Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2009;148:321–329
DOI: 10.1159/000170386

Received: March 25, 2008
Accepted after revision: June 4, 2008
Published online: November 11, 2008

Effect of n–3 Polyunsaturated Fatty Acids in Asthma after Low-Dose Allergen Challenge

R. Schubert[a] R. Kitz[a] C. Beermann[b] M.A. Rose[a] A. Lieb[a] P.C. Sommerer[a]
J. Moskovits[a] H. Alberternst[a] H.J. Böhles[a] J. Schulze[a] S. Zielen[a]

[a]Department of Pediatrics/ZAFES, J.W. Goethe University, Frankfurt/Main, and [b]Numico Research Germany, Friedrichsdorf, Germany

Key Words
Allergic asthma · n–3 Polyunsaturated fatty acids · Nutrition · Low-dose allergen challenge · Exhaled nitric oxide

Abstract
Background: We investigated the anti-inflammatory potential of n–3 polyunsaturated fatty acids (PUFA) on specific bronchial inflammation. Allergic asthmatics were challenged using a low-dose allergen provocation model. ***Methods:*** Our parallel double-blinded study randomly assigned 23 house dust mite-allergic asthmatics (aged 22–29 years; 13 females, 10 males) to dietary supplementation with either an n–3 PUFA-enriched fat blend (0.69 g/day) or placebo for 5 weeks. After 3 weeks, the patients were challenged daily with low doses of mite allergen for 2 weeks. Primary outcome parameters were effects on lung function (forced expiratory volume in 1 s, FEV_1) and exhaled nitric oxide (eNO) as a marker of bronchial inflammation. ***Results:*** Even before the bronchial challenge, eNO was significantly lower in the n–3 PUFA group ($p = 0.014$). Levels of eNO increased during allergen exposure in both groups, but differences in means were significantly lower in the n–3 PUFA group ($p = 0.022$). During the low-dose allergen challenge, there were no differences between the groups with regard to symptoms, FEV_1 or the allergen dose required to induce deterioration of lung function (PD_{20}). Numbers of sputum eosinophils did not differ significantly, while serum eosinophils (10.1 ± 0.1.84 vs. 5.79 ± 0.69%) as well as changes in eosinophilic cationic protein (20.5 ± 9.93 vs. –1.68 ± 4.36 ng/ml) and in vitro cysteinyl leukotriene release (2,889 ± 872 vs. 1,120 ± 173 ng/ml) were significantly lower in the n–3 PUFA group ($p < 0.05$ each). ***Conclusion:*** Our results provide evidence that dietary supplementation with n–3 PUFA is able to reduce bronchial inflammation even after low-dose allergen challenge.

Introduction

Asthma is a chronic inflammatory disorder of the airways leading to airway hyperreactivity and associated symptoms (e.g. wheezing and airflow obstruction). Despite variability between patients in the onset and clinical course of asthma, the disease almost always persists [1]. The inflammatory process is complex, involving a multitude of cell types and activities which define the early- and late-phase asthmatic responses.

R.S. and R.K. contributed equally to this work.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/09/1484–0321$26.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Richard Kitz
Department of Pediatrics/ZAFES
J.W. Goethe University, Theodor Stern Kai 7
DE–60590 Frankfurt/Main (Germany)
Tel. +49 69 6301 5754, Fax +49 69 6301 6061, E-Mail r.kitz@ckhf.de

Table 1. Subjects' characteristics

	PUFA	Placebo	p
Number	12	11	n.s.
Females/males, n	7/5	6/5	n.s.
Age, years	24.0 ± 2.5	23.7 ± 1.5	n.s.
FEV_1, % predicted	111.2 ± 12.5	105.8 ± 7.6	n.s.
$PD_{20}M$, mg	0.6 ± 1.0	0.6 ± 0.8	n.s.
Screening allergen dose[a], PNU	167.8 ± 180.1	300.5 ± 247.8	n.s.
Low-dose allergen dose[b], PNU	38.8 ± 42.9	56.6 ± 60.0	n.s.
Specific IgE to dust mite, IU/ml	98.1 ± 114.0	58.2 ± 189.6	n.s.

Values are shown as geometric means ± standard deviation except where indicated otherwise. Statistical evaluation of means revealed no significant differences. PNU = Protein nitrogen units; n.s. = not significant.

[a] Dose of mite allergen causing a 20% fall in FEV_1.

[b] Noncumulative dose of mite allergen used as a low dose for allergen challenge.

Inhaled corticosteroids are effective in reducing airway inflammation in severe and moderate asthma, but a stepwise approach requires additional therapeutic options. Considerable interest in the possible value of polyunsaturated fatty acids (PUFA) in asthma was sparked by the observation that these fatty acids possess a protective or even therapeutic effect on the pathogenesis of asthma due to their impact on mediators of inflammation [2–4].

Eicosapentaenoic acid (EPA) and docosahexaenoic acid are n–3 PUFA derived from fish oils that competitively inhibit inflammatory n–6 PUFA arachidonic acid metabolism. This interaction has the potential to reduce the generation of inflammatory leukotrienes and prostaglandins, as well as the production of cytokines from inflammatory cells [5–7].

Previous research has demonstrated that fish oil supplementation has a protective effect on exercise-induced bronchoconstriction [8, 9]. A fish oil diet can improve pulmonary function, with a concurrent reduction in bronchodilator use. Induced sputum differential cell counts and concentrations of leukotriene C_4-leukotriene E_4, prostaglandin D_2, IL-1β and TNF-α have been found to be significantly reduced on the fish oil diet. In addition, the consumption of fish oil resulted in partial replacement of arachidonic acid in inflamed cell membranes by EPA [6, 10]. This response alone is a potentially beneficial anti-inflammatory effect of PUFA.

Nevertheless, clinical data on the effect of fish oil supplementation in asthma have been contradictory [11]. A recent cross-sectional study found that plasma n–3 fatty acids were not associated with a reduced risk of asthma or atopy among young adults [12]. In keeping with this observation, no clinical improvement in asthmatic symptoms was detected in some interventional studies [13, 14]. However, other studies have demonstrated a benefit of n–3 PUFA supplementation in patients suffering from bronchial asthma [15–17]. The inconsistency among study results may be attributable to the heterogeneity of the populations (e.g. age, gender, clinical picture of asthma) and the amount of omega-3 fatty acid content supplied. In particular, only few data are available on the effect of fish oil supplementation on an experimentally induced allergic airway response in patients with asthma [18].

It is well known that repeated low-dose allergen exposure has a negative impact on preexisting allergic asthma [19]. This was demonstrated in previous studies by a stepwise increase in eosinophils in sputum and in the levels of exhaled nitric oxide (eNO; a marker of bronchial inflammation), which could be prevented by inhaled steroids [20, 21]. In order to investigate the anti-inflammatory effect of fish oil supplementation, we used this established model of low-dose allergen exposure.

The ideal type and dose of diet enriched with n–3 PUFA is a matter of debate. Some authors recommend a high pharmacological dose of 3 g per day to achieve stronger effects [18]. However, there have been at least 2 convincing studies which showed a beneficial effect on asthma symptoms using a daily intervention of less than 0.5 g of n–3 PUFA [15, 22]. Considering these data and the high costs of highly purified n–3 PUFA, we decided to examine a low dose and dietary intake instead of a pharmacological regimen.

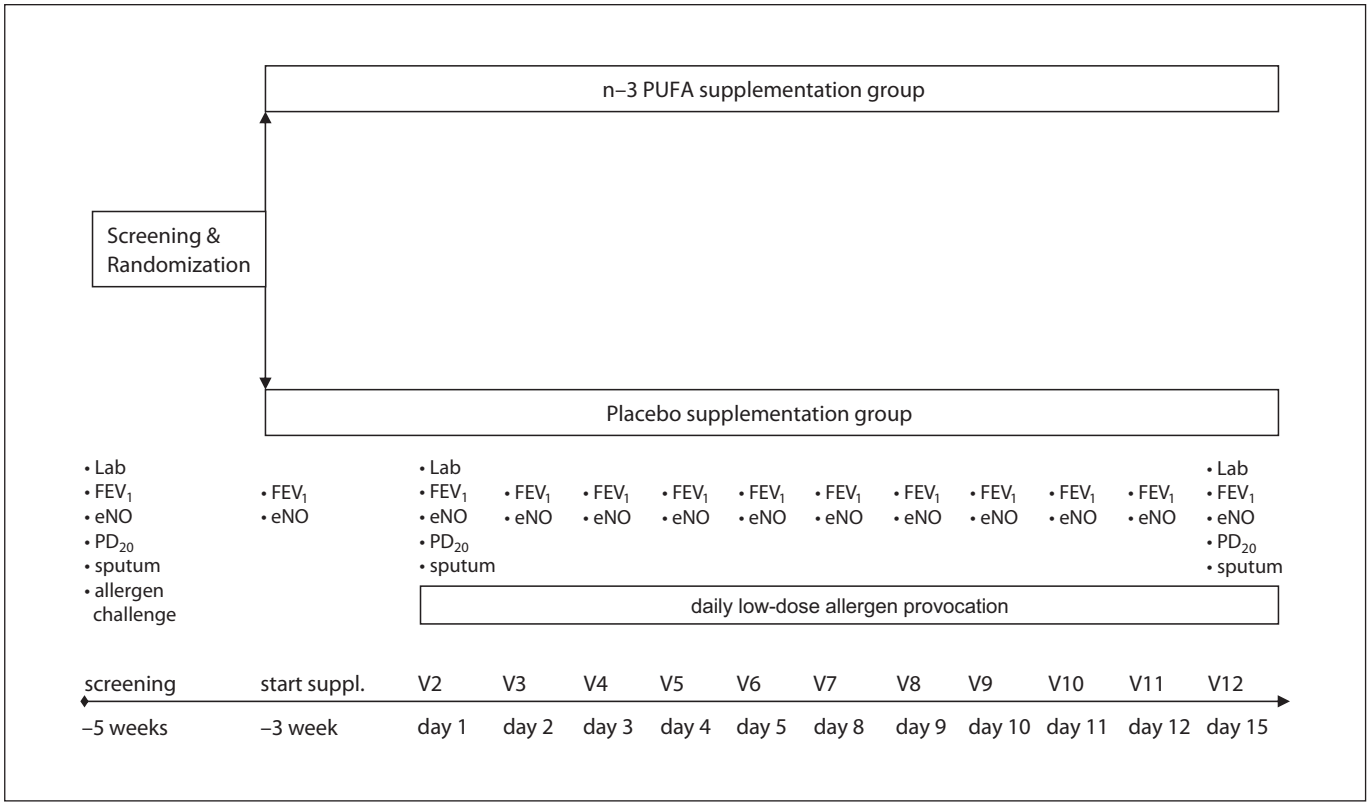

Fig. 1. Study design. The first determinations of eNO, lung function, $PD_{20}M$ and sputum eosinophils were performed prior to allergen challenge. Lab = Laboratory measurements (induced sputum, serum eosinophils and ECP, basophil activation test, PUFA concentrations in plasma and erythrocyte membranes).

Our study is the first to examine the impact of n–3 PUFA on eNO as a surrogate for bronchial inflammation in patients with preexisting allergic asthma during repeated low-dose allergen exposure.

Patients and Methods

Subjects and Study Design

Our prospective double-blind parallel study randomly assigned 23 house dust mite-allergic asthmatics (aged 22–29 years; 13 females, 10 males) to a PUFA-enriched fat blend (n = 12) or placebo for a 5-week period of dietary supplementation (table 1). The active fat blend contained EPA (20:5n3) 450 mg/day, docosahexaenoic acid 180 mg/day, gamma linoleic acid (18:3w6) 60 mg/day and stearidonic acid (C20-4w3) 60 mg/day; the placebo fat blend consisted mainly of unsaturated and monosaturated fatty acids (Numico Research Inc., Friedrichsdorf, Germany). A computerized randomization schedule was prepared by a biostatistician with allocation and dispensing of capsules by the distributor. The capsules were matched for size, shape and volume of content. Blinding was not abolished until the last subject had completed the study. After 3 weeks, patients were challenged daily with low doses of house dust mite allergen over a period of 2 weeks. Blood samples were taken before supplementation, after 3 weeks and after the challenge period (fig. 1).

Initially, each subject was evaluated by a questionnaire, skin prick test for common aeroallergens including house dust mite, lung function and methacholine testing. Only healthy subjects with a predicted forced expiratory volume in 1 s (FEV_1) and forced vital capacity $\geq$80% were challenged, as explained below. Participants were instructed to avoid fatty meals and the consumption of sea fish throughout the study.

Exclusion criteria were a history of smoking, predicted forced vital capacity <80%, FEV_1 <80%, age <18 years and β_2-agonist usage on demand more than twice a week, or any inhaled or systemic steroid use. Written informed consent was obtained from all participants. The study was approved by the local institutional ethics committee and performed in accordance with the Declarations of Helsinki (1975) and Edinburgh (2000).

Allergen Inhalation Challenge Protocol

For screening purposes, a bronchial allergen challenge was performed with a purified aqueous allergen extract of *Dermatophagoides pteronyssinus* (Allergopharma Inc., Reinbek, Germany). The solutions were delivered via a Medic-Aid nebulizer and

the aerosol provocation system APS® powered by compressed air (by Viasys Healthcare GmbH, Hoechberg, Germany). This system calculates the administered dose by breath from a constant flow and the inspiration time of any breath cycle, thus calculating the exact dose to be administered automatically. Mouthpieces and nebulizers were changed after each subject to avoid cross-contamination. The challenge test was stopped when the FEV_1 decreased by at least 20% from the individual baseline.

Then the noncumulative dose of allergen causing a fall in FEV_1 of 5% from the postdiluent baseline was calculated as the low dose (table 1) to be used for repeated allergen exposure on 5 consecutive days per week for 2 weeks according to the protocol of de Kluijver et al. [19].

Lung Function Tests

Baseline spirometry (before inhalation of 0.9% sterile saline and before allergen challenge) was performed with the Master Screen® body plethysmograph from Viasys Healthcare. Lung function testing was performed before and 10, 20 and 30 min after each challenge; peak flow measurements were performed hourly up to 10 h after the last inhalation. Subjects documented short-acting β_2-agonist usage and clinical complaints in diaries. Additionally, the unspecific bronchial hyperreactivity was estimated with stepwise inhalation of metacholine and defined by the required dose to cause a 20% decrease in FEV_1 ($PD_{20}M$).

Measurement of eNO

Measurements of eNO were performed using the NIOX® system (Aerocrine Inc., Solna, Sweden) according to American Thoracic Society guidelines [23]. This chemiluminescence gas analyzer is sensitive to measuring NO at concentrations from 1.5 to 200 ppb with a deviation from mean values of ±2.5 ppb for values of <50 ppb or ±5% of the measured value for values of >50 ppb. We controlled for intrasubject variability by using the mean values of 3 consecutive measurements.

Laboratory Measurements

Concentrations of eosinophilic cationic protein (ECP) and specific immunoglobulin E were routinely determined in our laboratory by chemiluminescence immunoassay (Biermann Inc., Bad Nauheim, Germany) [24].

Sputum Eosinophils

Sputum induction was carried out using hypertonic saline inhalation (4.5%) 1 h after the methacholine challenges [25, 26]. Specimens containing levels of squamous epithelial cells less than 50% of the total inflammatory cell number were considered adequate. At least 400 inflammatory cells were counted for each specimen. Eosinophils were expressed as percentages of the total cell count.

Cysteinyl Leukotrienes

Activation of leukocytes was measured by cellular antigen stimulation test (Buehlmann Laboratories Inc., Allschwil, Switzerland) [27]. Briefly, leukocytes were isolated by dextran sedimentation from EDTA blood. Then cells were stimulated with dust mite extract (2 ng/ml) for 40 min, and supernatants were decanted and stored at –80°C. Following leukocyte in vitro stimulation with house dust mite allergen, cysteinyl leukotrienes (cysLTs) were determined by the ELISA technique according to the manufacturer's instructions [28].

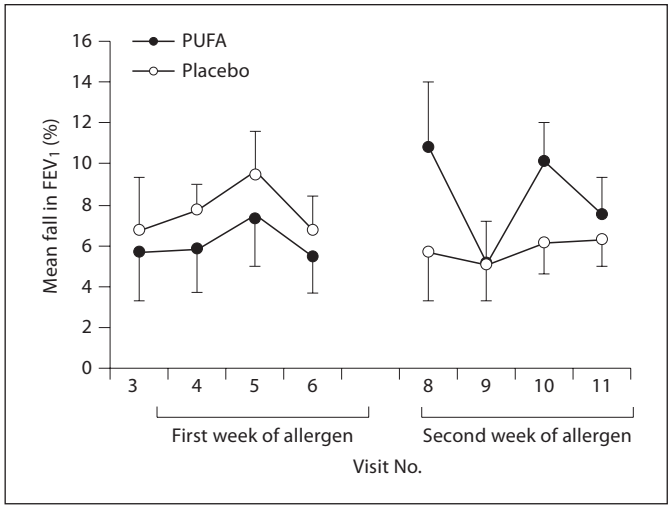

Fig. 2. Acute response (0–30 min) to low-dose allergen exposure presented as mean ± SEM falls in FEV_1 from prechallenge baseline levels.

Detection of PUFA in Plasma and Erythrocyte Membranes

In order to monitor compliance, total PUFA concentrations in plasma and erythrocyte membranes were measured by fatty acid methyl ester detection via capillary gas chromatography [29] by Numico Research. Values are expressed as weight percentage (wt%) of total fatty acids.

Statistical Analysis

Statistical data analysis was performed using the software package SPSS for Windows® 11.0 (SPSS Inc., Chicago, Ill., USA). All values are expressed as mean values ± SEM. Within groups, changes were analyzed using the paired Student's t test and between-group comparisons with the unpaired t test. Mixed-model ANOVA was used for baseline FEV_1, $PD_{20}M$ and eNO values to detect differences between and within groups and between study weeks. Additionally, the GLM procedure of repeated-measure analysis of variance (analysis of variance of contrast variables) was used, comparing the first visit with any following visit. Probability values <0.05 were considered significant. Paired and unpaired t tests were adjusted post hoc from multiple comparisons with a Bonferroni correction.

Results

Acute Response to Low Doses of Allergen Exposure

The procedures were well tolerated by all patients. During the study, no participant needed bronchodilators or anti-inflammatory medication. There were no between-group differences in FEV_1 baseline values. The biggest falls in the mean values of FEV_1 within 30 min after low-dose allergen exposure were 10.1 ± 1.92 and 9.63 ± 2.08% in the n–3 PUFA and placebo group, re-

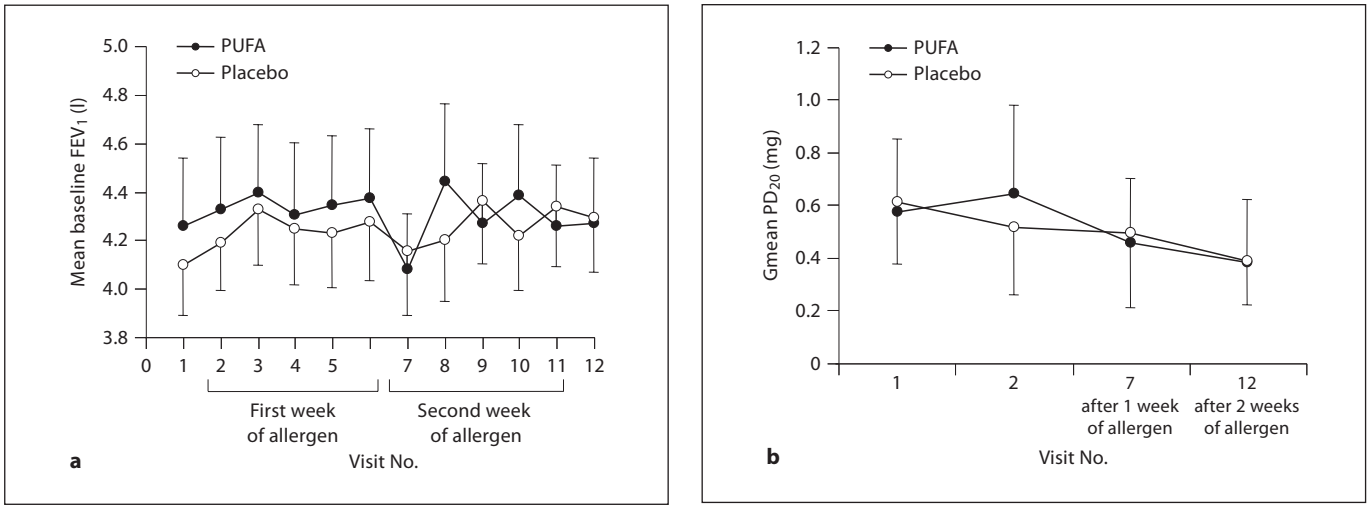

Fig. 3. a Baseline FEV_1 presented as mean ± SEM. Mixed-model ANOVA showed no significant differences between the groups during the study. **b** $PD_{20}M$ shown as geometric means (Gmean) ± geometric SEM.

spectively. There was no significant difference in the fall in FEV_1 between the groups at all visits (fig. 2). Within-group analysis at different times also revealed no significant differences. Only 4 consecutive challenges were analyzed since the first day of provocation each week (visits 2 and 7) was preceded by methacholine testing. Table 1 indicates that the 2 groups were well matched with regard to age, physical characteristics and initial response to inhalation.

Baseline FEV_1 and Bronchial Hyperreactivity

There was no significant difference in FEV_1 within or between the groups during the allergen challenge period (fig. 3a). $PD_{20}M$ decreased after allergen challenge in both groups but this change failed to reach significance (fig. 3b).

Exhaled NO

Mean changes in eNO (measured in parts per billion) significantly increased during low-dose allergen exposure in both groups, but were constantly lower in the n–3 PUFA group [n–3 PUFA: visit 2 (V2) –2.06 ± 3.6, V3 17.5 ± 4.5, V4 40.6 ± 8.4, V5 55.8 ± 11.0, V6 61.1 ± 12.6, V7 37.6 ± 8.3, V8 69.0 ± 13.3, V9 75.9 ± 12.4, V10 78.8 ± 12.3, V11 75.9 ± 11.8, V12 48.7 ± 12.6; placebo: V2 10.8 ± 3.1, V3 47.9 ± 11.9, V4 84.5 ± 17.2, V5 99.0 ± 11.8, V6 123.9 ± 17.68, V7 87.6 ± 14.8, V8 109.9 ± 14.7, V9 115.5 ± 15.2, V10 125.9 ± 18.3, V11 120.8 ± 19.2, V12 73.7 ± 12.1]. Mixed-effects ANOVA revealed significantly lower eNO

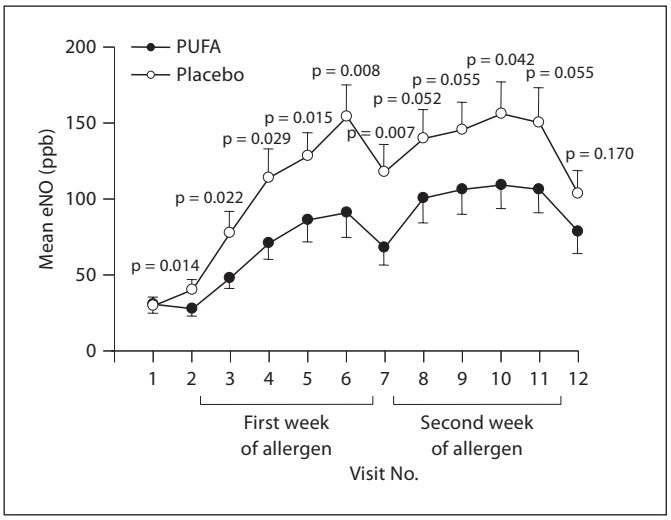

Fig. 4. eNO presented as mean ± SEM changes in eNO. Mixed-model ANOVA: * $p < 0.05$.

levels in the n–3 PUFA-supplemented group ($p = 0.022$; fig. 4), contradicting the hypothesis of parallel shapes of mean eNO courses for both treatments. Levels of eNO were even lower in the active group preceding the inhalation challenge (visit 2; $p = 0.014$). However, when calculating absolute data, the overall effect missed significance by a small margin ($p = 0.06$). When comparing the 2 weeks with each other, treatment effects just reached statistical

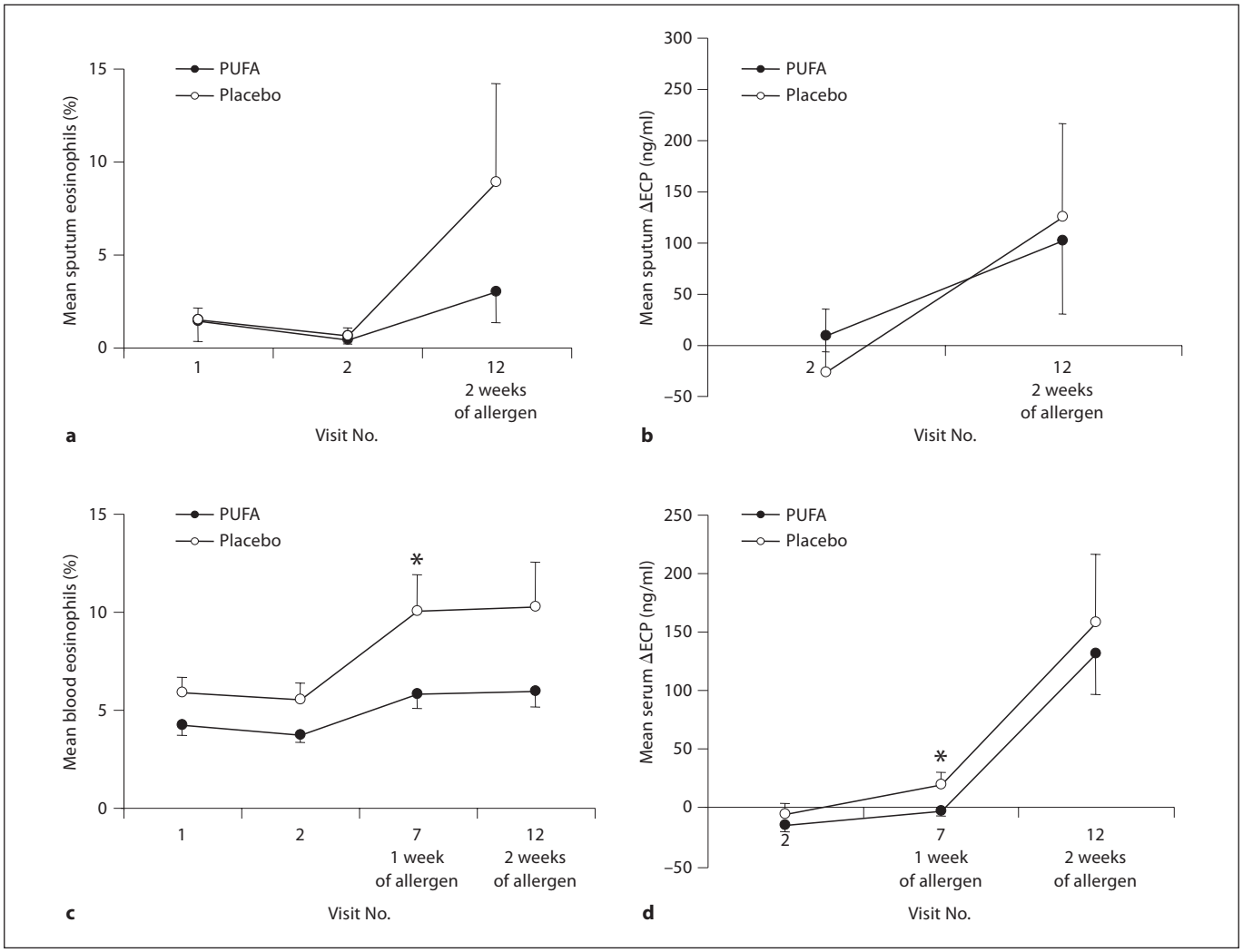

Fig. 5. Eosinophil counts in sputum (**a**) and serum (**c**) and change in ECP concentrations in sputum (**b**) and serum (**d**) are given as means ± SEM. Sputum was taken at visits 1, 2 and 12 (**a**, **b**) and serum at visits 1, 2, 7 and 12 (**c**, **d**). * $p < 0.05$.

significance ($p = 0.046$) and were stronger in the first week ($p = 0.0008$). This was confirmed by post hoc Bonferroni analysis after 1 week at visit 6 ($p = 0.049$).

Eosinophils and ECP in Sputum and Serum

The percentage of eosinophils in the sputum increased after allergen challenge in both groups after 2 weeks (fig. 5a). The increase tended to be greater in the placebo group, although without statistical significance (n–3 PUFA: 3.02 ± 1.63%; placebo: 8.91 ± 5.32%). Levels of ECP in sputum also rose after allergen challenge in both groups, but no differences were seen between the groups (fig. 5b).

The percentages of blood eosinophils increased during allergen exposure in the placebo group, whereas there was only a slight increase in the n–3 PUFA group (fig. 5c). These results were significant at visit 7 (placebo: 10.1 ± 0.1.84%; n–3 PUFA: 5.79 ± 0.69%; $p < 0.05$). Changes in ECP serum concentrations were significantly lower in the n–3 PUFA group compared to placebo at visit 7 (placebo: 20.5 ± 9.93 ng/ml; n–3 PUFA: –1.68 ± 4.36 ng/ml; $p < 0.05$), but not after 2 weeks of challenge (fig. 5d).

cysLT Release

cysLT release was low in unstimulated (basal) leukocytes and did not show any difference between the groups

(fig. 6). Stimulation with house dust mite extract induced a striking cysLT release in both groups that became significantly higher in the placebo group after 2 weeks of allergen challenge (visit 12) compared to the n–3 PUFA group (n–3 PUFA: 1,120 ± 173 ng/ml; placebo: 2,889 ± 872 ng/ml; $p < 0.05$; fig. 6).

PUFA Concentrations in Plasma and Erythrocyte Membranes

Concentrations of EPA (mean weight percentage of total fatty acids ± standard deviation) in plasma showed a significant increase in the n–3 PUFA group after supplementation compared to the placebo group. At visit 2, the mean values were 1.51 ± 0.82 wt% for the active group compared to 0.58 ± 0.28 wt% for the placebo group ($p < 0.001$). At visit 7, mean values were 1.43 ± 0.47 wt% for the active group versus 0.59 ± 0.20 wt% in the placebo group ($p < 0.001$). At visit 12, the mean concentration in the n–3 PUFA group was 1.61 ± 0.72 wt%, compared to 0.61 ± 0.43 wt% in the placebo group ($p < 0.001$). The n–3/n–6 ratio for the active group increased significantly after 3 weeks of supplementation (V1: 0.11 ± 0.01; V2: 0.15 ± 0.01, $p < 0.001$; V7: 0.15 ± 0.01, $p < 0.001$; V12: 0.16 ± 0.01, $p < 0.001$), whereas no changes were seen in the placebo group.

Similar effects were observed for EPA concentrations in erythrocyte membranes (n–3 PUFA: 0.61 ± 0.72 wt%; placebo: 0.28 ± 0.39 wt%; $p < 0.05$) as well as in the n–3/n–6 ratios (V1: 0.20 ± 0.02; V2: 0.22 ± 0.02; V7: 0.25 ± 0.02, $p < 0.01$; V12: 0.25 ± 0.03, $p < 0.01$). In the placebo group, the n–3/n–6 ratio had decreased significantly at V12 (0.17 ± 0.02; $p < 0.05$) compared to V1 (0.20 ± 0.02).

None of the other fatty acids showed a significant increase either in plasma or in erythrocyte membranes. We only found an overall trend for increasing docosahexaenoic acid values (data not shown).

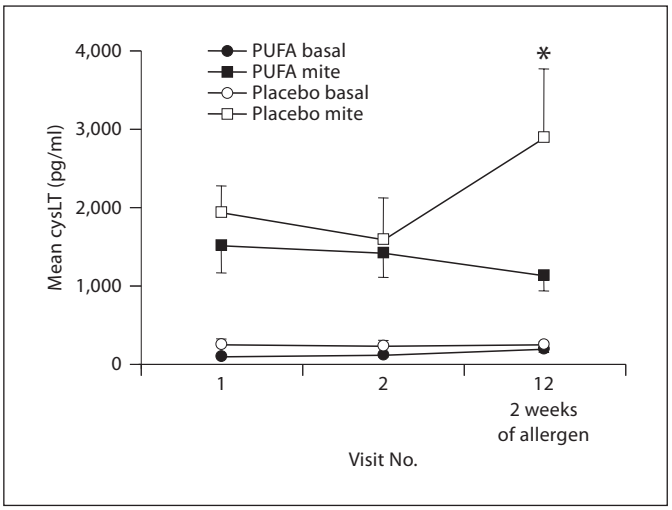

Fig. 6. cysLT production after in vitro stimulation with dust mite allergen. Data are presented as means ± SEM. Whole blood was taken at visits 1, 2 and 12, and cells were stimulated with dust mite extract (mite) or medium alone (basal). Activation of neutrophils was detected by release. * $p < 0.05$.

Discussion

Our study has demonstrated that allergen-induced airway inflammation was significantly attenuated by 5 weeks of dietary n–3 PUFA supplementation, verified by lower levels of eNO, serum and sputum eosinophils and ECP and suppression of the proinflammatory eicosanoids secreted by leukocytes. After PUFA supplementation, levels of eNO were significantly lower already on the first visit, indicating an effect even before an inhalation challenge.

Our observations support previous findings in patients with allergic disease in whom n–3 PUFA supplementation reduced the generation of inflammatory leukotrienes and prostaglandins [13, 16, 17, 30]. Repeated low-dose allergen challenge promotes features of airway inflammation, thus mimicking the natural course of allergic asthma more closely than a single high-dose allergen inhalation challenge [19]. It is an established model to study asymptomatic worsening of airway inflammation and the impact of anti-inflammatory therapies.

This anti-inflammatory effect of n–3 PUFA was less potent in the second week. However, the diminished efficacy of n–3 PUFA supplementation in the second week was not related to noncompliance, as verified by phospholipid fatty acid analysis in plasma and erythrocytes. Nevertheless, the effect on eNO is in contrast to the lack of an effect on allergen-associated bronchial hyperreactivity. This unexpected finding is difficult to explain. Mediator release by allergen-activated mast cells may contribute to both an increase in eNO and allergen-induced airway hyperreactivity. Allergen-induced mediator release from leukocytes, mainly basophils, can be inhibited to some extent by n–3 PUFA, but not blocked completely as shown by our in vitro data.

Similar to eNO, sputum eosinophils tended to increase less after 2 weeks of n–3 PUFA supplementation. At this time, eNO levels were also no longer significantly differ-

ent. An earlier determination of sputum eosinophils, which we did not perform for technical reasons, would have been able to prove effects also on this important marker of airway inflammation.

Our pilot study has the limitation of a relatively small sample size. Thus, we cannot exclude completely that our results are subject to a type I error. On the other hand, similar studies with PUFA supplementation or low-dose allergen challenge have been performed with similar sample sizes [8, 19].

Overall, a more potent anti-inflammatory treatment, e.g. corticosteroids, appears to be necessary to inhibit the development of allergen-induced airway hyperreactivity. The dissociation between airway inflammation and allergen-induced airway hyperreactivity has also previously been reported following pharmacological treatment with different doses of inhaled steroids [31]. Recent works suggest that indirect challenge methods (e.g. hypertonic saline) might be even more suitable to investigate airway inflammation after allergen provocation [32]. Since most of the studies performed on this subject used the direct method of metacholine testing, we chose this technique to facilitate comparability of the results. For future studies, we would consider performing direct and indirect testing methods.

In clinical trials, anti-inflammatory responses to n–3 PUFA were often limited. A current Cochrane review [11] including 9 randomized controlled trials comparing n–3 PUFA supplementation with placebo [13, 15, 30, 33–35] or with n–6 PUFA supplementation [14] could not find a consistent benefit of n–3 PUFA in the management of asthma.

A recent study by Mickleborough et al. [9] demonstrated a protective effect of fish oil supplementation (3 g per day) on exercise-induced bronchoconstriction. In addition, the same group showed that n–3 PUFA reduced airway narrowing, medication use and proinflammatory mediator generation in 20 nonatopic elite athletes with exercise-induced bronchoconstriction [8].

It is an ongoing debate, whether higher doses of n–3 PUFA than those applied in our study might exhibit greater anti-inflammatory capacity. A daily intervention of at least 3 g of omega-3 fatty acids, which is considered a high adult dose, has been used in many trials without convincing evidence that such a dose is superior to low-dose supplementation [18, 30, 35]. Theoretically, the most immediate outcome related to n–3 PUFA intake is a change in tissue levels of the fatty acids. However, the measurement and interpretation of the n–3 PUFA effect is complicated by the tissue distribution, sample sizes, type and dose of n–3 PUFA and the heterogeneity of asthma patients. Also, a control of any nutritional fatty acids independent of the study is important to avoid a distortion of the facts.

Nevertheless, the results of 2 further studies using low EPA doses have been very promising. Nagakura et al. [15] found that a 10-month intake of 120 mg of n–3 PUFA per day reduced asthma symptom scores and bronchial hyperreactivity to acetylcholine in children compared to controls. In addition, treatment with a lipid extract of New Zealand green-lipped mussel containing 100 mg of n–3 PUFA was recently studied in 46 patients with atopic asthma. There was a significant decrease in daytime wheeze and an increase in morning peak flow in the lipid extract group compared to the placebo group [22]. Such conclusions are supported by another study in which omega-3 fatty acid supplementation reduced arachidonic acid-derived inflammatory mediators, thereby reducing cough sensitivity in atopic disease [36].

In conclusion, our study provides further evidence that a 5-week dietary supplementation with 0.7 g of n–3 PUFA per day is able to reduce bronchial inflammation in allergic asthma. Based on the interesting finding that n–3 PUFA reduced eNO, we recommend a large randomized controlled study in patients with mild and moderate allergic asthma.

Acknowledgements

We are grateful to Gaby Gottwald, Katrin Krug and Petra Schoen for their technical assistance and laboratory advice. This study was supported by Numico Research Germany.

References

1 Morgan WJ, Stern DA, Sherrill DL, Guerra S, Holberg CJ, Guilbert TW, Taussig LM, Wright AL, Martinez FD: Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. Am J Respir Crit Care Med 2005;172:1253–1258.

2 Horrobin DF: Low prevalences of coronary heart disease (CHD), psoriasis, asthma and rheumatoid arthritis in Eskimos: are they caused by high dietary intake of eicosapentaenoic acid (EPA), a genetic variation of essential fatty acid (EFA) metabolism or a combination of both? Med Hypotheses 1987;22:421–428.

3 Stephensen CB: Fish oil and inflammatory disease: is asthma the next target for n–3 fatty acid supplements? Nutr Rev 2004;62:486–489.

4 Prescott SL, Calder PC: N–3 polyunsaturated fatty acids and allergic disease. Curr Opin Clin Nutr Metab Care 2004;7:123–129.
5 Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J 3rd, Spur BW, Robinson DR, Corey EJ, Lewis RA, Austen KF: Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 1985; 312:1217–1224.
6 Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC, et al: The effect of dietary supplementation with n–3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 1989;320:265–271.
7 Calder PC: Dietary fatty acids and the immune system. Lipids 1999;34(suppl):S137–S140.
8 Mickleborough TD, Murray RL, Ionescu AA, Lindley MR: Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. Am J Respir Crit Care Med 2003;168:1181–1189.
9 Mickleborough TD, Lindley MR, Ionescu AA, Fly AD: Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. Chest 2006;129:39–49.
10 Calder PC: N–3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. Lipids 2003;38:343–352.
11 Woods RK, Thien FC, Abramson MJ: Dietary marine fatty acids (fish oil) for asthma in adults and children. Cochrane Database Syst Rev 2002;(3):CD001283.
12 Kompauer I, Demmelmair H, Koletzko B, Bolte G, Linseisen J, Heinrich J: Association of fatty acids in serum phospholipids with hay fever, specific and total immunoglobulin E. Br J Nutr 2005;93:529–535.
13 Arm JP, Horton CE, Mencia-Huerta JM, House F, Eiser NM, Clark TJ, Spur BW, Lee TH: Effect of dietary supplementation with fish oil lipids on mild asthma. Thorax 1988; 43:84–92.
14 Hodge L, Salome CM, Hughes JM, Liu-Brennan D, Rimmer J, Allman M, Pang D, Armour C, Woolcock AJ: Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children. Eur Respir J 1998;11:361–365.
15 Nagakura T, Matsuda S, Shichijyo K, Sugimoto H, Hata K: Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. Eur Respir J 2000;16:861–865.
16 Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM: Reduced asthma symptoms with n–3 fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr 1997;65:1011–1017.
17 Okamoto M, Mitsunobu F, Ashida K, Mifune T, Hosaki Y, Tsugeno H, Harada S, Tanizaki Y: Effects of dietary supplementation with n–3 fatty acids compared with n–6 fatty acids on bronchial asthma. Intern Med 2000; 39:107–111.
18 Arm JP, Horton CE, Spur BW, Mencia-Huerta JM, Lee TH: The effects of dietary supplementation with fish oil lipids on the airways response to inhaled allergen in bronchial asthma. Am Rev Respir Dis 1989;139:1395–1400.
19 de Kluijver J, Evertse CE, Schrumpf JA, van der Veen H, Zwinderman AH, Hiemstra PS, Rabe KF, Sterk PJ: Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids. Am J Respir Crit Care Med 2002;166:294–300.
20 Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR: The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001; 164:738–743.
21 Peroni DG, Piacentini GL, Bodini A, Ress M, Costella S, Boner AL: Montelukast versus formoterol as second-line therapy in asthmatic children exposed to relevant allergens. Allergy Asthma Proc 2005;26:283–286.
22 Emelyanov A, Fedoseev G, Krasnoschekova O, Abulimity A, Trendeleva T, Barnes PJ: Treatment of asthma with lipid extract of New Zealand green-lipped mussel: a randomised clinical trial. Eur Respir J 2002;20: 596–600.
23 Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 1999;160:2104–2117.
24 Bez C, Schubert R, Kopp M, Ersfeld Y, Rosewich M, Kuehr J, Kamin W, Berg AV, Wahu U, Zielen S: Effect of anti-immunoglobulin E on nasal inflammation in patients with seasonal allergic rhinoconjunctivitis. Clin Exp Allergy 2004;34:1079–1085.
25 Spanevello A, Vignola AM, Bonanno A, Confalonieri M, Crimi E, Brusasco V: Effect of methacholine challenge on cellular composition of sputum induction. Thorax 1999; 54:37–39.
26 Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R: The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000;161:9–16.
27 Kopp MV, Brauburger J, Riedinger F, Beischer D, Ihorst G, Kamin W, Zielen S, Bez C, Friedrichs F, Von Berg A, Gerhold K, Hamelmann E, Hultsch, Kuehr J: The effect of anti-IgE treatment on in vitro leukotriene release in children with seasonal allergic rhinitis. J Allergy Clin Immunol 2002;110:728–735.
28 Van Rooyen C, Anderson R: Assessment of determinants of optimum performance of the CAST-2000 ELISA procedure. J Immunol Methods 2004;288:1–7.
29 Beermann C, Mobius M, Winterling N, Schmitt JJ, Boehm G: sn-position determination of phospholipid-linked fatty acids derived from erythrocytes by liquid chromatography electrospray ionization ion-trap mass spectrometry. Lipids 2005;40:211–218.
30 Kirsch CM, Payan DG, Wong MY, Dohlman JG, Blake VA, Petri MA, Offenberger J, Goetzl EJ, Gold WM: Effect of eicosapentaenoic acid in asthma. Clin Allergy 1988;18:177–187.
31 Gauvreau GM, Sulakvelidze I, Watson RM, Inman MD, Rerecich TJ, O'Byrne PM: Effects of once daily dosing with inhaled budesonide on airway hyperresponsiveness and airway inflammation following repeated low-dose allergen challenge in atopic asthmatics. Clin Exp Allergy 2000;30:1235–1243.
32 Cockcroft DW, Davis BE: Mechanisms of airway hyperresponsiveness. J Allergy Clin Immunol 2006;118: 551–559.
33 Stenius-Aarniala B, Aro A, Hakulinen A, Ahola I, Seppala E, Vapaatalo H: Evening primrose oil and fish oil are ineffective as supplementary treatment of bronchial asthma. Ann Allergy 1989;62:534–537.
34 Dry J, Vincent D: Effect of a fish oil diet on asthma: results of a 1-year double-blind study. Int Arch Allergy Appl Immunol 1991; 95:156–157.
35 Thien FC, Mencia-Huerta JM, Lee TH: Dietary fish oil effects on seasonal hay fever and asthma in pollen-sensitive subjects. Am Rev Respir Dis 1993;147:1138–1143.
36 Stone R, Barnes PJ, Fuller RW: Contrasting effects of prostaglandins E2 and F2 alpha on sensitivity of the human cough reflex. J Appl Physiol 1992;73:649–653.