

ORIGINAL ARTICLE

# Pilot Study of the Effects of n-3 Polyunsaturated Fatty Acids on Exhaled Nitric Oxide in Patients With Stable Asthma

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## ■ Abstract

**Background:** The anti-inflammatory effects of n-3 polyunsaturated fatty acids (n-3 PUFA) have been demonstrated both in vitro and in vivo. The results of epidemiological studies suggest that fish consumption has a beneficial effect on lung function and prevalence of asthma. However, data from intervention trials have not revealed a beneficial effect of n-3 PUFA supplementation in patients with established disease.

**Objective:** To study the effects of short-term n-3 PUFA supplementation in addition to maintenance therapy on exhaled nitric oxide in asthmatic patients.

**Methods:** A double-blind, placebo-controlled trial was undertaken in 20 women with asthma. Patients received either a combination of eicosapentaenoic acid and docosahexaenoic acid plus 10 mg vitamin E or placebo twice daily for 2 weeks. The primary outcome measure was the fraction of exhaled nitric oxide (FeNO) and the secondary outcomes were asthma control (score on the Asthma Control Questionnaire [ACQ]) and lung function (forced expiratory volume in 1 second [FEV<sub>1</sub>]).

**Results:** No significant differences were observed in FeNO, ACQ score, or FEV<sub>1</sub> between patients receiving n-3 PUFA supplementation and those receiving placebo.

**Conclusions:** Short-term dietary supplementation with n-3 PUFA in women with stable asthma was not associated with statistically significant changes in FeNO, asthma control, or lung function

**Key words:** Asthma. Airway inflammation. n-3 Polyunsaturated fatty acids. Eicosapentaenoic acid. Docosahexaenoic acid. Exhaled nitric oxide.

## ■ Resumen

**Antecedentes:** Los efectos antiinflamatorios de los ácidos grasos poliinsaturados n-3 (AGPI n-3) se han demostrado tanto in vivo como in vitro. Los resultados de estudios epidemiológicos realizados sugieren que el consumo de pescado tiene un efecto beneficioso sobre la función pulmonar y la prevalencia del asma. No obstante, los datos obtenidos a partir de estudios clínicos de intervención no han revelado efectos beneficiosos de la toma de suplementos AGPI n-3 en pacientes con la enfermedad establecida.

**Objetivo:** Estudiar los efectos de la suplementación con AGPI n-3 a corto plazo combinado con la terapia de mantenimiento sobre el óxido nítrico espirado en pacientes asmáticos.

**Métodos:** Se efectuó un estudio de doble ciego controlado con placebo en el que participaron 20 mujeres asmáticas. Se administró a las pacientes una combinación de ácido eicosapentaenoico y de ácido docosahexaenoico con 10 mg de vitamina E o placebo dos veces al día durante dos semanas. El principal criterio de valoración fue la fracción de óxido nítrico espirado (FeNO) y el secundario fue el control del asma (puntuación en el Cuestionario de control del asma [ACQ]) y la función pulmonar (volumen espiratorio forzado en el primer segundo [FEV<sub>1</sub>]).

**Resultados:** No se observaron diferencias significativas en el FeNO, la puntuación ACQ o el FEV<sub>1</sub> entre las pacientes que tomaron suplementos de AGPI n-3 y las que tomaron el placebo.

**Conclusiones:** Los suplementos dietéticos a corto plazo con AGPI n-3 en mujeres con asma estable no se asociaron con cambios estadísticamente significativos en los valores FeNO, de control del asma o de la función pulmonar.

**Palabras clave:** Asma. Inflamación de las vías respiratorias. Ácidos grasos poliinsaturados n-3. Ácido eicosapentaenoico. Ácido docosahexaenoico. Óxido nítrico espirado.

## Introduction

There is currently a lack of consensus regarding the proposed association between reduced intake of n-3 polyunsaturated fatty acids (n-3 PUFA) and increased prevalence of allergic diseases [1-3]. Although preventive effects of n-3 PUFA supplementation in terms of allergic sensitization or inflammatory variables have been reported [4-7], data generated from intervention trials have failed to show meaningful improvement in clinical or functional asthma outcomes once the disease is established [8].

The aim of this study was to determine whether airway inflammation assessed by exhaled nitric oxide is improved by short-term dietary n-3 PUFA supplementation in stable asthmatic patients receiving inhaled corticosteroids.

## Methods

### Subjects

Adult women diagnosed with stable persistent asthma who attended the allergy clinic of University Hospital São João were invited to participate. Stable asthma was defined as the absence of asthma exacerbations requiring oral prednisolone or an increased use of inhaled corticosteroids in the previous 4 weeks, use of rescue medication no more than 3 times a week, and the absence of clinical indications for alteration of treatment. Persistent asthma was defined by current use of inhaled corticosteroids. Patients were excluded if they were smokers, took antileukotrienes, had any chronic diseases other than asthma and rhinitis, were pregnant, or if they did not take contraceptive measures. The study was approved by the hospital ethics committee.

### Study Design

The study was a randomized, double-blind, placebo-controlled trial. General data were collected at the screening visit. After enrollment, at the baseline visit, subjects were randomly assigned to the intervention. Capsules of n-3 PUFA or placebo were labeled with study numbers according to a prepared blocked randomization list. Patients were allocated study numbers sequentially and, thus, randomly allocated trial supplement. The n-3 PUFA group (n=11) received 2 fish-oil capsules (Coolmar, Biopura, Lisbon, Portugal) containing a combination of 455 mg eicosapentaenoic acid (EPA) and 325 mg docosahexaenoic acid (DHA) plus 10 mg vitamin E each, taken once daily for 2 weeks; the placebo group (n=9) received 2 capsules of amide daily for the same period. The final visit was performed 3 days after the intervention. Compliance was monitored by counting returned capsules.

### Outcomes

The primary outcome measure was the fraction of exhaled nitric oxide (FeNO) and the secondary outcomes included lung function and control of asthma. FeNO was measured using the NIOX system (Aerocrine, Stockholm, Sweden) [9]. Asthma control was assessed using the 6-item Asthma

Control Questionnaire (ACQ) [10]. The ACQ was developed to measure the adequacy of clinical asthma control with a score ranging from 0 (good) to 6 (poor). Forced expiratory volume in the first second ( $FEV_1$ ) was determined by spirometry using a Vitalograph 2120 spirometer (Vitalograph Ltd, Buckingham, UK). A baseline Asthma Life Quality (ALQ) test [11] was administered. The ALQ test has a score ranging from 0 (good) to 20 (poor). Dietary intake was measured using a semi-quantitative food frequency questionnaire (FFQ) administered by a nutritionist and analyzed using Food Processor Plus software (ESHA Research, Salem, USA).

### Statistical Analysis

Analysis was conducted on an intention-to-treat basis, with subjects defined as all randomized patients who took at least 1 dose of the intervention. The estimated sample size required to detect a 33% effect of the intervention using FeNO as the main outcome was 20 subjects [12]. Clinical characteristics of placebo and n-3 PUFA groups were compared by Student *t* test for continuous variables and proportions were compared with  $\chi^2$  tests. Comparisons between baseline and final visits were made with paired *t* test. Comparisons between groups were made by covariance analysis.

The covariance analysis considers the initial value of the parameter as a covariate, allowing a better estimation of the effect of the intervention. Statistical significance was set at  $P < .05$ .

## Results

Of the 23 patients screened and randomized, 20 were analyzed (Table 1). Three withdrew before taking any supplement, having indicated that they were unable to attend visits. No side-effects, such as bleeding or menstrual problems, were reported. Patient compliance was above 75% in all cases. No differences were observed between n-3 PUFA and placebo groups in terms of the change in FeNO ( $P = .373$ ),  $FEV_1$  ( $P = .533$ ), or ACQ score ( $P = .978$ ) (Table 2). A significant increase in FeNO was observed in the placebo group ( $P = .041$ ) and an improvement in ACQ score was observed in the placebo ( $P = .033$ ) and n-3 PUFA ( $P = .021$ ) groups (figure).

## Discussion

In asthma, FeNO is used as an indirect marker of airway inflammation and is correlated with inflammatory biomarkers such as blood eosinophils, IgE level, and allergic sensitization [15]. Elevated levels of FeNO result from increased expression and activity of the inducible nitric oxide synthase (iNOS) in airway epithelial and inflammatory cells following stimulation by proinflammatory cytokines such as interleukin-1, tumor necrosis factor  $\alpha$ , and interferon- $\gamma$  [16,17]. However, the effects of n-3 PUFA on iNOS are unclear. In human osteoblastic cells, EPA prevented the increase in iNOS gene expression [18] while in rat vascular smooth muscle cells, DHA potentiated

**Table 1.** Baseline Characteristics of the Patient Group\*

	Placebo, n=9	n-3 PUFA, n=11	<i>P</i>
Age, y	34 (24 – 44)	41 (37 – 46)	.222
BMI, kg/m <sup>2</sup>	25 (19 – 31)	28 (20 – 36)	.553
Atopic, n (%)	6 (66.7)	6 (54.5)	.670
Inhaled corticosteroids, µg†	686 (274 – 1097)	1000 (486 – 1514)	.251
PUFA n-6:n-3, mean ± SD	8.7 ± 2.6	10.1 ± 2.2	.252
FeNO, ppb	20.4 (10.0 – 30.1)	27.6 (16.6 – 38.6)	.309
ACQ score	1.7 (1.0 – 2.5)	1.4 (0.8 – 2.1)	.488
FEV <sub>1</sub> , % predicted	90.9 (75.9 – 105.8)	96.7 (85.4 – 108.0)	.478
AQL score	13.9 (11.6 – 16.2)	13.5 (10.9 – 16.2)	.222

\*Data are shown as means (95% confidence interval) unless otherwise indicated. FEV<sub>1</sub> indicates forced expiratory volume in the first second; ACQ, Asthma Control Questionnaire; AQL, Asthma Quality of Life questionnaire; FeNO: fraction of exhaled nitric oxide; PUFA, polyunsaturated fatty acids; BMI, body mass index.

†Budesonide equivalent dose. Groups were compared by  $\chi^2$  or *t* test where appropriate.

iNOS expression [19].

Because of the exploratory nature of our study, the number of patients could have been insufficient to establish statistical significance. Additionally, duration or dosage of the supplement may have been inadequate to achieve the hypothesized antiinflammatory effects. Higher ratios of n-6:n-3 PUFA have been associated with an increased risk of asthma [20]. However, our strategy—supplementation at a dose known to carry no risk of adverse events and over a short period of time—is more prone to increase compliance in real life. We restricted inclusion to women based on previous observations that the relationship between asthma measures and diet or obesity is sex dependent [21,22]. Almost all of the subjects were within the normal range for baseline FeNO,

with values probably representing optimum FeNO levels, due to the current use of inhaled corticosteroids. It can be argued that the likelihood of a meaningful change would therefore be limited. However, the aim of our study was not to determine whether n-3 PUFA supplementation would be better than inhaled corticosteroids in terms of exhaled nitric oxide, but rather to assess if any further benefit would appear from n-3 PUFA supplementation in addition to regular maintenance treatment.

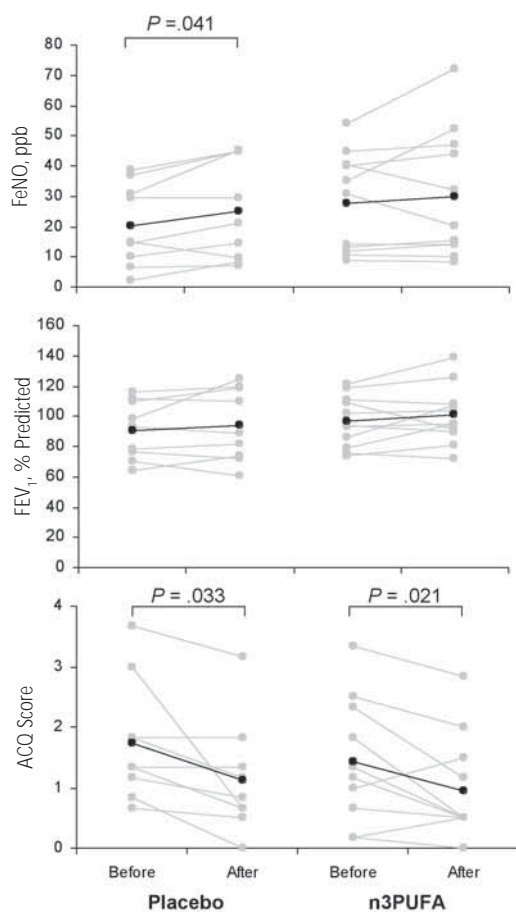
Previous studies failed to show any consistent effect of n-3 PUFA on FEV<sub>1</sub>. Doses from 200 to 3000 mg per day of EPA/DHA from 1 month to 1 year had no significant effect on lung function [23-25], while in 2 studies, 10 to 20 g per day of perilla-seed oil for 1 month in 14 asthma patients [26] and

**Table 2.** Changes in Exhaled Nitric Oxide, Lung Function, and Asthma Control After Supplementation With n-3 PUFA Compared to Placebo\*

	Placebo, n=9			n-3 PUFA, n=11†			Placebo vs n3PUFA‡
	Baseline	Post	Change† intervention	Baseline	Post	Change† intervention	
F <sub>E</sub> NO, ppb	20.4 (10.0 – 30.1)	25.0 (12.3 – 37.7)	4.6 (0.2 – 8.9); <i>P</i> = .041	27.6 (16.6 – 38.6)	30.0 (15.8 – 44.2)	2.4 (–3.5 – 8.3); <i>P</i> = .386	.373
FEV <sub>1</sub> , %	90.9 (75.9 – 105.8)	94.5 (75.9 – 113.0)	3.7 (–4.6 – 12.9); <i>P</i> = .336	96.7 (85.4 – 108.0)	100.7 (87.9 – 113.6)	4.0 (–3.7 – 11.7); <i>P</i> = .276	.533
ACQ score	1.7 (1.0 – 2.5)	1.1 (0.4 – 1.8)	–0.6 (–1.2 – –0.1); <i>P</i> = .033	1.4 (0.8 – 2.1)	1.0 (0.4 – 1.5)	–0.5 (–0.9 – –0.1) <i>P</i> = .021	.978

\*Data are shown as means (95% confidence interval). PUFA indicates polyunsaturated fatty acids; F<sub>E</sub>NO, fractional exhaled nitric oxide; ppb, parts per billion; FEV<sub>1</sub>, forced expiratory volume in 1 second; ACQ, asthma control questionnaire.

†Paired samples *t* test; ‡Analysis of covariance, baseline value as covariate.



Fractional exhaled nitric oxide (FeNO), forced expiratory volume in 1 second (FEV<sub>1</sub>), and asthma control questionnaire (ACQ) score before and after supplementation for placebo and n-3 PUFA groups. No differences were observed between the groups. Individual and mean values are shown in grey and black scatterplots respectively. ppb indicates parts per billion; PUFA, polyunsaturated fatty acids.

1000 mg per day of EPA/DHA for 1 year in 12 asthma patients [27] improved FEV<sub>1</sub>. Seven of our patients had an FEV<sub>1</sub> below 80% of predicted and 5 had an ACQ score above 2, leaving little room for improvement in these measurements.

In this pilot study, we failed to show any clinically meaningful or statistically significant effect of n-3 PUFA supplementation in women with asthma. Trials involving larger numbers of subjects over a longer period of time are needed to fully assess the effect of EPA/DHA on asthma.

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