in the past year; history of a life-threatening asthma exacerbation;  $\geq$  4 courses of systemic corticosteroids in the past year; respiratory tract infection, asthma exacerbation, systemic corticosteroid use, or an emergency department treatment for asthma within 1 month, prior intubation for asthma, or hospitalization for asthma within 3 months; cigarette smoking within the past year; pregnancy or lactation; failure to practice abstinence or use a medically acceptable birth control method; bleeding disorder and/or currently receiving a medication known with significant anticoagulant effects; hypersensitivity to milk, fish products, borage oil, citrus, or artificial sweeteners; concurrent use of nutritional and/or health food supplements (fish oil, borage oil, evening primrose oil, herbal supplements), vitamin E, and/or vitamin C, other than a daily multivitamin, and/or calcium and vitamin D within the past 4 weeks; morbid obesity (>95th percentile); and inability to tolerate or unwillingness to take the full amount (237 mL) of the novel nutritional formula (NNF) during the first week of the run-in period were also excluded.

The study was approved by the NJH Institutional Review Board before patient enrollment. Parents/guardians provided informed consent, with verbal assent given by children <7 years of age, and written assent from older children. It is registered with ClinicalTrials.gov, number NCT01087710.

## Study formulas

The NNF (Abbott Nutrition, Abbott Laboratories) (300 calories/237 mL) is not a complete nutritional formula but is a ready-to-drink liquid supplemented with a novel oil blend (similar to that in Oxepa<sup>®</sup>) containing fish oil (3.0 g EPA and 1.6 g DHA per 237 mL) and borage oil (3.0 g GLA per 237 mL) and a mix of antioxidant vitamins and minerals (Vitamins E, C,  $\beta$ -carotene, taurine, zinc, copper,

Table 1. Composition and ingredients of study formulas

| Nutrient                                  | NNF (237 mL)                   | Control (237 mL)               |
|-------------------------------------------|--------------------------------|--------------------------------|
| <b>Protein</b>                            |                                |                                |
| % of total calories                       | 10.7                           | 10.7                           |
| G                                         | 8                              | 8                              |
| Source                                    | 80% milk protein isolate       | 80% milk protein isolate       |
|                                           | 20% whey protein concentration | 20% whey protein concentration |
| <b>Carbohydrate</b>                       |                                |                                |
| % of total calories                       | 5.3                            | 5.3                            |
| G                                         | 6                              | 6                              |
| Source                                    | 100% sucrose                   | 100% sucrose                   |
| <b>Lipids</b>                             |                                |                                |
| % of total calories                       | 84                             | 84                             |
| G                                         | 28                             | 28                             |
| Source                                    | 51% borage oil                 | 96% high-oleic safflower oil   |
|                                           | 45% sardine oil                | 4% soy lecithin                |
|                                           | 4% soy lecithin                |                                |
| Energy (cal)                              | 300                            | 300                            |
| Sucralose (mg)                            | 45.2                           | 45.2                           |
| Acesulfame potassium (mg)                 | 36.2                           | 36.2                           |
| Water (g)*                                | 194                            | 194                            |
| Taurine (mg)                              | 100                            | 0                              |
| Vitamin A (IU)                            | 500                            | 0                              |
| $\beta$ -carotene ( $\mu$ g) <sup>†</sup> | 600                            | 0                              |
| All-natural Vitamin E (mg)                | 100                            | 0                              |
| Mixed tocopherols (mg) <sup>‡</sup>       | 100                            | 0                              |
| Vitamin C (mg)                            | 200                            | 0                              |
| Calcium (mg)                              | 200                            | 200                            |
| Copper (mg)                               | 1.0                            | 0                              |
| Zinc (mg)                                 | 12                             | 0                              |
| Selenium ( $\mu$ g)                       | 40                             | 0                              |
| Molybdenum ( $\mu$ g)                     | 50                             | 0                              |
| Sodium (mg)                               | 84                             | 84                             |
| Potassium (mg)                            | 336                            | 336                            |
| Chloride (mg)                             | 24                             | 24                             |
| Phosphorus (mg)                           | 151                            | 151                            |
| Magnesium (mg)                            | 10                             | 10                             |

\*1 g water = 1 mL water = 1 cm<sup>3</sup> water.

<sup>†</sup>600  $\mu$ g/237 mL  $\beta$ -carotene = 795 IU/237 mL vitamin A activity.

<sup>‡</sup>Mixed tocopherols consist of 56%  $\gamma$ , 30%  $\delta$ , 12%  $\alpha$ , and 2%  $\beta$ .  
NNF, novel nutritional formula.

selenium, molybdenum, and calcium) (Table 1). The control formula contained the same amount of calories, protein, carbohydrate, and calcium as the NNF with an oil blend (100% high-oleic safflower oil) and no antioxidant vitamins or minerals added. High-oleic safflower oil contains predominately oleic acid and very small amounts of linoleic acid (18:2n6) and linolenic acid (18:3n3). It was assumed that the small amounts of 18:2n6 and 18:3n3 would not affect either the n-6 or n-3 fatty acid pathways thus producing a neutral control group in terms of fatty acid profile. The flavours of NNF and the control formula were identical. Patients randomized to either study group

were instructed to drink one 237 mL tetra-pak per day for 12 weeks. Patients were instructed to maintain their routine diet throughout the study and to drink the NNF at mealtime or as a between-meal snack. Daily diary cards were given to each patient and they were instructed to keep a log of their formula intake to the nearest 1/4 tetra-pak. Treatment compliance was also assessed by measuring plasma and PBMC phospholipid fatty acids and plasma levels of retinol,  $\beta$ -carotene and  $\alpha$ -tocopherol after 2, 4, and 12 weeks of study duration.

### Outcome variables

The primary outcome selected for this study was the number of asthma-free days during the 12-week treatment period. An asthma-free day was defined as a day with no night awakenings, morning and evening peak flow rates  $\geq 80\%$  of personal best, no use of albuterol or prednisone for symptoms, no absence from school or contact with a physician because of asthma symptoms, and no episode of wheezing, coughing, chest tightness, or shortness of breath.

Daily diary cards with information on day and night-time symptoms, bronchodilator use, and twice daily peak flows were collected at each visit. Percutaneous puncture skin testing was performed to aeroallergens using the Multi Test II (Lincoln Diagnostics Inc., Decatur, IL, USA) device. Serum eosinophil cationic protein (UniCAP<sup>®</sup> 250, Phadia, Uppsala, Sweden), peripheral eosinophil count (Beckman Coulter<sup>®</sup> HmX<sup>™</sup>, Miami, FL, USA), and serum total IgE (UniCAP<sup>®</sup> 250, Phadia) were obtained at baseline and serially. Exhaled nitric oxide (eNO) concentration was measured by restricted-breath analysis (Aerocrine NIOX, Stockholm, Sweden) using a chemiluminescence analyser before spirometry at screening, randomization, and weeks 2, 4, 8, and 12. Spirometry (Jaeger, VIASYS Healthcare Respiratory Technologies, Yorba Linda, CA, USA) was measured at least 4 h after the last use of short-acting bronchodilator. Methacholine challenges were performed by administering doubling concentrations of methacholine using the Wright nebulizer-tidal breathing technique during the run in, and at 4 and 12 weeks after randomization. The concentration that induced a 20% fall from baseline FEV<sub>1</sub> was obtained by linear interpolation of the logarithmic dose-response curve (PC<sub>20</sub>). A 12-min sputum induction similar to the protocol adopted by Fahy et al. [39] using nebulized sterile 3% saline delivered via a Devilbiss ultrasonic nebulizer (Wright, Vancouver, BC, Canada) at a rate of 2.5 mL/min was conducted at baseline, 6 and 12 weeks post-randomization. Following collection of sputum, an equal volume of dithiothreitol 10% (Sputolysin; Behring Diagnostic Inc., Somerville, NJ, USA) was added. The samples were then gently vortexed and placed in a shaking water bath at 37 °C for 15 min to ensure adequate homogenization. A differential cell count was performed with 1 mL sputum. A sample was considered to be inadequate if sputum induction was

tolerated for <4 min or sputum volume was <1 mL, or squamous cell percentage was >80%.

The following variables were used to determine that NNF was safe: height, weight, serum biochemistries, liver function panel, haematology and coagulation indices, plasma and PBMC phospholipid fatty acid profiles, and plasma  $\beta$ -carotene, retinol, and  $\alpha$ -tocopherol levels. Plasma and PBMC phospholipids were isolated from the total lipids by thin-layer chromatography and quantitated by gas chromatography as described previously [40]. Plasma levels of  $\beta$ -carotene, retinol, and  $\alpha$ -tocopherol were measured using high-performance liquid chromatography with programmed wavelength ultraviolet detection [41].

After randomization, subjects were to discontinue the study treatment if the following occurred: hospitalization, hypoxic seizure, or intubation; requirement for a second prednisone burst for an exacerbation, a significant adverse event related to study medication, subject finding the formula intolerable, if the study physician determined that it was not in the best interest of the subject to continue, subject was lost to follow-up, withdrawal of consent or assent; or pregnancy.

### Statistical analysis

Sample size was estimated based on variability in asthma-free days in similar study populations [42, 43]. These studies indicated that similar groups of subjects could be expected to have on average 2.3 asthma-free days per week improvement in symptom control with treatment. Assuming that the control group would not have a change in asthma-free days, it was determined that 20 subjects in each arm could detect a 0.6 increase or greater in the average asthma-free days between the two groups using a two-sided  $\alpha$  of 0.05 with 80% power. This would be equivalent to increasing the average asthma-free days from 2.3 per week in the control group to 2.9 per week in the treatment group over the period of the study.

Characteristics at baseline were summarized using descriptive statistics and compared between treatment groups using ANOVA for continuous measures and Fisher's exact test for categorical measures. The intent-to-treat approach was applied to this study.

Primary analysis was based on a test of differences between Poisson regression parameters using reported asthma-free days over the period of time on study since randomization as the outcome variable and the treatment used as the primary exposure variable. The effects of covariates, age, gender, and severity of disease, were examined using a linear mixed model with asthma-free days per week as the outcome variable. Bronchodilator response and PC<sub>20</sub> were used as stratification variables and analysed accordingly. Baseline imbalance between the arms for a variable determined its inclusion as a covariate. Treatment group and week and their interaction

were also included in this model. To find the best model for these *a priori* variables, stepwise, backward regression with a  $P > 0.05$  to eliminate was used. A secondary analysis was performed as a stepwise, forward regression with a  $P \leq 0.05$  to enter. The first covariate in this model was NNF, and other variables examined based upon their putative importance to the outcome variable.

A subgroup analysis of 13 NNF compliant subjects who completed the study and had plasma phospholipid EPA values above 2.5% at weeks 2, 4, and 12 was also performed. Plasma phospholipid EPA levels below 2.5% represent <1 g of EPA consumed (<50% of NNF consumed) as previously observed with chronic feeding studies [44]. Children who consumed an average of 80% of NNF had EPA values close to 5%. Subjects who were excluded from the compliant group and/or who did not complete the study procedures were analysed to ascertain if they differed in an identifiable, systematic fashion from subjects who completed the protocol.

The analyses were performed using SAS PROC GENMOD and PROC MIXED [45].

### Results

Out of 43 randomized participants, 19 participants receiving NNF and 18 on control formula completed the 12-week trial. Four participants in the NNF group did not complete the study (one due to a hospitalization for asthma immediately after randomization, one disliked the taste of the formula within a month after randomization, one due to physician discretion, and one withdrew consent) while there were two in the control group who finished early (one due to formula taste intolerance within a month after randomization and another one due to elevated serum potassium within 6 weeks after randomization).

Participants had similar proportion of asthma-free days at baseline ( $23 \pm 6$  and  $28 \pm 6$  for NNF and control groups, respectively), consistent with frequency found for mild to moderate persistent asthma, average of two asthma-free days per week. Comparisons between participants randomized to the two treatment groups at baseline showed higher percentages of atopic patients ( $P = 0.02$ ) [with trend towards higher total serum IgE level ( $P = 0.08$ )] and lower symptom scores ( $P = 0.01$ ) [with trend towards higher albuterol rescue use for symptoms ( $P = 0.09$ )] in the control group compared with the NNF group (Table 2). Although participants in the NNF and control groups had no significant airflow limitation based on FEV<sub>1</sub>% predicted and only minimal obstruction based on FEV<sub>1</sub>/forced vital capacity (FVC), they had elevated eNO (geometric mean: 24.5 and 33.8 p.p.b., respectively) and moderate bronchial hyperresponsiveness (BHR) (geometric mean FEV<sub>1</sub> PC<sub>20</sub>: 1.75 and 1.54 mg/ml, respectively) based on methacholine FEV<sub>1</sub> PC<sub>20</sub> at baseline (Table 3).

### Intent-to-treat analyses

Over the 3-month study period, both groups demonstrated improvement in asthma-free days ( $P = 0.04$ ) with

Table 2. Baseline characteristics

|                                          | NNF (23)   | Control (20) | P-value     |
|------------------------------------------|------------|--------------|-------------|
| Age (years)                              | 10.4±0.44  | 10.9±0.47    | 0.5         |
| Gender (M/F)                             | 13/10      | 12/8         | 0.8         |
| Height (cm)                              | 142.4±2.88 | 143.7±2.73   | 0.9         |
| Weight (kg)                              | 40.7±3.09  | 42.2±4.83    | 0.8         |
| Body mass index (kg/m <sup>2</sup> )     | 19.3±0.85  | 19.6±1.39    | 0.8         |
| Percent atopic*                          | 78%        | 100%         | <b>0.02</b> |
| No. of positive percutaneous skin test   | 3.6±0.56   | 5.6±0.6      |             |
| Diary cards (scale 0–3) <sup>†</sup>     |            |              |             |
| Symptom score                            | 1.97±0.49  | 1.39±0.38    | <b>0.01</b> |
| Activity score                           | 0.19±0.09  | 0.14±0.09    | 0.34        |
| Albuterol (inhalations per day) used for |            |              |             |
| Exercise                                 | 0.39±0.16  | 0.35±0.19    | 0.66        |
| Symptoms                                 | 1.07±0.29  | 0.80±0.32    | 0.09        |
| Daytime                                  | 1.42±0.35  | 1.12±0.35    | 0.10        |
| Night-time                               | 0.09±0.08  | 0.06±0.07    | 0.53        |

\*One or more positive skin prick tests to aeroallergens (dog, cat, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, bermuda grass, tree mix, German roach, roach mix, *Alternaria tenuis*, *Cladosporium herbarum*, weed mix, *Penicillium notatum*, timothy grass).

<sup>†</sup>Severity increases with increase in score.

Data expressed as mean±SEM, unless otherwise specified. Bold values signify P-values <0.05.

NNF, novel nutritional formula.

no difference between the two treatments (Fig. 1a,  $P = 0.93$ ).

There were significant differences in log eNO concentration between the two groups at weeks 4, 8, and 12 (significant overall group effect  $P = 0.03$ ; Fig. 1b), with the control group demonstrating higher concentrations. In addition, there was a marginal overall group difference in log FEV<sub>1</sub> PC<sub>20</sub> ( $P = 0.05$ ) (Fig. 1c). However, there were no significant differences in these markers along with FEV<sub>1</sub>% predicted (Fig. 1d) and sputum eosinophil count over time (Fig. 1e), total serum IgE and ECP over time in either group (i.e. group vs. time interaction) compared with baseline (Table 3).

### Study formula compliance

Patients who completed the study reported drinking similar daily volumes (80–85% of 237 mL tetra-pak per day or 190–201 mL) of study formula over the 12-week period. Significantly lower levels of plasma phospholipid AA was found in the NNF group at weeks 4 and 12 compared with the control group (Fig. 2a), with a trend towards a decrease in PBMC phospholipid AA in the NNF group compared with controls at week 4 (Fig. 2b). In contrast, higher plasma and PBMC phospholipid % EPA levels (Figs 2c and d), EPA/AA (Figs 2e and f), and DGLA plus EPA/AA (Figs 2g and h) ratios were observed in patients consuming NNF as early as 2 weeks and remained elevated throughout the 12-week study period indicating

good compliance and acceptance of study formulations. In addition, no significant changes in phospholipid % EPA levels and in the ratios of EPA/AA and DGLA plus EPA/AA were found in patients consuming the control formula.

There was a significant group vs. time interaction in plasma  $\beta$ -carotene concentration ( $P = 0.01$ ), with the control group having a decrease and the NNF group having an increase in concentration by the second week (Fig. 3a). A marginal group vs. time interaction for plasma  $\alpha$ -tocopherol concentration was found ( $P = 0.08$ ), mostly driven by a change in concentration in the treatment group (differences between groups at weeks 4 and 12 at  $P = 0.02$  and  $0.08$ , respectively) (Fig. 3b). There was no significant difference in retinol levels between the two groups (Fig. 3c).

### Comparisons between compliers and control analyses

Based on the analysis of plasma phospholipid fatty acids, 13 participants in the NNF group (68%) were considered 'compliers', i.e. plasma phospholipid EPA values were consistently above 2.5%. No differences in the proportion of asthma-free days, FEV<sub>1</sub>% predicted, FEV<sub>1</sub>/FVC ratio, and sputum eosinophilia were observed between the compliers and the control group over time (Table 3). In contrast to the comparison between NNF and control groups, the analysis comparing the 'compliers' and the control groups increased the level of significance between groups for log eNO ( $P = 0.01$ ) and FEV<sub>1</sub>, PC<sub>20</sub> ( $P = 0.01$ ). In addition, the 'compliers' also had lower log ECP value at week 4 ( $P = 0.04$ ) and higher FEV<sub>1</sub>/FVC ratio at week 12 ( $P = 0.03$ ) compared with the control group. The total eosinophil counts were already significantly different between the 'compliers' and control group at baseline and remained so throughout the study, but no group change over time was found.

With respect to differences in plasma vitamin levels, statistically significant differences at  $P < 0.05$  were apparent between 'compliers' and control groups, respectively, for  $\beta$ -carotene at week 12 ( $0.17 \pm 0.02$  vs.  $0.11 \pm 0.02$ ,  $P = 0.03$ );  $\alpha$ -tocopherol at weeks 2 ( $10.88 \pm 0.96$  vs.  $7.99 \pm 0.44$ ,  $P = 0.001$ ), 4 ( $11.15 \pm 0.85$  vs.  $8.15 \pm 0.55$ ,  $P = 0.001$ ), and 12 ( $10.54 \pm 0.63$  vs.  $8.06 \pm 0.52$ ,  $P = 0.01$ ); and retinol at week 12 ( $0.41 \pm 0.02$  vs.  $0.33 \pm 0.02$ ,  $P = 0.01$ ).

### Safety variables and adverse events

At baseline and at 4 and 12 weeks of study, the control and NNF groups had similar mean values for serum chemistries, electrolytes, liver function, haematology, and blood coagulation indices, with all variables remaining within the normal range (data not shown). Despite the addition of 300 calories/day, there were no significant differences in body weight, height, or body mass index changes (baseline to 12 weeks) between the study groups. Adverse events, which included respiratory tract infections, colds, sore throat, viral illness, diarrhoea, vomiting, nummular

Table 3. Effects of a novel nutritional formula (NNF) vs. control on clinical, physiologic and airway inflammation outcome measures

|                                               | NNF      |            | Control  |            |                  | Compliers |            |                   |
|-----------------------------------------------|----------|------------|----------|------------|------------------|-----------|------------|-------------------|
|                                               | <i>n</i> | Mean       | <i>n</i> | Mean       | <i>P</i> -value* | <i>n</i>  | Mean       | <i>P</i> -value** |
| Asthma-free days (%)                          |          |            |          |            |                  |           |            |                   |
| Baseline                                      | 21       | 23±6       | 19       | 28±6       | 0.55             | 13        | 21±7       | 0.49              |
| Week 2                                        | 21       | 25±6       | 20       | 28±7       | 0.71             | 12        | 31±9       | 0.86              |
| Week 4                                        | 21       | 26±7       | 18       | 31±7       | 0.63             | 13        | 23±8       | 0.46              |
| Week 6                                        | 19       | 27±7       | 19       | 33±8       | 0.54             | 13        | 25±10      | 0.51              |
| Week 8                                        | 17       | 33±7       | 18       | 40±9       | 0.55             | 12        | 30±9       | 0.44              |
| Week 12                                       | 19       | 34±8       | 18       | 45±9       | 0.32             | 12        | 34±10      | 0.40              |
|                                               |          | Group      |          |            | 0.31             |           |            | 0.38              |
|                                               |          | Week       |          |            | <b>0.04</b>      |           |            | 0.12              |
|                                               |          | Group×week |          |            | 0.93             |           |            | 0.84              |
| FEV <sub>1</sub> (L)                          |          |            |          |            |                  |           |            |                   |
| Baseline                                      | 23       | 2.04±0.12  | 20       | 2.04±0.13  | 0.96             | 13        | 2.02±0.17  | 0.92              |
| Week 2                                        | 22       | 2.05±0.14  | 20       | 1.99±0.14  | 0.78             | 13        | 2.02±0.19  | 0.91              |
| Week 6                                        | 20       | 2.04±0.15  | 18       | 1.92±0.15  | 0.61             | 13        | 2.03±0.21  | 0.68              |
| Week 12                                       | 19       | 2.17±0.15  | 18       | 2.06±0.14  | 0.52             | 13        | 2.15±0.20  | 0.61              |
|                                               |          | Group      |          |            | 0.80             |           |            | 0.85              |
|                                               |          | Week       |          |            | 0.05             |           |            | <b>0.01</b>       |
|                                               |          | Group×week |          |            | 0.52             |           |            | 0.39              |
| FEV <sub>1</sub> % predicted                  |          |            |          |            |                  |           |            |                   |
| Baseline                                      | 23       | 96.09±2.03 | 20       | 94.85±2.82 | 0.72             | 13        | 95.62±2.41 | 0.85              |
| Week 2                                        | 22       | 94.82±2.06 | 20       | 92.20±3.00 | 0.47             | 13        | 94.31±2.72 | 0.63              |
| Week 6                                        | 20       | 93.70±2.81 | 18       | 88.22±2.90 | 0.18             | 13        | 92.92±3.34 | 0.30              |
| Week 12                                       | 19       | 96.11±2.17 | 18       | 92.06±3.04 | 0.30             | 13        | 97.62±2.42 | 0.20              |
|                                               |          | Group      |          |            | 0.33             |           |            | 0.48              |
|                                               |          | Week       |          |            | 0.18             |           |            | <b>0.04</b>       |
|                                               |          | Group×week |          |            | 0.72             |           |            | 0.74              |
| FEV <sub>1</sub> /FVC                         |          |            |          |            |                  |           |            |                   |
| Baseline                                      | 23       | 78.6±1.5   | 20       | 76.6±1.7   | 0.35             | 13        | 78.2±2.2   | 0.59              |
| Week 2                                        | 22       | 77.8±1.6   | 20       | 75.3±2.0   | 0.25             | 13        | 79.2±1.9   | 0.19              |
| Week 6                                        | 20       | 78.6±2.0   | 18       | 75.9±2.4   | 0.39             | 13        | 78.9±2.3   | 0.39              |
| Week 12                                       | 19       | 81.5±1.9   | 18       | 76.6±1.5   | 0.04             | 13        | 82.4±2.3   | <b>0.03</b>       |
|                                               |          | Group      |          |            | 0.16             |           |            | 0.16              |
|                                               |          | Week       |          |            | 0.45             |           |            | 0.22              |
|                                               |          | Group×week |          |            | 0.70             |           |            | 0.25              |
| Log FEV <sub>1</sub> PC <sub>20</sub> (mg/mL) |          |            |          |            |                  |           |            |                   |
| Baseline                                      | 23       | 0.56±0.24  | 20       | 0.43±0.28  | 0.8              | 13        | 0.64±0.37  | 0.65              |
| Week 4                                        | 20       | 0.79±0.31  | 19       | −0.11±0.37 | 0.07             | 12        | 1.09±0.32  | <b>0.03</b>       |
| Week 12                                       | 19       | 0.78±0.30  | 18       | 0.09±0.37  | 0.17             | 13        | 1.13±0.39  | 0.07              |
|                                               |          | Group      |          |            | 0.05             |           |            | <b>0.01</b>       |
|                                               |          | Week       |          |            | 0.72             |           |            | 0.66              |
|                                               |          | Group×week |          |            | 0.60             |           |            | 0.75              |
| Log serum total IgE (kU/L)                    |          |            |          |            |                  |           |            |                   |
| Baseline                                      | 23       | 4.81±0.30  | 18       | 5.56±0.26  | 0.08             | 13        | 4.24±0.40  | <b>0.01</b>       |
| Week 4                                        | 21       | 4.72±0.33  | 17       | 5.65±0.25  | 0.03             | 13        | 4.29±0.44  | <b>0.01</b>       |
| Week 12                                       | 19       | 4.77±0.37  | 18       | 5.55±0.28  | 0.10             | 13        | 4.28±0.46  | <b>0.02</b>       |
|                                               |          | Group      |          |            | 0.05             |           |            | <b>0.007</b>      |
|                                               |          | Week       |          |            | 0.86             |           |            | 0.97              |
|                                               |          | Group×week |          |            | 0.95             |           |            | 0.91              |
| Log exhaled nitric oxide (p.p.b.)             |          |            |          |            |                  |           |            |                   |
| Baseline                                      | 23       | 3.20±0.18  | 20       | 3.52±0.17  | 0.21             | 13        | 3.11±0.22  | 0.15              |
| Week 2                                        | 21       | 3.15±0.20  | 20       | 3.64±0.20  | 0.10             | 12        | 2.99±0.27  | 0.05              |
| Week 4                                        | 21       | 3.19±0.15  | 19       | 3.66±0.15  | 0.03             | 13        | 3.02±0.19  | <b>0.01</b>       |
| Week 8                                        | 15       | 3.05±0.23  | 15       | 3.77±0.20  | 0.02             | 10        | 2.85±0.25  | <b>0.01</b>       |
| Week 12                                       | 19       | 3.04±0.17  | 18       | 3.81±0.14  | 0.002            | 13        | 2.93±0.21  | <b>0.001</b>      |

Table 3. continued

|                                             | NNF      |              | Control  |             | <i>P</i> -value* | Compliers |             | <i>P</i> -value** |
|---------------------------------------------|----------|--------------|----------|-------------|------------------|-----------|-------------|-------------------|
|                                             | <i>n</i> | Mean         | <i>n</i> | Mean        |                  | <i>n</i>  | Mean        |                   |
|                                             |          | Group        |          |             | 0.03             |           |             | 0.01              |
|                                             |          | Week         |          |             | 0.64             |           |             | 0.94              |
|                                             |          | Group × week |          |             | 0.06             |           |             | 0.08              |
| Total eosinophil count per mm <sup>3</sup>  |          |              |          |             |                  |           |             |                   |
| Baseline                                    | 22       | 309 ± 250    | 19       | 411 ± 218   | 0.19             | 12        | 233 ± 156   | 0.02              |
| Week 4                                      | 22       | 327 ± 237    | 19       | 453 ± 241   | 0.10             | 13        | 254 ± 145   | 0.01              |
| Week 12                                     | 19       | 289 ± 238    | 19       | 411 ± 197   | 0.10             | 13        | 254 ± 133   | 0.02              |
|                                             |          | Group        |          |             | 0.10             |           |             | 0.004             |
|                                             |          | Week         |          |             | 0.44             |           |             | 0.76              |
|                                             |          | Group × week |          |             | 0.96             |           |             | 0.82              |
| Log eosinophil cationic protein (mcg/L)     |          |              |          |             |                  |           |             |                   |
| Baseline                                    | 21       | 2.79 ± 0.18  | 19       | 2.64 ± 0.16 | 0.53             | 12        | 2.59 ± 0.17 | 0.87              |
| Week 4                                      | 21       | 2.61 ± 0.17  | 16       | 2.90 ± 0.20 | 0.28             | 12        | 2.36 ± 0.12 | 0.04              |
| Week 12                                     | 18       | 2.88 ± 0.19  | 18       | 2.96 ± 0.20 | 0.69             | 12        | 2.76 ± 0.24 | 0.46              |
|                                             |          | Group        |          |             | 0.62             |           |             | 0.22              |
|                                             |          | Week         |          |             | 0.17             |           |             | 0.16              |
|                                             |          | Group × week |          |             | 0.10             |           |             | 0.06              |
| Sputum eosinophil, % of non-squamous cells  |          |              |          |             |                  |           |             |                   |
| Baseline                                    | 12       | 0.5 ± 0.2    | 11       | 0.8 ± 0.4   | 0.4              | 7         | 0.3 ± 0.3   | 0.3               |
| Week 6                                      | 9        | 1.1 ± 0.6    | 7        | 1.4 ± 0.8   | 0.7              | 5         | 0.7 ± 0.3   | 0.5               |
| Week 12                                     | 10       | 1.1 ± 0.5    | 11       | 3.6 ± 1.7   | 0.2              | 7         | 0.7 ± 0.2   | 0.2               |
|                                             |          | Group        |          |             | 0.13             |           |             | 0.14              |
|                                             |          | Week         |          |             | 0.1              |           |             | 0.26              |
|                                             |          | Group × Week |          |             | 0.38             |           |             | 0.46              |
| Sputum neutrophils, % of non-squamous cells |          |              |          |             |                  |           |             |                   |
| Baseline                                    | 12       | 60.5 ± 8.2   | 11       | 56.0 ± 7.1  | 0.7              | 7         | 51.2 ± 11.4 | 0.7               |
| Week 6                                      | 9        | 51.8 ± 8.2   | 7        | 59.4 ± 9.3  | 0.5              | 5         | 37.0 ± 8.4  | 0.12              |
| Week 12                                     | 10       | 66.6 ± 7.4   | 11       | 51.7 ± 6.6  | 0.15             | 7         | 69.6 ± 8.0  | 0.1               |
|                                             |          | Group        |          |             | 0.44             |           |             | 0.88              |
|                                             |          | Week         |          |             | 0.43             |           |             | 0.07              |
|                                             |          | Group × week |          |             | 0.16             |           |             | 0.02              |

\**P*-value is for a 2 group *t*-test between NNF and control for each week separately in the main body of the table and for an ANOVA for the factors Group, Week and Group × Week over the time periods in the table.

\*\**P*-value is for a 2 group *t*-test between NNF Compliers and Control for each week separately in the main body of the table and for an ANOVA for the factors Group, Week and Group × Week over the time periods in the table.

Data expressed as mean ± SEM. Bold values signify *P*-values < 0.05.

FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity.

eczema, myalgia, headache, elbow bruise, head injury, mild elevation in liver enzyme, hyperkalemia, and retained baby tooth, were reported in 16 NNF and 10 control patients; of the 42 adverse events reported, 26 were in the NNF, and 16 in the control group. The only 'severe' event, an upper respiratory infection probably not related to the study formula, occurred in the control group. There were three possibly related events, one in the NNF group (mild elevation of liver enzymes) and two in the control group (one mild elevated potassium and one moderate episode of vomiting).

## Discussion

This prospective, randomized, controlled feasibility study evaluating the efficacy of a NNF containing EPA, DHA,

GLA, and antioxidants compared with a control formula did not reveal a difference in our primary outcome of proportion of asthma-free days. Daily consumption of both NNF and the control formula for 3 months demonstrated improvements in the proportion of asthma-free days over time (*P*-value week = 0.04, Fig. 1a). Although no statistically significant differences in the patterns of several secondary outcomes over time between the two groups were found, lower eNO concentrations and higher log FEV<sub>1</sub> PC<sub>20</sub> values during the study were seen in the NNF compared with the control group (Figs 1b and c). The level of significance for the differences in eNO and BHR at different visits was more pronounced when 'compliers' and the control group were compared.

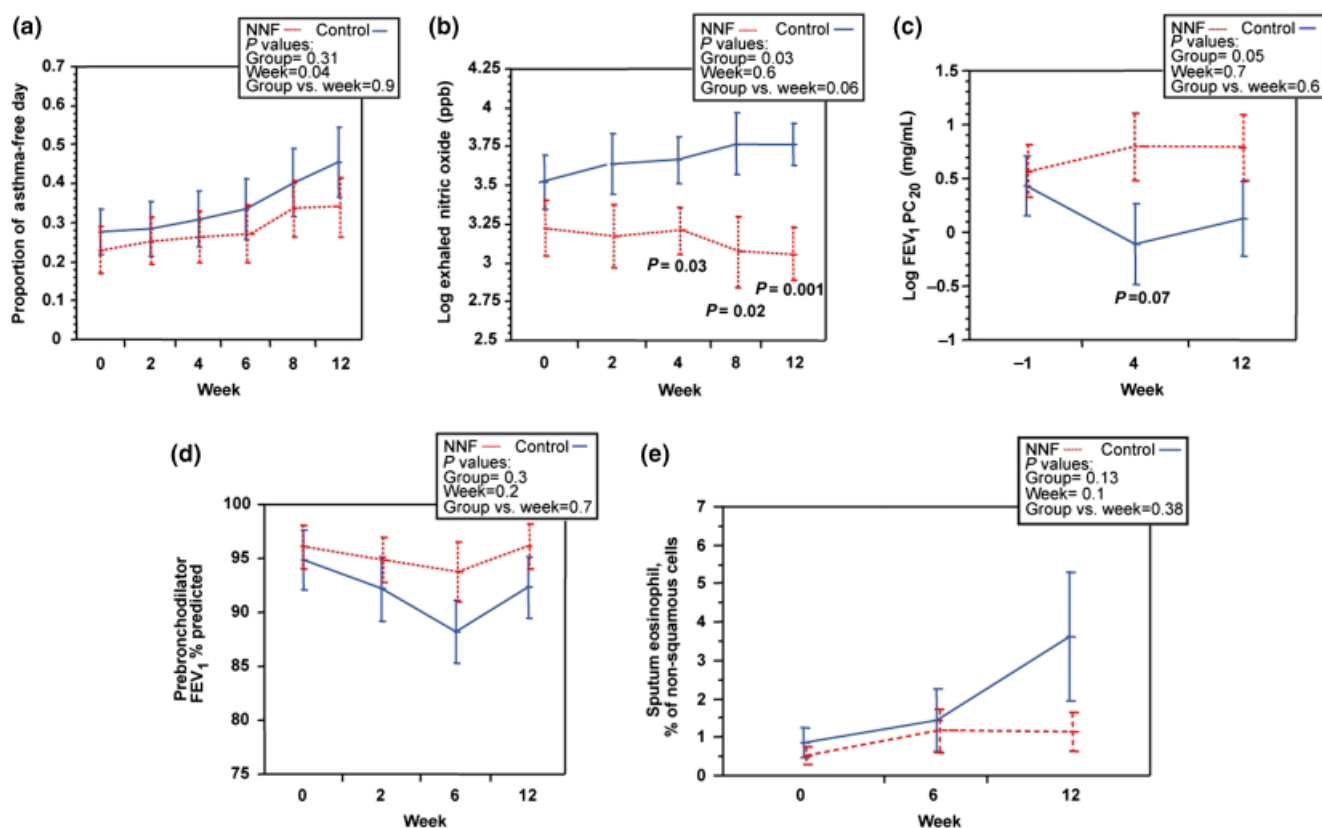


Fig. 1. Comparison of asthma-free days (a), log exhaled nitric oxide (b), methacholine FEV<sub>1</sub> log PC<sub>20</sub> (c), pre-bronchodilator FEV<sub>1</sub>% predicted (d), and sputum eosinophil (% of non-squamous cells) (e) in the NNF and control groups. FEV<sub>1</sub>, forced expiratory volume in 1 s; NNF, novel nutritional formula.

The lack of effect on the primary outcome chosen for this study (asthma-free days) could be related to the low level of asthma severity selected for this group, in order to initially test safety and adherence for NNF. Alternatively, the 12-week treatment duration may have been inadequate to reach full treatment effect. Of note, the participants in the control group had features consistent with worsening asthma control in relation to lung function and airway inflammation, while the NNF group remained stable. In general, prior clinical trials utilizing dietary supplement with EPA and DHA of varying dosages have not shown consistent outcomes with respect to symptoms, lung function, BHR, and asthma medication usage. However, individual studies have demonstrated effects on specific parameters. In particular, Mickleborough et al. [28] showed that 3-week daily fish oil supplement containing 3.2 g EPA and 2.2 g DHA reduced post-exercise fall in FEV<sub>1</sub>. Another study with seven patients receiving 3 g of fish oil daily (0.96 g EPA+DHA) for a month noted a significant decrease in airway responsiveness and residual volume with no change in FEV<sub>1</sub> [25]. In a 10-month intervention study in 29 children who received either fish oil capsules with 0.084 g EPA and 0.036 g DHA or 300 mg olive oil as control under close supervision, a reduction in asthma

scores and acetylcholine thresholds was observed [23]. Another double-blind, placebo-controlled study of 46 adults with mild-moderate asthma, randomized to receive two capsules twice daily for 2 months of either a lipid extract capsule containing 0.05 g EPA and DHA (total daily 0.2 g) and 100 mg olive oil (total 400 mg daily) or placebo capsule containing 150 mg olive oil (total 600 mg daily), had reduced daytime wheezing and exhaled H<sub>2</sub>O<sub>2</sub> and increase in peak flow rates in the supplemented group with no change in FEV<sub>1</sub>, compared to the reference group. [29]. Given the diverse effects of the dietary supplement on several asthma outcomes, larger studies with adequate power, perhaps of longer duration, are necessary.

The anti-inflammatory potential of NNF was observed in our study by significant increases in plasma and PBMC EPA levels and in the ratios of EPA/AA and DGLA plus EPA/AA and decrease in plasma AA in patients consuming NNF. Similar increases in EPA and decreases in AA in immune cell phospholipids have been observed in  $\omega$ -3 fatty acid supplementation studies in mild to moderate persistent asthma patients with exercise-induced bronchoconstriction (EIB) [28, 29]. Such changes seen with the fish oil diet in a randomized, double-blind, placebo-controlled crossover trial over 8 weeks, were also

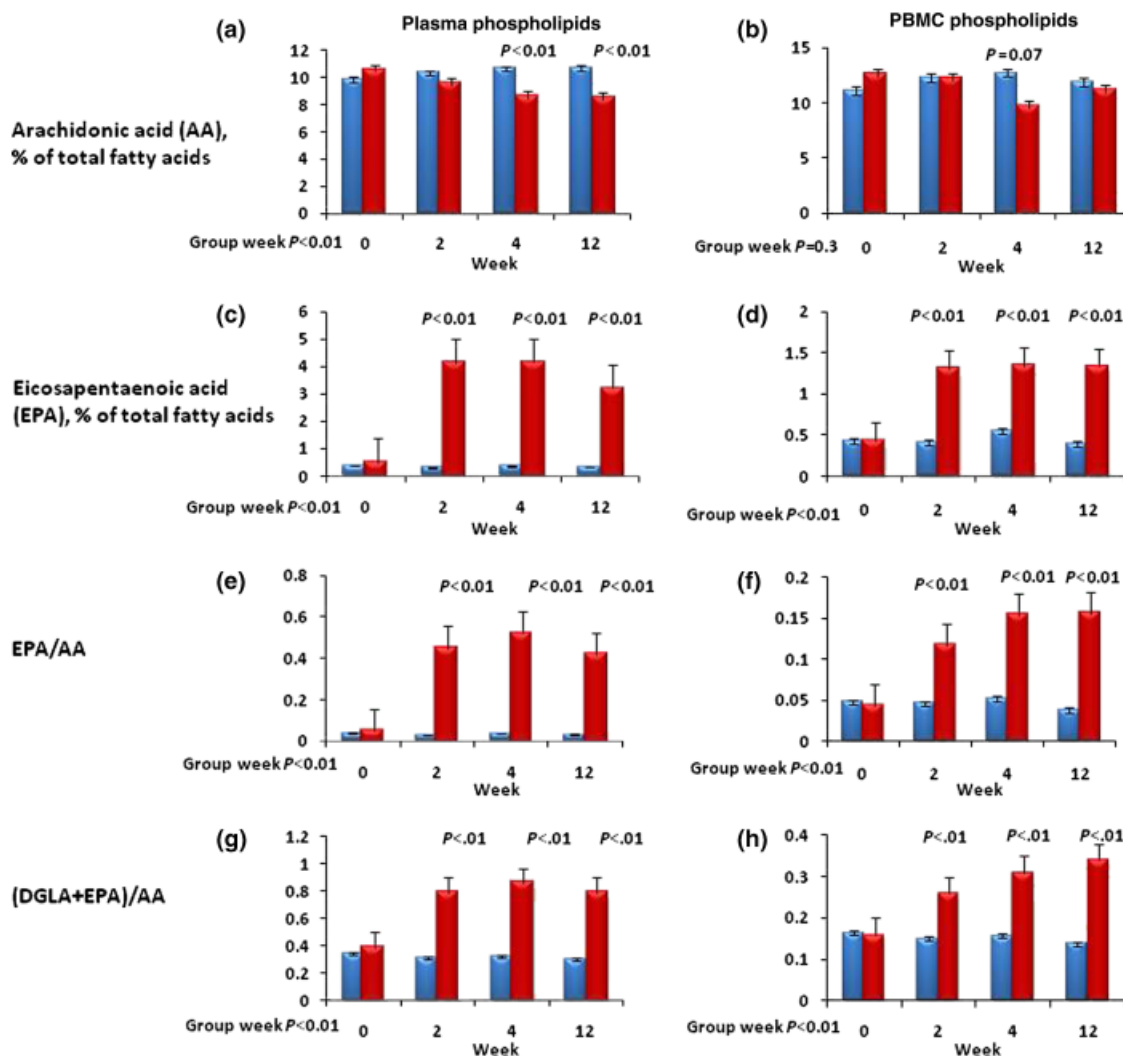


Fig. 2. Levels of arachidonic acid (AA), eicosapentaenoic acid (EPA) as % of total fatty acids, and EPA/AA and dihomono- $\gamma$ -linolenic acid (DGLA+EPA)/AA ratios in plasma (a, c, e, g) and peripheral blood mononuclear cell phospholipids (b, d, f, h) in the novel nutritional formula (red bars) and control groups (blue bars).

associated with an amelioration of the exercise-induced airway obstruction and significant suppression of the sputum eosinophil and neutrophil counts and sputum supernatant eicosanoid ( $\text{LTC}_4$ – $\text{LTE}_4$ , and  $\text{PGD}_2$ ) and cytokine (IL-1 $\beta$  and TNF- $\alpha$ ) levels [29].

In addition to the plasma and PBMC changes in fatty acids, there were significant and sustained increases in the plasma levels of  $\beta$ -carotene and  $\alpha$ -tocopherol concentrations for NNF patients found in this study. It was impossible in this study to assess the benefits of the antioxidants alone. Reactive oxygen metabolites such as superoxide, hydroxyl radicals, and hydrogen peroxide are produced by activated PBMC, neutrophils, and macrophages and are believed to contribute to the airway damage seen in asthma [46]. One hypothesis to explain the increasing prevalence of childhood asthma is that as a child's diet becomes progressively more deficient in antioxidants, this

leads to an increased susceptibility of the pulmonary airways to reactive oxygen metabolites resulting in airway inflammation and asthma. Considering a compromised antioxidant state of the asthmatic airway, we postulated that the provision of antioxidants in NNF would be beneficial by maintaining or increasing airway protection against reactive oxygen metabolites. The shift in the fatty acid concentrations towards an anti-inflammatory profile combined with antioxidant supplementation may have contributed in part to the prevention in the deterioration in several objective features of asthma such as BHR and airway inflammation.

Nineteen children randomized to NNF and 18 control children completed the 12-week trial with both groups consuming on average 80–85% of their 8 oz drink. Thus children given NNF consumed approximately 2.4–3.0 g GLA from borage oil and 2.4–3.0 g EPA and 1.28–1.60 g

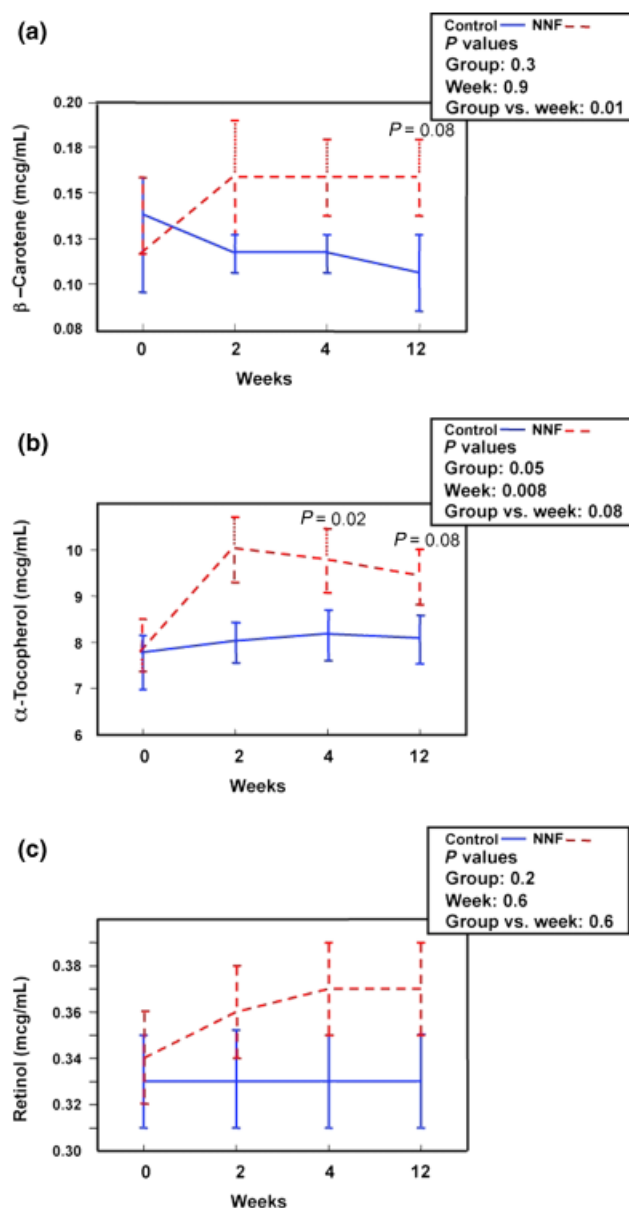


Fig. 3. Levels of  $\beta$ -carotene (a),  $\alpha$ -tocopherol (b), and retinol (c) in children who received the novel nutritional formula (NNF) and the control formula.

DHA from sardine oil. None of the adverse events reported were deemed to be 'Probable formula (control or NNF) related' with no abnormalities observed in serum biochemistries, liver function, haematology indices, coagulation indices, and plasma and PBMC phospholipid fatty acid profiles (which reflect the metabolism of the elevated levels of EPA and GLA intake) for the 12-week feeding period.

The limitations of our study should be addressed in future studies of NNF, including a careful assessment of the duration, primary outcome, and design. Longer duration of treatment could provide insight on greater changes in BHR or other measures of inflammation that could

eventually impact significant clinical outcomes, such as exacerbations and health care utilization. Similar to the Hodge study [19], changes in biomarkers (i.e. TNF- $\alpha$  production and eosinophils) may not be maximal at 3 months, and although speculative, the changes in inflammation could eventually impact asthma severity or symptoms similar to the observations found by Mickleborough *et al.* [29] with respect to attenuation of EIB. While this dietary therapy may not exceed the benefits provided by inhaled corticosteroid therapy, it could serve as adjunctive therapy and limit the dose of inhaled corticosteroids necessary to control asthma and reduce the duration and total exposure to inhaled corticosteroid and supplemental long-term control therapy. Alternatively, as indicated by studies with inhaled corticosteroids or combination inhaled corticosteroid and long-acting  $\beta$ -agonist in which a proportion of patients remain inadequately controlled [1], this intervention may provide a promising option which can lend support to a different disease mechanism involved.

In summary, while no difference in asthma-free days was found between the active treatment and control groups over the course of this 3-month study, there is now evidence of a potential effect of NNF on alleviating inflammatory biomarkers in children with persistent asthma. Dietary manipulation of fatty acids and antioxidants offer a potential strategy for the prevention of airway inflammation associated with persistent asthma.

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