

available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/rmed

Omega-3 polyunsaturated fatty acids and bronchial inflammation in grass pollen allergy after allergen challenge

Richard Kitz^{a,c}, Markus A. Rose^{a,*,c}, Ralf Schubert^a,
Christopher Beermann^b, Annika Kaufmann^a, Hans Josef Böhles^a,
Johannes Schulze^a, Stefan Zielen^a

^a Children's Hospital, Goethe University, Frankfurt, Germany

^b NUMICO Research Germany, Friedrichsdorf, Germany

Received 23 December 2009; accepted 25 June 2010

Available online 15 July 2010

KEYWORDS

Grass pollen allergy;
Omega-3
polyunsaturated fatty
acids (PUFAs);
Nutrition;
single-step allergen
challenge;
Exhaled NO (eNO)

Summary

Ratio: Asthma is a major public health problem, with bronchial inflammation as the therapeutic target. The role of dietary fish oil derived polyunsaturated fatty acids (PUFAs) in allergic inflammation is controversial. Most asthmatics suffer from mild disease and non-pharmacologic interventions are attractive. This study investigates the anti-inflammatory potential of nutritional PUFAs in an experimentally induced bronchial inflammation.

Methods: We examined 38 grass pollen allergic asthmatics and 19 controls. History of dietary PUFA intake was compared with levels of PUFAs in erythrocyte membranes, and stratified according to low (25th quartile; Q25) and high (75th quartile; Q75) ratios of omega-3 (n-3) to omega-6 (n-6) PUFAs as a surrogate for anti-inflammatory (Q75) or proinflammatory (Q25) effects. Bronchial inflammation was simulated with one-step inhalation of grass pollen. Bronchial response (exhaled nitric monoxide, eNO as surrogate for inflammation, decrease of FEV₁) was correlated with levels of PUFAs in erythrocyte membranes.

Results: Ratios of n-3/n-6 PUFA were significantly lower in asthmatics than in healthy controls. Levels of eNO were significantly higher in Q25 asthmatics than in Q75 asthmatics ($p = 0.040$). There was a trend of higher bronchial hyperreactivity in Q25 asthmatics (median PD₂₀ 0.27 vs. 0.14; n.s.), induced by specific bronchial challenge with grass pollen (FEV₁ decrease 16.7 vs. 23.1%; n.s.).

* Corresponding author. Children's Hospital, J.W. Goethe University, Theodor Stern Kai 7, D-60590 Frankfurt, Germany. Tel.: +49 69 6301 5754; fax: +49 69 6301 6061.

E-mail address: Markus.Rose@kgu.de (M.A. Rose).

^c Both authors contributed equally to this work and should therefore be considered as first authors.

Conclusion: When stratifying for erythrocyte membrane PUFA content as a surrogate for alimentary intake, we found mild effects on bronchial allergic inflammation. Future intervention studies with pharmacological PUFA doses appear suitable to clarify dietary PUFA role as an adjunctive intervention to the established treatment of asthma.

ClinicalTrials.gov No. **NCT00519740**.
© 2010 Elsevier Ltd. All rights reserved.

Introduction

The prevalence of asthma continues to increase despite improved treatment options. There is accumulating evidence that dietary modifications have the potential to influence the severity of asthma and reduce the dose requirements of drug treatment. Possibly contributing to the increasing incidence of asthma in industrialized societies may be the consumption of a proinflammatory diet. In the typical Western diet, omega (n)-6 polyunsaturated fatty acids (PUFAs) dominate, resulting in the release of proinflammatory arachidonic acid-derived metabolites. Considerable interest in the possible value of polyunsaturated fatty acids (PUFAs) was sparked by ecological studies showing beneficial associations between intake of oily fish rich in n-3 fatty acids, and allergic airway diseases.^{1–4} Additional impetus for research came from observations that n-3 fatty acids might have a protective or even therapeutic effect. This may be attributable to their impact on mediators of inflammation thought to be related to the pathogenesis of asthma.^{5,6} The dietary intake of fatty acids affects the production of eicosanoids, which are potent immune mediators being mainly synthesized from eicosapentaenoic acid (EPA; C20:5 n-3) and arachidonic acid (AA; C20:4 n-6). As a correlate of a dysregulated Th2 response in atopic disease, eicosanoids may interfere with immune cells shifting the pattern of the cytokine production from Th1 to Th2. Since EPA and other n-3 PUFAs seem to have the potential to antagonize the effects of AA, a high dietary input of EPA may be associated with a decreasing risk of allergic asthma. In this context, the ratio of n-6/n-3 PUFA appears as a predictive marker. Docosahexaenoic acid (DHA) and EPA are n-3 PUFAs mainly derived from fish oils that competitively inhibit n-6 PUFA arachidonic acid metabolism, thus reducing the generation of inflammatory leukotrienes and prostaglandins as much as the production of cytokines from inflammatory cells.^{7,8} In those studies, a fish oil diet improved pulmonary function, with a concurrent reduction in asthma medication use. Induced-sputum differential cell counts and concentrations of LTC₄-LTE₄, PGD₂, IL-1beta, and TNF-alpha were significantly reduced on the fish oil diet, supporting the presumed anti-inflammatory effects.

Nevertheless, the efficacy of fish oil supplementation in asthma has been a matter of debate.⁹ 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.¹⁰ In keeping with this observation, no clinical improvement in asthmatic symptoms was detected in some interventional studies.^{11,12} However, other studies on patients suffering from bronchial asthma have demonstrated a benefit of n-3 PUFA supplementation.^{13–15} The inconsistency among study results may be attributable

to the heterogeneity in definitions of the study populations (e.g., age, gender, clinical picture of asthma including its severity), and the type of intervention (e.g., amounts of oil and omega-3 fatty acid contents). Only few data are available on the effect of fish oil supplementation on an experimentally induced allergic airway response in patients with allergic diseases.¹⁶

It is well known that allergen exposure has a negative impact on preexisting allergic asthma.¹⁷ This was demonstrated by a stepwise increase of eosinophils in sputum and of the levels of exhaled nitric oxide (eNO, a marker of bronchial inflammation).^{18,19} In order to investigate the anti-inflammatory effect of fish oil supplementation, we used this established model of a standardized allergen exposure.

In a preceding study on allergic asthmatics and following dietary EPA supplementation, we detected a 30% reduction of the eNO increase.²⁰ Thus, we challenged patients sensitized to grass pollen with a standardized dose of grass pollen preseasonally and correlated it with their plasma and erythrocyte PUFA contents. Our study is the first to examine the impact of nutritional n-3 PUFAs on bronchial inflammation and hyperreactivity in patients with preexisting allergic disease after single-dose allergen exposure.

Methods

Subjects and study design

Our study examined 38 grass pollen allergic asthmatics and 19 age-matched healthy controls. Initially, each subject was evaluated by a questionnaire referred to allergic and/or asthmatic symptoms, skin prick test for common aeroallergens including grass pollen, lung function, and methacholine testing. Only subjects who were positive for grass pollen as determined by positive skin prick test or specific IgE and with an FEV₁ and FVC \geq 80% were challenged with grass pollen extract on a separate visit day as explained later. Controls were screened in the same way from medical school students. Exclusion criteria were age <18 or >45 years, regularly β 2-agonist and/or inhaled corticosteroid or anti-histamine usage, chronic disease conditions, pregnancy, lactation, and the incapability to understand and follow instructions. 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).

Lung function tests

Spirometry was performed with the MasterScreen[®] spirometer by Cardinal Health Inc., Hoechberg, Germany.

FEV₁, FVC, PEF, and MEF₂₅ were recorded as representative lung function parameters. Peak flow measurements were performed with the Mini-Wright peak flow meter (60–800 L per min measuring range). The best of three consecutive attempts was recorded by the participants on a diary card.

Bronchial metacholine provocation test

To assess airway hyperreactivity, metacholine testing was performed according to American Thoracic Society (ATS) recommendations²¹ and was stopped when the FEV₁ decreased at least 20% from individual baseline. PD₂₀ metacholine was then calculated from the cumulative dose resulting in a 20% fall of FEV₁.

Allergen inhalation challenge protocol

Patients and controls were challenged with 150 mg of purified aqueous allergen extract of grass pollen (5000 mg/ml; Allergopharma Inc., Reinbek, Germany). The solutions were delivered via a medic aid nebulizer and the aerosol provocation system APS[®] powered by compressed air (Cardinal Health Inc., Hoechst, Germany.).

Measurement of exhaled NO (eNO)

Measurements of eNO were done using the NIOX[®] system (Aerocrine Inc., Solna, Sweden) according to ATS guidelines.²² This chemiluminescence gas analyzer is sensitive to measure NO at concentrations from 1.5 ppb to 200 ppb with a deviation from mean values of ± 2.5 ppb < 50 ppb or $\pm 5\%$ of the measured value > 50 ppb. We controlled for intra-subject variability using mean values of three consecutive measurements.

Laboratory measurements

Blood samples were taken before the challenge. Red and white blood cell count, concentrations of total IgE, and of specific IgE (chemiluminescence-immunoassay, Biermann Inc., Bad Nauheim, Germany) were determined in our laboratory. Fatty acids in plasma and erythrocyte membrane (red blood cells; RBCs) were determined at the NUMICO research laboratory by HPLC/fatty acid methyl ester (FAME) detection via capillary gas-chromatography.²³

Values are expressed in weight percent (wt%) of total fatty acids and the ratio of omega-3/omega-6 PUFAs.

Statistical analysis

Erythrocyte membrane fatty acids (in % FAME) were categorized in quartiles according to the distribution in the total study population. Data analysis was performed using the software package SPSS for Windows[®] 11.0 (SPSS Inc., Chicago, Illinois/USA). All values are expressed as median values \pm Standard Errors of Means (SEM). Since data were not distributed normally, we chose a non-parametric test (Mann–Whitney and Wilcoxon test). Probability values < 0.05 were considered as significant.

Results

Clinical and laboratory characteristics of the study population

Our study examined 38 grass pollen allergic asthmatics (classified according to the Global Initiative for Asthma (GINA) as “mild intermittent”, GINA I^o; median age 25.1 years) and 19 healthy controls (median age 22.5 years; Table 1). When stratifying for levels of PUFA in erythrocyte membranes and limiting the analysis to low (25th quartile; Q25) vs. high (75th quartile; Q75) ratios of omega-3 (n-3) to omega-6 (n-6) PUFAs, analysis encompassed 16 asthmatics. There was a trend of a more frequent sea fish consumption in Q75 subjects compared to the Q25 group ($p = 0.07$).

PUFA concentrations in plasma and erythrocyte membranes

Ratios of n-3/n-6 PUFA were significantly lower in asthmatics (0.29, SEM 0.063) than in healthy controls (0.33, SEM 0.038; $p = 0.038$; see Fig. 1).

Bronchial hyperreactivity to metacholine and single-dose grass pollen challenge

The procedures were well tolerated by all subjects. There was a trend of a higher bronchial hyperreactivity to metacholine in Q25 asthmatics (median PD₂₀ 0.15, SEM 0.08) compared to Q75 asthmatics (median 0.27, SEM 0.03,

Table 1 Subjects' baseline characteristics. Q25 = first quartile and Q75 = fourth quartile of n-3/n-6-ratio of PUFAs in erythrocyte membranes. Inter-group comparison between controls and asthmatics or Q25 and Q75 asthmatics revealed no statistical significant differences unless indicated otherwise.

	Controls	Asthmatic		
		Total	Q25	Q75
Gender	5 m/14 f	12 m/26 f	1 m/7 f	2 m/6 f
Height [cm]	171.1 (0.40)	174.4 (0.28)	173.0 (1.18)	170.0 (1.64)
Body weight [kg]	63.0 (5.40)	68.2 (4.10)	73.8 (2.38)	61.5 (1.60)
IgE [IU/ml]	77.4 (10.9)*	271.2 (9.63)*	147.5 (17.6)	353.5 (83.5)
Spec. IgE [IU/ml]	0.10 (0.00)**	212.7 (8.26)**	169.6 (27.6)	147.4 (43.1)

IgE = Immunoglobulin E; spec. IgE = IgE specific for grass pollen; * $p = 0.04$; ** $p = 0.005$.

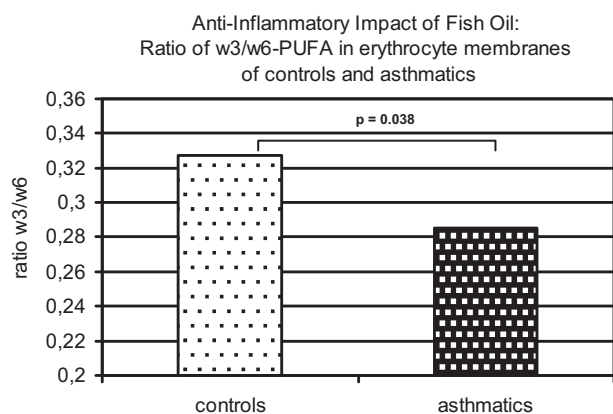


Figure 1 Ratio of n-3/n-6 PUFA levels in erythrocyte membranes in allergic asthmatics compared to healthy controls.

$p = 0.37$). This was confirmed by the results from the specific bronchial challenge with grass pollen: FEV₁ decrease in Q25 asthmatics 20.9 (SEM 2.11) vs. 19.3 (SEM 2.18) in Q75 asthmatics; $p = 0.84$.

Exhaled nitric oxide (eNO)

Levels of eNO were significantly higher ($p = 0.040$) in Q25 asthmatics (median 35.4, SEM 5.95) than in Q75 asthmatics (13.0, SEM 2.09; Fig. 2).

Discussion

Epidemiological studies support the thesis of a positive impact of n-3 PUFAs on atopic and asthmatic airway disease. A case-control study of the dietary intakes of Spanish adult subjects with asthma ($n = 54$) showed a positive correlation of n-3 fatty acid intake with FEV₁.²⁴ In a nested case-control study, Murray and coworkers examined 3–5 year old children with recurrent wheeze. Young sensitized wheezy children had a significantly higher total polyunsaturated fat intake compared to non-sensitized

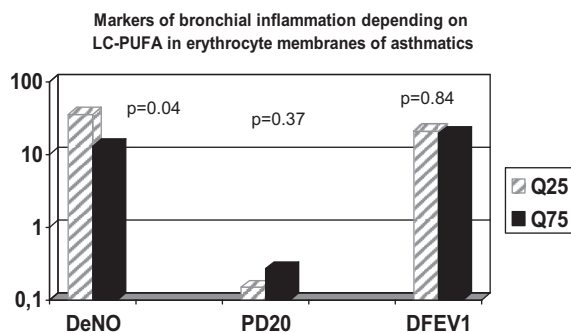


Figure 2 Markers of bronchial inflammation depending on LC-PUFA in erythrocyte membranes of asthmatics. LC-PUFA-long-chain polyunsaturated fatty acids; DeNO = change of exhaled nitric oxide; PD₂₀ = dosage of grass pollen challenge causing a 20% decrease of FEV₁; D FEV₁ = change of forced expiratory volume within 1 s.

children as calculated from patients nutrition diaries.²⁵ A cross sectional population-based study German study examined 568 adults, detecting a high content of n-3 fatty acids in erythrocyte membranes or in the diet as associated with a decreased risk of allergic sensitization and allergic rhinitis.²⁶ A recent study on 2112 North-American and Canadian students detected low dietary n-3 fatty acid intake as associated with lower pulmonary function and increased respiratory symptoms.²⁷

Thus, an intervention supplementing food with n-3 PUFAs appears reasonable. In a recent study, we examined allergen-induced airway inflammation developing over two weeks of repeated low-dose allergen inhalation in sensitized allergic asthmatics.²⁰ Here, bronchial inflammation was significantly attenuated by five weeks of 0.7 g dietary n-3 PUFA supplementation, verified by lower levels of exhaled NO, serum and sputum eosinophils, Eosinophilic Cationic Protein, and a suppression of the proinflammatory eicosanoids secreted by leukocytes. This observation supported previous findings in patients with allergic disease where n-3 PUFA supplementation reduced the generation of inflammatory leukotrienes and prostaglandins.^{13–16,28}

Other studies on PUFAs and allergic airway disease mainly relied on dietary intake data, being subjected to recall bias. Our approach was to use the fatty acid composition of erythrocyte membranes, reflecting the biologically available amount of PUFA in cellular membranes. With respect to the RBCs life span of 120 days, these values can be considered as representative for a relatively long time frame. On the other hand, the fatty acid analysis of serum phospholipids, which is often performed to assess the alimentary fatty acid intake within the near past, does hardly represent the biologically relevant source of precursors for eicosanoid formation, i.e., membrane PUFAs.

The potential benefit of n-3 PUFA in the management of asthma is a matter of debate; a recent meta analysis review²⁹ encompassing randomized controlled trials comparing n-3 PUFA supplementation with placebo^{12,13,30,31,38} or with n-6 PUFA supplementation¹⁵ could not find consistent benefit. The childhood asthma prevention study group (CAPS)³² reported that a dietary intervention with approximately 184 mg omega-3 fatty acid daily significantly reduced cough in atopic infants. Other authors suggest that omega-3 fatty acid supplementation reduced arachidonic acid-derived inflammatory mediators, thereby reducing cough sensitivity in atopic disease.³³ The findings from CAPS could not be confirmed by follow-up investigations.³⁴ A recent study of Mickleborough et al.³⁵ demonstrated a protective effect of fish oil supplementation (3 g/d) on exercise-induced bronchoconstriction. In addition, the same group showed that n-3 PUFA reduced airway narrowing, medication use, and proinflammatory mediator generation in non-atopic elite athletes with exercise-induced bronchoconstriction.³⁶ A comparison of these studies to our results showed equal reactions to bronchial stimulation in placebo and Q25 subjects, but only significant suppression of bronchial reactions in high dose of EPA supplementation. The underlying mechanism can be explained in part by a recent *in vitro* study, where human alveolar macrophages pretreated with EPA demonstrated a significantly weaker inflammatory response when

stimulated with lipopolysaccharides.³⁷ These findings suggest that dietary fish oil supplementation may be a viable treatment modality and/or adjunct therapy but it is an ongoing debate, whether higher doses or long-term supplementation of n-3 PUFA might exhibit more 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 several short-term trials.^{16,27,38} 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 the n-3 PUFA, and the heterogeneity of asthma patients.

Nevertheless, even results of studies using low-doses have been promising. Nagakura et al.¹³ found that a 10-month intake of 120 mg n-3 PUFA per day reduced asthma symptoms scores and bronchial hyperreactivity in children compared to controls. In another placebo-controlled study on 60 children from Egypt with moderate persistent asthma, six weeks of food supplementation with 1 g of triglyceride oil (containing 30% EPA/DHA) resulted in a significant improvement of lung functions.³⁹ A lipid extract of New Zealand green-lipped mussel containing 100 mg 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 Expiratory Flow in the lipid extract group compared to the placebo group.⁴⁰ Also the duration of the dietary manipulation might explain the discrepancy between epidemiological findings and the often weak effects of short-term PUFA supplementation.

A potential pitfall in studies investigating effects of fish oil on allergies is the phenomenon of reverse causation, i.e., allergic subjects might avoid fish intake by themselves due to food allergies or fear of sensitization. In such a situation, low PUFA levels in their organisms would be the consequence of allergic conditions, and not vice versa. Our study's concept tried to avoid this by not only documenting fish consumption of our patients, but also stratifying for PUFA content of erythrocyte membranes.

Conclusions

This study used PUFA distribution in erythrocyte membranes reflecting dietary intake to assess biologically available PUFAs at the cellular level and its association with markers of pulmonary function and inflammation. Our non-interventional study revealed lower n-3 PUFA levels in allergic asthmatics than in non-allergic healthy controls. The increase of exhaled nitric oxide following experimentally induced allergic bronchial inflammation was significantly higher in asthmatics with low n-3/n-6 PUFA levels, implying a mild anti-inflammatory effect of nutritional fish oil. Overall, regular dietary consumption of fish oil does not seem sufficient to suppress specific bronchial reactivity. On the basis of available data, no final recommendation on the preventive or therapeutic use of PUFAs in allergic diseases can be given. Further studies with pharmacological doses of

n-3 PUFAs are needed to clarify their role in regulating allergic asthmatic conditions.

Conflict of interest

RK, MAR, RS, AK, HJB, JS, and SZ have no financial or personal relationships that may inappropriately influence their actions. CB was associated with Numico Research Inc. when the study was performed. The study was supported in part by Numico Research Inc.

Acknowledgements

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

References

- 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;22:421–8.
- Peat JK, Salome CM, Woolcock AJ. Factors associated with bronchial hyperresponsiveness in Australian adults and children. *Eur Respir J* 1992;5:921–9.
- Hodge L, Salome MM, Peat JK, Haby MM, Xuan W, Woolcock AJ. Consumption of oily fish and childhood asthma risk. *Med J Aust* 1996;164:137–40.
- Haby MM, Peat JK, Marks GB, Woolcock AJ, Leeder SR. Asthma in preschool children: prevalence and risk factors. *Thorax* 2001;56:589–95.
- Stephensen CB. Fish oil and inflammatory disease: is asthma the next target for n-3 fatty acid supplements? *Nutr Rev* 2004;62:486–9.
- Prescott SL, Calder PC. N-3 polyunsaturated fatty acids and allergic disease. *Curr Opin Clin Nutr Metab Care* 2004;7:123–9.
- Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese 3rd J, 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;312:1217–24.
- Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC. 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;320:265–71.
- Woods RK, Thien FC, Abramson MJ. Dietary marine fatty acids (fish oil) for asthma in adults and children. *Cochrane Database Syst Rev*; 2002:CD001283.
- 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;93:529–35.
- 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;3:84–92.
- 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;11:361–5.
13. 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;16:861–5.
 14. 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;65:1011–7.
 15. 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;39:107–11.
 16. 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;139:1395–400.
 17. de Kluijver J, Evertse CE, Schrupf 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.
 18. Jones SL, Kitelson 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;164:738–43.
 19. Peroni DG, Piacentini GL, Bodini A, Ressa M, Costella S, Boner AL. Montelukast versus formoterol as second-line therapy in asthmatic children exposed to relevant allergens. *Allergy Asthma Proc* 2005;26:283–6.
 20. Schubert R, Kitz R, Beermann C, Rose MA, Lieb A, Sommerer PC. Effect of n-3 polyunsaturated fatty acids in asthma after low-dose allergen challenge. *Int Arch Allergy Immunol* 2009;148(4):321–9.
 21. American Thoracic Society. Guidelines for metacholine and exercise challenge testing-1999. *Am J Respir Crit Care Med* 2000;161:309–29.
 22. American Thoracic Society. 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. Official statement, adopted by the ATS Board of directors. *Am J Respir Crit Care Med* July 1999;160:2104–17.
 23. Beermann C, Mobius M, Winterling N, Schmitt JJ, Boehm G. Senior position determination of phospholipid-linked fatty acids derived from erythrocytes by liquid chromatography electrospray ionization ion-trap mass spectrometry. *Lipids* 2005;40:211–8.
 24. de Luis DA, Armentia A, Aller R, Asensio A, Sedano E, Izaola O, Cuellar L. Dietary intake in patients with asthma: a case control study. *Nutrition* 2005;21:320–4.
 25. Murray CS, Simpson B, Kerry G, Woodcock A, Custovic A. Dietary intake in sensitized children with recurrent wheeze and healthy controls: a nested case-control study. *Allergy* 2006;61:438–42.
 26. Hoff S, Seiler H, Heinrich J, Kompauer I, Nieters A, Becker N, Nagel G, Gedrich K, Karg G, Wolfram G, Linseisen J. Allergic sensitisation and allergic rhinitis are associated with ω -3 polyunsaturated fatty acids in the diet and in red blood cell membranes. *Eur J Clin Nutr* 2005;59(9):1071–80.
 27. Burns JS, Dockery DW, Neas LM, Schwartz J, Coull BA, Raizenne M, Speizer FE. Low dietary nutrient intakes and respiratory health in adolescents. *Chest* 2007;132:238–45.
 28. 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;18:177–87.
 29. Reisman J, Schachter HM, Dales RE, Tran K, Kourad K, Barnes D, Sampson M, Morrison A, Gaboury I, Blackman J. 2006 Treating asthma with omega-3 fatty acids: where is the evidence? A systematic review. *BMC Complement Altern Med* 2006;19(6):26.
 30. 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;95:156–7.
 31. 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;62:534–7.
 32. Peat JK, Mirshahi S, Kemp AS, Marks GB, Tovey ER, Webb K, Mellis CM, Leeder SR. Three-year outcomes of dietary fatty acid modification and house dust mite reduction in the childhood asthma prevention study. *J Allergy Clin Immunol* 2004;114:807–13.
 33. 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;73:649–53.
 34. Almquist C, Garden F, Xuan W, Mirshahi S, Leeder SR, Oddy W, Webb K, Marks GB. Omega-3 and omega-6 fatty acid exposure from early life does not affect atopy and asthma at age 5 years. *J Allergy Clin Immunol* 2007;119(6):1438–44.
 35. 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.
 36. 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;168:1181–9.
 37. Mickleborough TD, Tecklenburg SL, Montgomery GS, Lindley MR. Eicosapentaenoic acid is more effective than docosahexaenoic acid in inhibiting proinflammatory mediator production and transcription from LPS-induced human asthmatic alveolar macrophage cells. *Clin Nutr* 2009;28(1):71–7.
 38. 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;147:1138–43.
 39. Biltagi MA, Baset AA, Bassiouny M, Kasrawi MA, Attia M. Omega-3 fatty acids, vitamin C and Zn supplementation in asthmatic children: a randomized self-controlled study. *Acta Paediatr* 2009;98(4):737–42.
 40. 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.