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Dietary n-3 long chain polyunsaturated fatty acids in allergy prevention and asthma treatment

Linette E.M. Willemsen

Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands

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ABSTRACT

The rise in non-communicable diseases, such as allergies, in westernized countries links to changes in lifestyle and diet. N-3 long chain polyunsaturated fatty acids (LCPUFA) present in marine oils facilitate a favorable milieu for immune maturation and may contribute to allergy prevention. N-3 LCPUFA can suppress innate and adaptive immune activation and induce epigenetic changes. Murine studies convincingly show protective effects of fish oil, a source of n-3 LCPUFA, in food allergy and asthma models. Observational studies in human indicate that high dietary intake of n-3 LCPUFA and low intake of n-6 PUFA may protect against the development of allergic disease early in life. High n-6 PUFA intake is also associated with an increased asthma risk while n-3 LCPUFA may be protective and reduce symptoms. The quality of the marine oil used has impact on efficacy of allergy prevention and several observations link in particular n-3 LCPUFA DHA to allergy suppression. Randomized controlled trials indicate that optimal timing, duration and dosage of n-3 LC-PUFA is required to exert an allergy protective effect. Supplementation during early pregnancy and lactation has shown promising results regarding allergy prevention. However these findings should be confirmed in a larger cohort. Although clinical trials in asthma patients reveal no consistent clinical benefits of n-3 LCPUFA supplementation on lung function, it can suppress airway inflammation. Future food-pharma approaches may reveal whether adjunct therapy with dietary n-3 LCPUFA can improve allergy prevention or immunotherapy via support of allergen specific oral tolerance induction or contribute to the efficacy of drug therapy for asthma patients.

1. Dietary fatty acids and allergic disease

Over the last decades the prevalence of allergic disease has steadily been rising and recent figures indicate that in the western world 10–25% of people are affected with allergic diseases ranging from food allergy, atopic dermatitis/ eczema, allergic rhinitis, hay fever to asthma (Levy et al., 2007; Platts-Mills, 2015). In early life food allergy and atopic dermatitis are the first occurring allergic diseases, peaking at one year of age. 2-8% Of young infants and 5% of adults are affected with food allergy (Sanchez-Garcia et al., 2015; Sicherer and Sampson 2014), while the prevalence of asthma reaches 5% in western countries (Barros et al., 2015). In the majority of cases children out grow food allergy by the age of three to five years however later in life allergic rhinitis and asthma can develop as a consequence of the atopic constitution (Malmberg et al., 2010; Alduraywish et al., 2015). Changing environmental conditions such as urbanization, sedentary lifestyle and dietary alterations have been suggested to impair immune maturation and increase immunological disorders such as allergic diseases, fitting in the category of non-communicable diseases (Prescott, 2013; Haahtela et al., 2015; Dunstan and Prescott, 2005; Schroder et al., 2015). Increased fat, sugar and salt intake and reduced fiber and antioxidant intake have been suggested to contribute to allergic sensitization and/or severity of allergic symptoms (Nagel et al., 2010; Ellwood et al., 2013; Barros et al., 2015; Trak-Fellermeier et al., 2004) and fish, fruit and vegetable intake have been associated with allergy protection (Barros et al., 2015; Magnusson et al., 2013; Shaheen et al., 2001; Lumia et al., 2011; Woods et al., 2003; Calvani et al., 2006). Besides the increase in quantity also

Abbreviations: AA, arachidonic acid; ALA, alpha linolenic acid; aOR, adjusted odds ratio; aRR, adjusted risk ratio; BLG, beta-lactoglobulin; CCL, chemokine ligand; cysLT, cysteinyl leukotrienes; DC, dendritic cell; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; eNO, exhaled nitric oxide; FA, Fatty Acids; FADS, Fatty Acid Desaturase; FEV_1, Forced Expiratory Volume; HR, Hazard Ratio; LA, linoleic acid; LCPUFA, long chain poly unsaturated fatty acids; LPS, lipopolysaccharide; MLN, mesenteric lymph nodes; OVA, ovalbumin; PA, palmitic acid; PBMC, peripheral blood mononuclear cells; SFA, saturates fatty acids; SPT, skin prick test; Treg, regulatory T cell; T_H, T helper cell; Tr1, type 1 regulatory T cell

E-mail address: L.E.M.Willemsen@uu.nl

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the quality of dietary fat has changed over the decades. N-6 polyunsaturated fatty acid (PUFA) linoleic acid (LA; 18:2, n-6) is increasingly consumed since it is abundantly present in vegetable oils such as sunflower, corn, and soybean oil which are present in margarine, while the increased consumption of meat enhances arachidonic acid intake (AA; 20:4, n-6). In female increased intake of saturated fatty acid (SFA) and mono-unsaturated FA for example has been associated with increased risk of allergic sensitization while an increasing intake of PUFA over SFA was associated with protection (Trak-Fellermeier et al., 2004). However in the same study cohort increasing intake of n-6 PUFA over n-3 PUFA enhanced the risk of developing atopic eczema in females and the same was shown for an increasing intake of n-6 LA over n-3 alpha-linolenic acid (ALA; 18:3, n-3) (Trak-Fellermeier et al., 2004). LA and ALA are essential FA that need to be acquired via the diet and can slowly be converted in biologically active long chain poly-unsaturated fatty acid (LCPUFA), AA or eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3). Intake of n-3 LCPUFA EPA and DHA, which are present in fatty fish (such as salmon, herring, mackerel and tuna), algae, krill and marine oils, is traditionally low in westernized countries. ALA from vegetable sources only allows for 0.1-10% of the n-3 LCPUFA pool depending on the conversion rate which is determined in part by polymorphisms in the fatty acid desaturase (FADS) gene (Lattka et al., 2012). Similar applies for n-6 LCPUFA AA derived from LA (Koletzko et al., 2014; Lattka et al., 2012). In the cell membrane n-6 and n-3 PUFA compete for incorporation into phospholipids. n-3 LCPUFA will replace AA resulting in altered FA composition of the cell membrane and an altered pattern of eicosanoid and resolvin production (Calder, 2015). Intake of n-3 LCPUFA during pregnancy or postnatally, has often been suggested to be beneficial for the cognitive and visual outcome of the infants and although randomized controlled trial outcomes currently are inconclusive the data are encouraging (Koletzko et al., 2014; De Giuseppeet al., 2014). The European Food Safety Authority advises 100–200 mg DHA during pregnancy and 100 mg DHA/day for young infants, with the addition of 140 mg/day AA during the first 6 months of life, and 250 mg EPA plus DHA/day after two years of age (WHO, 2010). Also the Nutrition Academy for the Asian population advices a minimum intake of 200 mg DHA per day (can be achieved by eating two portions of fatty fish per week) during pregnancy while higher amounts (600–800 mg DHA/day) are preferable to prevent preterm birth (Koletzko et al., 2014). Also during breast-feeding women are advised to acquire at least 200 mg DHA per day to allow for 0.3% DHA in the FA pool of human milk and the supply of 100 mg DHA should be provided during infancy (Koletzko et al., 2014). Beyond their function in neurological development and vision n-3 LCPUFA may affect allergy development (De Giuseppeet al., 2014). It has been suggested that the FA composition and n-3 over n-6 LCPUFA ratio of breast milk influence the susceptibility of the infant to develop allergic disease (Friedman and Zeiger, 2005; Wijga et al., 2006). Indeed some studies indicate that marine oil, such as fish oil, intake during pregnancy and lactation, may positively affect allergy development in the offspring, which may apply in particular for those having low endogenous n-3 LCPUFA precursor conversion rates (Koletzko et al., 2014). This review aims at describing the known effects of n-3 versus n-6 PUFA on sensitization and allergy development with a focus on food allergy and asthma.

2. Allergy and effects of PUFA on innate, adaptive and effector immune cells

In IgE mediated allergic disease a T helper cell (T_H) 2 polarized adaptive immune response towards otherwise tolerized proteins (allergens) occurs. Allergens from for example food can induce food allergy and airborne allergens such as present in birch pollen or house dust mite (HDM) can induce allergic asthma. In general it is thought that AA derived eicosanoids such as prostaglandin (PGE)-2 contribute to allergic sensitization since PGE_2 contributes to a T_H2 polarized immune response and is responsible for IgE isotype switching in B-cells (McIlroy et al., 2006; Fedyk and Phipps, 1996; Kay et al., 2013; Roper et al., 1990; Roper et al., 1995). In addition, depending on its concentration PGE_2 can dampen or enhance the allergic effector response (Church et al., 2012; Safholm et al., 2015). Antigen presenting cells (APC) like dendritic cells (DC) present the allergens to T-cells which polarize into T_H2 effector cells instead of being deleted, rendering anergic or develop into regulatory T-cells (Treg). These T_H2 cells drive B-cell isotype switching and maturation into allergen specific IgE-producing plasma cells. Allergen specific IgE binds the FcεRI on mast cells and basophils (sensitization). Allergic symptoms occur upon allergen challenge which crosslinks membrane-bound IgE resulting in effector cell degranulation and mediator release like histamine, PGD_2 and cytokines which cause the allergic symptoms. T_H2 biased immune responses can be restored via the installation of allergen specific Treg ($CD4^+CD25^{high}FoxP3^+$, Tr1 or T_H3 cells) that produce suppressive cytokines like IL-10 and/or TGF-β or T_H1 cells that produce IFN-γ which counteracts T_H2 activation and survival.

2.1. Effect of PUFA on DC-T-cell interaction

Tolerogenic DC can instruct T-cell anergy or Treg, while activated DC can induce effector T-cell responses. Inflammatory mediators like lipopolysaccharide (LPS) activate DC which induce maturation resulting in increased expression of major histocompatibility complex II, costimulatory molecules and T-cell polarizing cytokine release. Draper et al. studied the effects of EPA and DHA on LPS induced murine bone marrow derived DC maturation and showed EPA and DHA (25μM added during differentiation and maturation) to suppress MHCII and costimulatory molecule expression and T_H1 driving IL-12p70 release while increasing IL-10 secretion by DC (Draper et al., 2011). EPA and DHA suppressed nuclear factor κB (NF-κB) activation in these cells. By contrast, LA did not suppress DC maturation (Draper et al., 2014). In human monocyte derived DC (moDC) EPA as well as AA (20μM) were found to suppress basal expression of costimulatory molecules on DC when added during differentiation and/or maturation. These remained reduced upon LPS induced maturation while IL-12p40 and TNFα were reduced in a dose dependent manner (Zeyda et al., 2005). Palmitic (PA) and oleic (OA) acid had no effect. EPA and AA exposed DC also suppressed T-cell proliferation in a mixed lymphocyte reaction (Zeyda et al., 2005). EPA and AA did not reduce activation of NF-κB or MAPK signaling cascades in this study and cyclooxygenase and lipoxygenase inhibitors did not interfere with their effect either (Zeyda et al., 2005). Although in these studies both n-3 LCPUFA EPA as well as n-6 LCPUFA AA inhibited LPS induced DC activation, a dietary intervention study with fish oil in healthy volunteers showed that supplementation of 0.3–2.0 g fish oil (EPA: DHA, 2:1) per day for four weeks lowered basal and/or LPS induced TNFα and IL-6 release by peripheral blood mononuclear cells (PBMC) (Trebble et al., 2003). Also in a study in elderly humans, consuming oil rich in ALA (2 g), GLA (720 mg), AA (680 mg), DHA (720 mg) or fish oil (1 g EPA and DHA) for four weeks, only in the GLA and fish oil groups PBMC proliferation was reduced, however no effects on IFN-γ production were shown (Thies et al., 2001). In a study by Kew et al. in human volunteers receiving a daily diet enriched with either 9.5 g ALA or 1.7 g EPA and DHA for a period of 6 months LPS induced TNFα, IL-1β, IL-6 or IL-10 release by PBMC remained unaltered (Kew et al., 2003). However *in vitro* exposure of human PBMC shows LCPUFA

to affect inflammatory responses. When provided two hour prior to LPS stimulation, DHA or docosapentaenoic acid (DPA, C22:5, n-6) (rich in algae) (100 μM), but not EPA or AA, reduced IL-1β and PGE_2 release by human PBMC (TNFα showed a similar pattern) (Nauroth et al., 2010). Hence marine oil may be capable of reducing LPS induced inflammatory responses and DHA and algae oil derived DPAn-6 may be more effective than EPA and AA, which may relate to the length of the carbon chain and number of double bonds.

2.2. Effect of PUFA on mast cells

PUFA also have been shown to affect the mast cell phenotype. AA is a precursor for PGD_2 upon conversion by cyclo-oxygenase. EPA and DHA suppressed PGD_2 release by mast cells without affecting degranulation or histamine release (Obata et al., 1999; van den Elsen et al., 2013b). 24 h Of pre-exposure of human mast cells with 1 μM EPA was found to reduce IgE induced PGD_2 release upon mast cell degranulation compensated with a limited induction of PGD_3 (Obata et al., 1999). In a recent study 100 μM EPA, DHA or AA was found to reduce mast cell degranulation in murine bone marrow derived mast cells but AA increased FcεRI expression in lipid rafts while this was decreased by EPA and DHA (Wang et al., 2015). Furthermore EPA reduced inflammatory cysteinyl leukotrienes (cysLT) and TNF-α secretion while this was enhanced by AA, and DHA reduced chemokine ligand (CCL) 2 release (Wang et al., 2015). Also in another study AA (25 μM) increased PGD_2 and TNFα, while EPA and DHA decreased PGD_2 release by a human mast cell line (van den Elsen et al., 2013b). At 100 μM AA, EPA and DHA reduced IL-13 release by HMC-1 cells. However EPA and DHA were more effective than AA, and DHA was already lowering IL-13 at 25 μM in association with reduced generation of reactive oxygen species in these cells (van den Elsen et al., 2013b). Hence n-6 LCPUFA AA enhanced secretion of inflammatory mediators by mast cells, while n-3 LCPUFA DHA was most effective in reducing T_H2 type IL-13 release. These *in vitro* observations show that PUFA can affect the innate as well as the adaptive immune response and cells responsible for allergic sensitization as well as symptom elicitation.

3. PUFA and allergic sensitization and symptoms in mice

3.1. N-3 LCPUFA supplementation and allergic sensitization and food allergy in mice

In a murine study it was observed that the type of dietary fat could influence the susceptibility to develop food allergy. A diet containing 13% salmon oil, but not diets with soybean oil, sunflower oil, linseed oil or beef tallow, suppressed allergic symptoms in an ovalbumin (OVA) induced food allergy model while enhancing the vaccination response in an influenza model (Hogenkamp et al., 2011). Relevance of n-3 over n-6 PUFA in protection against allergic sensitization and symptoms was studied in a murine model for orally induced cow's milk allergy (using cow's milk protein whey for sensitization). Mice were fed a n-6 PUFA rich 10% soybean oil (consisting of 5.6% ALA and 53.1% LA) diet or a fish oil diet in which 6% of the soybean oil was replaced by tuna oil rich in n-3 LCPUFA (consisting of 7.0% EPA and 27.8% DHA). The DHA content of erytrocyte membranes increased from appproximately 6 to 12% and AA decreased from approximately 14 to 7% (van den Elsen et al., 2013c). This change in type of oil largely prevented allergic sensitization and symptoms. The tuna oil fed whey allergic mice had low serum whey specific IgE and a suppressed allergic effector response in association with an increased frequency of tolerogenic DC in the mesenteric lymph nodes (MLN) and Foxp3+Treg in the spleen and intestinal lamina propria (van den Elsen et al., 2013c). The splenic Treg of fish oil fed sensitized mice, were capable of transferring their protective effect to control diet fed naïve mice when transferred prior to sensitization (van den Elsen et al., 2013a, 2013c), indicating that the fish oil diet had generated functional Treg. Beyond increasing Treg cell frequency the tuna oil diet was found to suppress both the T_H2 as well as T_H1 cell frequency in the MLN and/or the spleen in sham as well as in whey sensitized mice (van den Elsen et al., 2013c). Also when comparing mice that were sensitized subcutaneously and orally challenged with OVA, while fed a 7% soybean oil (containing 4.8% ALA and 55.8% LA) or fish oil (containing 15.9% EPA and 7.9% DHA) diet directly after weaning, the fish oil diet reduced OVA specific IgE and IgG_1 levels. Furthermore, amongst others the number of eosinophils in the proximal jejunum was reduced in the fish oil group (de Matos et al., 2012). Beyond the benefits of n-3 LCPUFA over n-6 PUFA also the quality of the fish oil and the allergen used may impact the efficacy by which fish oil supplementation is capable of suppressing food allergy. Dietary supplementation with tuna oil (EPA: DHA∼1:4) was compared to maruha oil (EPA: DHA∼2:1) and only a 6% exchange of soybean oil by tuna oil but not maruha oil suppressed sensitization for whey protein (van den Elsen et al., 2014). Oral peanut sensitization could not be prevented by either of the oils. However both fish oils suppressed the acute allergic skin response, indicative for suppression of effector cell induced symptoms. Also for the latter purpose tuna oil was more effective than maruha oil. This was confirmed in passively sensitized mice fed the fish oil diets. The allergen specific allergic effector response was suppressed in the fish oil group compared to the soybean oil fed control group, indicating that fish oil can suppress allergic symptoms beyond modulation of the sensitization cascade (van den Elsen et al., 2014). By contrast, increasing dietary intake of n-6 PUFA was shown to enhance allergic symptoms and break oral tolerance induction in food allergic mice (van den Elsen et al., 2015). Hence these studies indicate that replacement of n-6 PUFA rich soybean oil by n-3 LCPUFA rich fish oil may not only suppress the onset of sensitization for certain food proteins but also the allergic effector response and consequently the allergic symptoms. It was hypothesized that an increased ratio of DHA over EPA in the fish oil contributes to the enhanced efficacy of tuna oil over maruha oil in this mouse model (van den Elsen et al., 2014). Thang et al. studied the effect of supplementation with different n-3 over n-6 PUFA ratio's (1:10, 1:4, 4:1) in mice. The diets had a 35% fat content mimicking a western style high fat diet and safflower oil was used as n-6 PUFA source while menhaden oil was the n-3 LCPUFA source. Mice were fed the diets and subsequentially intraperitoneally sensitized with beta-lactoglobulin (BLG), a major allergen in whey. In these studies the EPA over DHA ratio remained almost equal between the comparisons and the total amount of n-3 LCPUFA was increased at the expense of LA (Thang et al., 2013). Upon oral BLG challenge only the mice fed the high n-6 PUFA diet (1:10) developed a significant drop in temperature as indicative for systemic anaphylaxis as a consequence of food allergy. The levels of BLG-specific immunoglobulins were the highest in the n-3 LCPUFA supplemented groups during sensitization but at the endpoint measurement in these groups serum BLG-IgE and splenic IL-4 decreased. Only in the sensitized group fed the highest amount of n-3 LCPUFA, IL12p40, IL-10 and IFNγ increased in restimulated MLN cells (Thang et al., 2013). Hence also in a high fat diet the increase of n-3 LCPUFA at the expense of n-6 PUFA suppressed T_H2 polarization and ameliorated the allergic response. n-3 LCPUFA are effective in food allergy prevention by suppressing sensitization and ameliorating allergic symptoms in mice and a high DHA over EPA ratio may enhance its efficacy.

3.2. N-3 LCPUFA supplementation and asthma in mice

Other proof of preferential effects of n-3 PUFA over n-6 PUFA in allergy prevention comes from a murine asthma model using Fat-1 transgenic mice that are capable of converting n-6 PUFA into n-3 PUFA rendering the ratio of n-6 over n-3 PUFA of 2.9 in their erythrocytes when fed a n-3 PUFA deficient diet while in wild type mice this was 46.6 (Jang et al., 2014; Kang et al., 2004). These mice were intraperitoneally sensitized with OVA and challenged in the lung while fed a 10% corn oil (rich in n-6 PUFA) diet. Allergen specific T_H cell activation and proliferation was suppressed in the MLN and spleen, and T_H1 and T_H2 cytokine levels were reduced in the Fat-1 transgenic mice compared to wild type mice as a consequence of reduced T-cell receptor signaling (Jang et al., 2014). In OVA challenged allergic mice lung eosinophil and lymphocyte influx was completely abolished in Fat-1 transgenic mice and pulmonary T_H cell activation and cytokine levels and serum OVA-IgE were significantly suppressed (Jang et al., 2014). These Fat-1 mice had an AA over EPA and DHA ratio of 1.6 in their lung tissue, while in wild type mice this was 6.3. Fat-1 transgenic OVA allergic mice showed a strong reduction in inflammatory cell influx and airway eosinophilia in broncho alveolar lavage fluid upon OVA airway challenge and the airway hyperresponsiveness was suppressed along with reduced concentrations of inflammatory cytokines and chemokines (Bilal et al., 2011). Previous studies in the same model had suggested that increased levels of n-3 PUFA derived protectin D_1 and resolvin E_1 in Fat-1 mice contribute to the asthma protective effects in these mice (Bilal et al., 2011). Protectin D_1 and resolvin E_1 are known to dampen airway inflammation and asthma exacerbations are known to be associated with inadequate protectin D_1 generation in human (Haworth et al., 2008; Levy et al., 2005; Levy et al., 2007). The latter was observed in exhaled breath condensates from healthy volunteers compared with asthmatics having an exacerbation (Levy et al., 2007). In OVA asthmatic mice protectin D_1 reduced airway hyper responsiveness and eosinophilia and lymphocyte influx, and lowered airway IL-13, cysLT, LXA_4 and PGD_2 (Levy et al., 2007). Resolvin E_1 was found to promote resolution of airway inflammation in OVA asthmatic mice by suppression of IL-17, IL-23 and IL-6 while increasing IFN-γ and LXA_4 in lung tissue(Haworth et al., 2008). Indeed in human asthmatics the LXA_4 over CysLT ratio was positively correlated with long function (forced expiratory volume, FEV_1) and LXA_4 levels are significantly reduced in severe asthmatics (Levy et al., 2005). Indicating the importance of LXA_4 in dampening airway inflammation and the importance of n-3 PUFA derivate resolvin E_1 in regulation of LXA_4 levels. Beyond protectin D_1 and resolvin E_1 also DHA derived maresin-1 was recently shown to suppress allergic airway inflammation in mice by means of inducing de novo Treg that suppress innate lymphoid type 2 cells via release of TGFβ (Krishnamoorthy et al., 2015). Also dietary intervention studies in asthmatic mice show encouraging results. In OVA asthmatic mice intervention with a fish oil (6.3% menhaden oil containing 13% EPA, 12% DHA and 0.7% soybean oil) diet prevented OVA induced airway inflammation, hyper responsiveness, eosinophilia and airway fibrosis while reducing serum OVA-IgE and T_H2 and T_H17 type cytokines in the lung when compared to a diet containing 7% soy oil (Bargut et al., 2013). Hence also in asthmatic mice fish oil effectively suppressed allergic sensitization and the consequent allergic airway inflammation and hyper reactivity. Studies in mice convincingly show protective effects of fish oil in prevention of sensitization and allergic symptoms. However mice have the same genetic background, are housed in a controlled environment and have standardized diets. Effects of dietary PUFA in the heterogeneous human population with a huge genetic diversity with different dietary habits and living conditions have been investigated in observational and intervention studies.

4. Effects of dietary n-6 and n-3 PUFA on allergic disease in humans

4.1. Effects of dietary PUFA during gestation, lactation and infancy on allergic outcome

Observational studies in human have indicated a link between dietary fatty acid composition and susceptibility to develop allergic disease. In a Finnish cohort FA intake during pregnancy was calculated based on dietary intake questionnaires and low intake of ALA (hazard ratio, HR 1.70) and total n-3 PUFA (HR 1.66) and high intake of total n-6 PUFA (HR 1.41) was associated with increased risk of asthma in offspring at the age of five. The mean calculated intake of ALA, EPA and DHA was 2.6 g/d, 92 mg/day, 300 mg/day respectively and in this study n-3 LCPUFA EPA or DHA alone were not associated with reduced asthma risk. On the other hand low intake of AA (HR 0.52), and high intake of palmitic acid (PA) (HR 0.50) or total saturated FA (HR 0.55) were associated with a decreased risk (Lumia et al., 2011). The main source of saturated FA being meat, milk, butter and cheese and the main source of n-6 PUFA (mostly linoleic acid (LA)) being oils (LA), meat (LA, AA) and margarine (LA); beyond LA oils also contain n-3 ALA. However butter and a high ratio of n-6 over n-3 PUFA was found to increase the risk of allergic rhinitis (HR 1.33) at the age of five (Lumia et al., 2011). When studying the impact of dietary FA intake during lactation a positive association with the risk of developing asthma was observed for margarines (HR 1.96) but not butter (Lumia et al., 2012; Nwaru et al., 2012). This was in children with enhanced genetic risk of developing Type 1 Diabetes based on human leukocyte antigen typing and may therefore not properly reflect the general population. In the German LISA birth cohort study high maternal intake of margarine or vegetable oils during the last four weeks of pregnancy was associated with increased risk of eczema during the first two years in the offspring (adjusted odds ratio (aOR) 1.48, 1.49), while high fish intake reduced this risk (aOR 0.75) (Sausenthaler et al., 2007). Furthermore dietary questionnaires of 2582 children at the age of two revealed a positive association between margarine, and not butter, intake in the life prevalence of doctor-diagnosed eczema (aOR 2.10) and allergic sensitization for inhalant allergens (aOR 2.10). This was particularly clear in infants at high risk of developing allergic disease (Sausenthaler et al., 2006). In a Japanese study that included mother child pairs from the general population (N=1354), high maternal intake of n-3 LCPUFA EPA and DHA was associated with reduced risk of wheeze (adjusted risk ratio (aRR) of 0.70 in the upper quartile) but not eczema in offspring at age 2-2.5 years. The study group had an estimated mean daily intake of EPA and DHA of 170 mg and 290 mg respectively (Miyake et al., 2013). By contrast in a cohort in Singapore no association between dietary FA intake during pregnancy and offspring allergy risk at the age of 18 months was observed (Yu et al., 2015). In the above mentioned studies FA intake was calculated based on dietary questionnaires. In a Spanish study maternal and cord blood plasma FA of non-atopic women and their offspring were measured and linked to the prevalence of self-reported recurrent (atopic) eczema of the infant at 6 and 14 months of age. In this study the LCPUFA plasma content was negatively correlated with atopic eczema, this was much stronger for DHA or total n-3 LCPUFA levels in cord blood (aRR of 0.50). However lifestyle factors may have confounded these observations since for example the level of parental education was much higher in the butter group while avoidance of dairy products or smoking during pregnancy was much higher in the margarine group (Sausenthaler et al., 2006). Typically the ratio n-6/n-3 PUFA in maternal plasma was 12–13, while in cord blood plasma this was 6.5-7, indicating the preferential availability of n-3 over n-6 PUFA in the blood supply of the baby (Montes et al.,

2013). The authors conclude that cord blood plasma may provide a better reflection of the FA status of the baby than maternal plasma. The Dutch KOALA birth cohort study linked maternal (34-36 weeks of gestation) plasma FA status to allergic outcome in the offspring until 7 years of age. In this cohort a high n-6 versus n-3 LCPUFA ratio was associated with a lower risk of eczema (aOR 0.60 at a ratio >2.85), with AA being protective in the first 7 months. No further associations with sensitization or airway disease were observed (Notenboom et al., 2011). Cord blood LCPUFA and FA ratios have been shown to be influenced by the FADS genotype of both mother and child (Lattka et al., 2013). Therefore dietary FA can result in different membrane FA composition depending on individual FADS variation. Pooled plasma samples of children of the KOALA and LISA birth cohort study were analyzed for genetic variation in FADS at two years of age. There was a significant association between FADS variants and plasma PUFA levels except for ALA and EPA but no link with IgE (Rzehak et al., 2010). Also in another study by Standle et al. (LISAplus and GINIplus birth cohort) no association was found between FADS variants and allergic disease or sensitization at 10 years of age. However the n-3 PUFA over n-6 PUFA ratio was positively associated with an increased risk of hay fever in homozygous allele carriers of six FADS variants and margarine intake and asthma in homozygous major allele carriers (Standl et al., 2011). In the LISA birth cohort study the FADS variants were associated with eczema whereas the PUFA levels were not (Rzehak et al., 2010). Indeed FADS variation may have impact on the immune response of the infant. Muc et al. measured breast milk LCPUFA content at 4 weeks and AA was found to negatively correlate with IL-10, IL-17, IL-5 and IL-13, while EPA correlated positively with blood Treg and decreased T_H cell counts in PBMC of 6 months old babies (Muc et al., 2015). If in the latter study mothers had the minor allele of FADS single-nucleotide-polymorphism rs174556, the breastmilk AA and EPA levels were reduced and the children had higher IL-10, IL-17 and IL-5 production by PBMC (Muc et al., 2015). Another study (LISAplus and GINIplus birth cohort) showed that breastfeeding was protective for the development of asthma in heterozygous (aOR 0.37) and homozygous (aOR 0.47) carriers of the minor allele while there was no effect in homozygous major allele carriers (Standl et al., 2012). Beyond the FADS genotype also current allergy is linked with altered PUFA composition. In a recent Dutch study the mean n-6/n-3 PUFA ratio in maternal plasma was 6.06 and in this study maternal total PUFA and n-6 PUFA were associated with a decreased risk of developing childhood asthma at 6 years of age (OR 0.76 and 0.71 respectively), while there was an increased risk of eczema (OR 1.16 and 1.21)(Rucci et al., 2015). In another study the LCPUFA status or ratio of cord blood serum was not found to correlate with eczema at 2 years of age or asthma, hay fever or allergic rhinitis at 6 and 10 years of age. However, the n-3 LCPUFA serum content in children with eczema at 2 years of age was lower and the n-6/n-3 ratio was higher compared to controls. Furthermore, at 6 years of age children with asthma and aeroallergen sensitization had lower levels of serum n-3 LCPUFA than controls (Standl et al., 2014). So current allergy was associated with reduced n-3 LCPUFA levels. In the International Study of Asthma and Allergies in Childhood (ISAAC) study cohort of children aged 8-11 years a positive correlation for ALA or AA levels with asthma was found (fourth quartile OR 3.35 and 4.54 respectively), and high AA levels were associated with a reduced FEV_1. By contrast, LA was associated with reduced asthma risk (fourth quartile OR 0.34) and increased FEV_1 (Bolte et al., 2006). No protective effect of n-3 PUFA was observed but analysis of DHA was omitted in this study. In a UK cohort of 865 term born 6 year old children a skin prick test (SPT) and wheezing scores were correlated with fatty acids in the maternal plasma at 34 weeks of gestation (Pike et al., 2012). Although no clear associations were observed for transient and persistent wheeze in the general group, when data were split in a non-atopic group scoring negative in the SPT and an atopic group, maternal total n-3 PUFA, EPA and DHA all reduced the risk of having persistent wheeze (adjusted relative risk 0.57–0.74) in both groups, also a high n-3 over n-6 PUFA ratio was protective (0.73). However in the non-atopic group only, AA was protective (0.76), while ALA (1.46) increased the risk. LA levels were associated with reduced risk on skin sensitization (0.86) (Pike et al., 2012). Oddy et al. studied an Australian birth cohort of children of parents affected with atopic disease (Childhood Asthma Study cohort) and no associations were observed with FA present during lactation and the allergic outcome of the children at 6 months and 5 years of age. However, an increased ratio of n-6 over n-3 PUFA was associated with higher risk of non-atopic eczema at 6 months of age ($P<0.005$) (Oddy et al., 2006). In the ALSPAC study in the United Kingdom the cord blood red blood cell ratio of AA/EPA and LA/ALA were positively correlated with eczema and later-onset wheeze respectively and ALA/n-3 PUFA was negatively correlated with later-onset wheeze but these lost significance upon adjustment for multi-comparisons (Newson et al., 2004). Hence these observational studies show inconsistency in results and may have been biased by dietary, socioeconomic or lifestyle factors. From a number of studies it appears that a high dietary intake of EPA or DHA or low n-6 PUFA may protect from the development of allergic disease such as atopic dermatitis and asthma early in life. Variation in FADS genotype and current allergy are factors to take into account since these can interfere with the effect of dietary PUFA on FA composition.

4.2. N-3 LCPUFA status and effects on cord blood

Based on these observational studies it was suggested that the availability of n-3 LCPUFA during pregnancy may be associated with a reduced chance of developing allergic disease. In a US birth cohort cord blood plasma FA status was correlated with allergen specific proliferation and cytokine responses of the cord blood mononuclear cells (Gold et al., 2006). In this study EPA but not DHA levels were found to correlate negatively with proliferation upon exposure to cockroach, HDM and OVA and the same result was observed for AA regarding HDM. EPA and AA levels correlated negatively with allergen induced T_H1 type IFN-γ production. For DHA this was only the case for HDM but in this case the same tendency was observed for IL-13 (Gold et al., 2006). No association between EPA, AA and allergen induced IL-13 was observed, whereas levels of LA were positively correlated with allergen induced IL-13 (Gold et al., 2006). These data indicate that LA, AA and EPA may increase the T_H2 over T_H1 balance of cord blood mononuclear cells to environmental allergens while DHA does not. However, in particular EPA was capable of reducing proliferation induced by these environmental allergens. In Mexican women, supplemented with 400 mg algal DHA (N=131) or olive oil placebo (N=130) in the last 4-5 months of pregnancy, was found that DHA balanced out DNA methylation of the IL-13 and IFN-γ promotor sites in cord blood mononuclear cells. Low IL-13 gene methylation was compensated with low methylation of the IFN-γ locus (Lee et al., 2013). This indicates that n-3 LCPUFA like DHA in the maternal diet can have impact on the immunological outcome of the infant later in life via induction of epigenetic changes.

4.3. N-3 LCPUFA supplementation during gestation, lactation and/or early life and offspring allergy

In recent years the effects of n-3 LCPUFA in allergic disease have been studied using dietary intervention in human mainly during pregnancy and in early infancy (Table 1). Intervention as early as 20 weeks in gestation is thought to be most optimal since

Table 1
Double blind placebo controlled intervention studies on allergy prevention in early life.

Study group	Study population	Intervention period and dose	LCPUFA incorporation before-after supplementation	Clinical effect of n-3 PUFA in infants
		Pregnancy		
Dunstan et al. JACI 2003 Clin Exp All 2003	N=40 vs 43 (placebo) Atopic pregnant women Australian	Week 20- delivery Daily: 3.7g n-3 LCPUFA (fish oil) or olive oil	Cord blood red blood cells > EPA 0.37-1.33%, DHA 7.30-10.21% < AA 17.45-15.02%	Reduced plasma IL-13 At one year: = AD, food allergy, wheeze Reduced severity AD
Palmer et al. BMJ 2012 Allergy 2013	N=368 vs 338 (placebo) At risk Australian	Week 21- delivery Daily: 100 mg EPA, 800 mg DHA (fish oil) or rapeseed/sunflower/palm oil mix	Cord blood plasma > EPA 0.27-0.54%, DHA 6.2-7.5% < AA 16.4-14.6%	At one year: reduced egg sensitization At three years: = food allergy, AR, wheeze
		Pregnancy and lactation		
Furuhjelm et al. Acta Ped 2009 PAI 2011	N=52 vs 65 (placebo) Pregnant women with allergy in family Swedish	Week 25- 3-4 months breastfeeding Daily: 1.6 g EPA, 1.1 g DHA (fish oil) or soybean oil	Cord blood plasma > EPA 0.5-2.7%, DHA 8.3-10.1% < AA 16.0-12.6% (effect remains after one year but is lost at two years)	At one year: strong reduction in sensitization for egg, wheat, milk and more than 50% reduction in AD and food allergy At two years: effect remains but no prevention of novel cases of food allergy
CAPS team Mihrshahi et al. JACI 2003 Mihrshahi et al. PAI 2004 Peat et al. JACI 2004 Marks et al. JACI 2006 Almquist et al. JACI 2007	N=275 vs 279 (placebo) Pregnant women with asthma in family Australian	Week 36- and directly from 6 months of age (if not prior breastfed) or when starting bottle feeding Daily: 30 mg EPA, 128 mg DHA (tuna fish oil) or sunola oil and adapted cooking oil and fat spreads	18 Months infant plasma Total n-3 PUFA of total FA Lowest quartile 2.14-4.40% Highest quartile 7.29-11.71% Average 18 months-5 year in infant plasma > Total n-3 PUFA 4.99-6.15% < Total n-6 PUFA 35.26-33.15%	At 18 months: reduction in cough during sleep and bronchodilator use and 9.8% reduction in prevalence of any wheeze. =sensitization for egg, peanut and inhaled allergen At 3 years: 10% reduction in atopic cough At 5 years: no effect on wheeze, atopy, eczema, asthma
		After birth		
D'Vaz et al. Clin Exp All 2012 Pediatr 2012	N=218 vs 202 (placebo) Children from allergic pregnant women Australian	Birth- 6 months squired in mouth baby Daily: 110 mg EPA, 280 mg DHA (tuna fish oil) or olive oil	6 Months infant erythrocytes > EPA 0.76-0.92%, DHA 6.22-6.82% < AA 14.6-13.6% 6 Months infant plasma > EPA 0.88-1.23%, DHA 4.53-5.29% < AA 11.0-10.6%	At one year: = AD, food allergy, wheeze In a subgroup of 150 infants: Reduced HDM specific IL-13 upon fish oil supplementation, also when comparing group with high DHA versus low DHA incorporation (*ex vivo*).

Abbreviations: AD, atopic dermatitis; AR allergic rhinitis; HDM, house dust mite.

this is prior to establishment of detectable T-cell responses to possible allergens in the infant (Dunstan et al., 2003a, 2003b). Dietary intervention studies indeed reveal allergy protective effects, but the study designs and effects reported differ considerable. Dunstan et al. provided 40 pregnant atopic women with 3.7 g n-3 LCPUFA (fish oil) from week 20 of gestation to delivery; the control group received olive oil. Neonatal cord blood analyses of erythrocyte membranes revealed a considerable increase in EPA and DHA incorporation (see Table 1), while AA reduced (Dunstan et al., 2003b). The supplemented group had lower levels of T_H2 associated plasma IL-13 which was inversely correlated with incorporated DHA levels. Allergen specific cytokine responses of neonatal cord blood mononuclear cells (IL-13, IFN-γ and IL-10) towards OVA (allergen in hen's egg) and cat hair extract tended to be lower in the supplemented group and at one year of age the risk for allergic sensitization for egg tended to be three times lower ($p=0.055$). In general no effect was observed on the occurrence of food allergy, atopic dermatitis or asthma or related symptoms, but since the study was not designed to assess clinical effects it may have been underpowered to do so. Typically the number of cases with severe atopic dermatitis was significantly lower in the supplemented group (Dunstan et al., 2003a). Another study performed by Palmer et al., provided 100 mg EPA and 800 mg DHA (fish oil) daily from week 21 of gestation to birth in 368 women bearing a child with a family history of allergic disease, the control group obtained a mixture of rapeseed, sunflower and palm oil. At one year of age the children in the fish oil group tended to be protected for atopic eczema (aOR 0.64, p=0.06) and were protected against egg but not peanut sensitization (aOR 0.62, p=0.02) nor food allergy triggered by egg (Palmer et al., 2012). At three years of age the protective effect on eczema with sensitization showed the same tendency (aRR 0.75, p=0.10), but in general no effects were found for food protein sensitization, aeroallergens nor HDM or cat allergens and food allergy, asthma or allergic rhinitis (Palmer et al., 2013). In a Swedish study by Furuhjelm et al., an at risk population of 52 women was daily supplemented with fish oil containing 1.6 g EPA and 1.1 g DHA from week 25 of gestation and also during 3-4 months of breastfeeding, the control group received soybean oil containing 2.5 g LA and 0.28 g ALA. During the first year 30% of the children in the placebo group had a positive skin prick test for egg, milk or wheat while this was 15% in the fish oil group in which egg sensitization was particularly suppressed having an aOR of 0.31 (Furuhjelm et al., 2009). Also the cumulative incidence of IgE associated eczema (from more than 20% to below 10%) and food allergy (from 15% to 2%) was significantly reduced in fish oil group (aOR 0.09). After two years the cumulative incidence for a positive skin prick for egg and IgE mediated food allergy and eczema were still significantly reduced by over 50% although food allergen specific IgE levels remained unaffected and no differences between the placebo and fish oil group were found in the point prevalence of all allergy indicators at 24 months (Furuhjelm et al., 2011b). Similar to the studies of Dunstan et al. (2003b), neonatal cord blood analyses of erythrocyte membranes revealed effective EPA and DHA incorporation, at the expense of AA. At three and 12 months of age the children still had increased levels of EPA and DHA and a reduced AA/EPA ratio however this was lost at 24 months of age (Furuhjelm et al., 2011b). Typically higher plasma EPA or DHA levels or a lower AA/EPA ratio of the mother or the child at 3 months of age, and higher DHA levels in the infant at 12 and 24 months of age significantly protected against development of multiple allergic symptoms (Furuhjelm et al., 2009). This indicates that maternal fish oil supplementation during pregnancy and lactation can be beneficial for infants in the first year of life. The prolonged detection of high plasma levels of CCL17 was observed in allergic versus healthy children and may indicate hampered immune maturation and consequently a prolonged T_H2 dominated immune response (Furuhjelm et al., 2011a). In the study of Furuhjelm et al., fish oil supplementation was found to enhance immune maturation since the CCL17/CCL11 ratio (CCL11 being indicative for a T_H1 prone immune response) was significantly lower in the infants of the fish oil group compared to the control group at 2 and 12 months. However this was only the case in the offspring of non-allergic mothers. In addition, only in these children the CCL17/CCL11 ratio at 12 months was inversely correlated with maternal plasma EPA and DHA levels (Furuhjelm et al., 2011a). Hence these data indicate that the maternal allergy status may interfere with the immunological effect of maternal fish oil supplementation on the offspring (Furuhjelm et al., 2011a). Beyond affecting plasma chemokine levels, supplementation of fish oil during pregnancy has been shown to alter several other immune markers. Like mentioned before in the study of Dunstan et al. allergen specific cytokine responses of in neonatal cord blood mononuclear cells such as IL-13 tended to be lower in the fish oil group (Dunstan et al., 2003b). Also in a study by Krauss-Etschmann et al. daily intake of 0.5 g DHA and 0.15 g EPA (fish oil) from week 22 of gestation was found to lower T_H2 associated IL-13 and also IL-4 and CCR4 (mRNA) in cord blood and IL-1 and IFNγ in maternal blood while enhancing mRNA levels of regulatory TGFβ in maternal and cord blood (Krauss-Etschmann et al., 2008). Lauritzen et al. supplemented mothers during lactation with 4.5 g fish oil for a period of three months and determined effects on the immune system of the infants at 2.5 years. LPS stimulated whole blood samples of the children showed increased IFN-γ responses in the fish oil supplemented group (Lauritzen et al., 2005). In the Childhood asthma prevention study (CAPS) 616 pregnant women were included at 36 weeks of gestation and the unborn child was at risk for developing asthma since family members were affected with asthma (Almqvist et al., 2007; Mihrshahi et al., 2004). The women were given 500 mg tuna oil daily and adapted fat spreads and cooking oils, controls were provided Sunola oil. At 18 months after birth the n-3 PUFA plasma levels were positively related with a reduced percentage of neonates affected with cough during sleep and reduced bronchodilator use ($P<0.001$), but no effects were found on atopy (serum IgE or SPT) or eczema (Mihrshahi et al., 2003; Mihrshahi et al., 2004). Also at 18 months of age the prevalence of wheeze was reduced with almost 10% and at three years of age a 10% reduction in the prevalence of cough in atopic children in the supplemented group was observed although no effects on sensitization, wheeze, eczema or asthma were found (Mihrshahi et al., 2003; Peat et al., 2004). At five years of age no effect of the interventions on atopic disease were observed despite a limited increase in plasma n-3 PUFA and lowering of n-6 PUFA in the supplemented group at all age groups (Almqvist et al., 2007; Marks et al., 2006). The difference between the study by Furuhjelm et al. and the CAPS study may relate to the timing (from wk21 of gestation and lactation versus from wk34 of gestation and lactation) and the daily LCPUFA dosage that was given to the mothers (2.7 g vs 158 mg LCPUFA). Hence in the CAPS study the intervention may have started too late in gestation with a too low a dose of fish oil in order to suppress allergy, although it did reduce the prevalence of wheeze and atopic cough at 18 months and 3 years of age. Also compared to Dunstan et al. (2003b), the study results of Furuhjelm et al. showed more prominent effects. The dosage given in the study by Dunstan et al. was higher than that of Furuhjelm et al. but dosing was not continued during breastfeeding, while Furuhjelm et al. did. This may in part explain the difference in effect size between the studies. However, in the study by Dunstan et al. atopic mothers were included while Furuhjelm et al. included subjects with a family history of allergy meaning that the allergy could also be present in the birth father or a child previously born from this mother. The authors state that children from atopic mothers may benefit less from the protective effect of

fish oil on allergic sensitization than those of non-allergic mothers (Furuhjelm et al., 2009). In another study cohort 131 healthy women were supplemented with 2.7 g fish oil from week 30 in gestation until delivery and 136 women received olive oil as a placebo control. In this cohort the risk of developing allergic asthma in the offspring at 16 years of age was reduced by 87%. However, it appeared that the fish oil group and non-supplemented group had a similar cumulative incidence of allergic asthma, rhinitis and atopic dermatitis while the olive oil control group appeared to have an increased risk, questioning the validity of olive oil as a control (Olsen et al., 2008). Hence intervention during pregnancy and lactation provides promising results and depending on the study design and fish oil dose may have future implications for allergy prevention. However more and larger intervention studies are needed to substantiate this finding. In addition to these studies also supplementation after birth has been studied. In a study by D'Vaz et al. infants at high risk for developing allergic disease were daily supplemented with 280 mg DHA and 110 mg EPA (fish oil) or olive oil as a control from birth up until 6 months of age. The oils were squeezed into the infant's mouth prior to feeding. The supplementation increased DHA and EPA while reducing AA by 1-2% in the erythrocyte membranes but since these changes were fairly limited the compliance was challenged. In this study no effect of n-3 PUFA was observed on eczema, FA, persistent cough and wheeze, but children with recurrent wheeze did have lower plasma DHA (4%) or n-3 LCPUFA (6%) than children without recurrent wheeze (5% and 7% respectively) at 1 year of age (D'Vaz et al., 2012b). Although no clear effects were shown in this study, fish oil supplementation did reduce HDM induced IL-13 and high plasma DHA levels were accompanied by reduced beta-lactoglobulin induced IL-13 and IL-5 secretion by PBMC (Schubert et al., 2009). Furthermore fish oil supplementation increased IFN-γ and TNF-α production by PBMC in response to general mitogen PHA (D'Vaz et al., 2012a). Hence from these intervention studies it becomes clear that optimal timing, duration and a minimal intake dosage of n-3 LCPUFA may be required to exert a robust allergy protective effect and an atopic condition of the mother may reduce the efficacy of n-3 LCPUFA. Supplementation during early pregnancy and lactation has shown promising results regarding allergy prevention but also supplementation after birth could reduce allergy biomarkers. Therefore continued supplementation to the child after weaning could be considered to maintain the increased n-3 LCPUFA membrane content and the possible beneficial effect that is being generated during the first year for a prolonged period.

4.4. Dietary PUFA intake and asthma in adults

Dietary fatty acid and antioxidant intake has been studied for its relation with asthma development (Barros et al., 2015; Shaheen et al., 2001; Woods et al., 2004). In the German EPIC prospective multicenter study comparing diets of adult asthma patients (N=105) with controls (N=420), high margarine and oleic acid intake was associated with asthma development (3rd tertile OR 1.73; for men this was 2.51 and for women 1.47). No association for dietary intake of oxidants nor n-6 or n-3 PUFA was observed in this study. In a study by Kitz et al., PUFA erythrocyte membrane contents of 38 grass pollen allergic asthma patients and 19 controls were compared and the n-6/n-3 PUFA ratio appeared higher in the asthmatics (Kitz et al., 2010). This was also the case in a study by Barros et al. in which dietary intake of 174 adults treated for asthma in relation to asthma symptom control was examined. N=40 controlled asthma versus N=134 non controlled asthma patients were characterized based on use of inhaled corticosteroids, exhaled nitric oxide (eNO) and FEV_1. A high n-6/n-3 PUFA ratio was associated with poor asthma control and the ALA, EPA and DHA intake was higher in the controlled group while inhaled corticosteroid use was lower (Barros et al., 2011). In this study increased intake of n-3 PUFA (OR 0.14) and in particular ALA (OR 0.18) but also SFA (OR 0.36) were found to associate positively with asthma control. No association was observed for antioxidant intake except for a midrange of retinoic acid (OR 0.36) (Barros et al., 2011). In a study cohort of 1601 young Australian adults with and without asthma, plasma n-3 PUFA levels were not associated with asthma while n-6 LCPUFA DGLA was (OR 1.25). Furthermore, plasma DGLA, total n-6 and the n-6/n-3 ratio correlated positively with bronchial reactivity. By contrast, for DHA and total n-3 PUFA this correlation was negative and total n-3 PUFA was also negatively correlated with the number of recent asthma attacks (Woods et al., 2004). Hence, also in adult asthmatics increased levels of n-3 PUFA do appear to reduce markers for asthma severity. From these observational studies it can be concluded that high dietary intake of n-6 PUFA over n-3 PUFA is associated with an increased risk of being affected with asthma while n-3 PUFA may be protective and may reduce symptoms.

4.5. n-3 LCPUFA supplementation and asthma treatment

In recent years a number of intervention studies in asthmatic patients have been performed with different success (Table 2). Nagakura et al. provided children treated for asthma with 6-12 capsules fish oil (84 mg EPA and 36 mg DHA per capsule) or olive oil a day depending on their body weight, for a period of 10 months (Nagakura et al., 2000). This resulted in a significant increase in plasma EPA concentrations and from months 6 onwards a reduction in asthma scores and a reduced sensitivity for acetylcholine induced drop in FEV_1. Implicating that fish oil supplementation had suppressed airway inflammation and improved airway function. However these patients were for 85% of time hospitalized and therefore their food intake and exposure to inhalant allergens was controlled. Medication at that time did not contain inhaled steroids (Nagakura et al., 2000). In a study by Thien et al. no effect of fish oil supplementation (3.2 g EPA and 2.2 g DHA daily from April to September) on bronchial hyper reactivity during the pollen season was observed, while the supplementation did effectively increase neutrophil EPA and decrease AA content (Thien et al., 1993). In this study no further markers of airway inflammation were studied. Hodge et al. supplemented children affected with asthma for 6 months with fish oil containing a daily dosage of 1.2 g n-3 LCPUFA (EPA: DHA, 3:2). Although plasma EPA levels significantly increased, clinical symptoms did not reduce in this study. However blood eosinophil counts and plasma TNFα tended to decrease (Hodge et al., 1998). Arm et al. supplemented nine atopic asthma patients for 10 weeks with 3.2 g EPA and 2.2 g DHA and allergen induced airway responsiveness was compared to placebo (olive oil) controls. Although acute allergic symptoms and histamine release were not suppressed, the fish oil supplemented group had reduced late asthmatic symptoms (improved airway conductance, but no improved peak expiratory flow or bronchodilator use) which was associated with reduced *ex vivo* chemotaxis and LBT_4 release by activated neutrophils from the blood of these patients (Arm et al., 1989). In a more recent study, HDM asthmatic adults were provided with a supplement containing amongst others 630 mg n-3 LCPUFA (450 mg EPA and 180 mg DHA) for 5 weeks. Also in this study no effects on FEV_1 decline were observed, however allergic airway inflammation upon low dose allergen exposure in asthmatics was ameliorated since eNO and HDM induced inflammatory eicosanoid cysLT release were reduced in the n-3 PUFA group compared to placebo controls and eosinophil numbers were reduced in the blood (Schubert et al., 2009). Fish oil supplementation can alter lipid metabolite formation. PUFA are

Table 2
Double blind placebo controlled intervention studies on asthma treatment.

Study group	Study population	Intervention period and dose	LCPUFA incorporation before-after supplementation	Clinical effect of n-3 PUFA on asthma symptoms
Arm et al. Am Rev Res Dis 1989	N=19 vs 20 (placebo) Atopic asthmatics (18-42 years) United Kingdom	10 Weeks Daily: 3.2g EPA and 2.2g DHA (Max-EPA fish oil) or olive oil	Neutrophil > EPA 0.4-3.9%, DHA 2.5-3.7% < AA 19.0-13.5%	= Challenge or histamine induced acute asthma reduced late phase response but no reduction in FEV_1 or medication
Hodge et al. Eur Resp J 1998	N=19 vs 20 (placebo) Children with atopic asthma (8-12 years) Australia	6 Months Daily: 0.72g EPA and 0.48g DHA (Max-EPA fish oil) plus canola oil margarine or sunflower oil plus margarine	Plasma at three months delta n-3 PUFA +3.18% delta n-6 PUFA -0.21% delta EPA +1.98%	= Histamine induced FEV_1 and FVC Tendency to decreased blood eosinophil number and plasma TNFα
Nagakura et al. Eur Resp J 2000	N=15 vs 14 (placebo) Children with bronchial asthma (4-17 years) Japan	10 Months Daily EPA 17.0-26.8 mg/kg BW, DHA 7.3-11.5 mg/kg BW	Plasma at 10 months EPA 24.19-55.76 μg/mL	Increased provocation dose of methacholine to induce 20% drop in FEV_1 from 6 months onwards
Biltagi et al. Acta Ped 2009	N= 60 (self controlled) Children with moderate asthma (7-10 years) Egypt	6 Weeks per period, two weeks rest, randomized Daily: 1g triglyceride containing EPA/DHA (2:1) and/or vitamin C or Zn or lactose as a placebo	ND	All interventions including EPA/DHA improved FEV_1 and FEV_1/FVC ratio, reduced induced sputum white blood cells and eosinophils, however the latter was also reduced in the placebo group.
Schubert et al. IAAI 2009	N=12 vs 11 (placebo) Atopic asthmatics (22-29 years) Germany	5 Weeks 0.69 g/day PUFA enriched fat blend with 450 mg EPA, 180 mg DHA) or control fat blend	ND	= Challenge induced FEV_1 reduced eNO at baseline and after challenge and sputum eosinophils reduced serum eosinophils and cysLT
Thien. Am Rev Res Dis 1993	N=12 vs 9 (placebo) Seasonal hay fever and asthma for pollen asthmatics (19-42 years) United Kingdom	6 Months Daily: 3.2g EPA and 2.2g DHA (Max-EPA fish oil) or olive oil	Neutrophil (post season) > EPA 0.13-2.02% < AA 11.16-8.83% Plasma (post season) > EPA 0.76-5.43% < AA 5.21-4.82%	= Histamine induced symptoms = PEF, wheeze and medication during pollen season
Mickleborough. Chest 2006	N=8 vs 8 (placebo) Exercise induced bronchoconstriction in asthmatics (mean 23 years) United States of America	3 Weeks Daily: 3.2g EPA and 2.0g DHA (fish oil) or olive oil	Neutrophil > EPA 0.16-4.01%, DHA 2.24-3.32% < AA 23.9-13.1%	Improved exercise induced drop in FEV_1 and FVC, reduced use of bronchodilator, reduced macrophages and reduced IL1β, TNFα, PGD_2, LTC_4-LTE_4 in induced sputum before and after exercise, reduced LTB_4 and increased LTB_5 in activated polymorphonuclear leukocytes

Abbreviations: cysLT, cysteinyl leukotriene; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; ND, not determined; PEF, peak expiratory flow.

converted via cyclooxygenase, lipoxygenase and/or cytochrome P450 enzymes and AA is a precursor for pro-inflammatory cysLT such as LTB_4, LTC_4 and LTE_4, bronchoconstrictive thromboxanes such as TXA_2 and prostaglandins such as PGD_2. EPA and DHA compete with AA for membrane incorporation and thus reduce the generation of pro-inflammatory LT (4-series) and prostanoids (2 series). By contrast EPA and/or DHA are precursors of less inflammatory LT (5-series) and anti-inflammatory and pro-resolving resolvins and maresins (Giudetti and Cagnazzo 2012). In a randomized double blind cross over study 16 patients with exercise-induced bronchoconstriction (often causing symptoms in asthmatics) were supplemented daily for 3 weeks with fish oil containing 3.2 g EPA and 2 g DHA or placebo. Already before exercise and also following exercise, a strong reduction in percentage eosinophils, neutrophils, lymphocytes was measured in the induced sputum, while the percentage of macrophages had increased and LCT_4, PGD_2, TNFα and IL-1β were decreased in the sputum supernatant. In addition, activated polymorphonuclear leukocytes contained increased EPA and DHA and reduced AA and produced lower amounts of LCT_4 and increased LTB_5 in the fish oil supplemented group. Furthermore, the percentage of FEV_1 decline upon exercise did not exceed 10% in the fish oil group while it reached 25% in the control groups (Mickleborough et al., 2006). Similar findings were shown using marine lipid fraction of the New Zealand green-lipped mussel (Perna canaliculus) PCSO-524 (Lyprinol/Omega XL) in hyperpnoea-induced bronchoconstriction in asthma (Mickleborough et al., 2013). Biltagi et al. studied supplementation of asthmatic hospitalized Egyptian children with ~330 mg EPA and DHA containing triglyceride oil or zinc or vitamin C or a combination of these for a consecutive period of 6 weeks and two weeks washout (randomized including a lactose control group). All interventions improved the subjective asthma scores and objective FEV_1 measures and the percentage eosinophils in induced sputum and LTC_4-E_4 and PGD_2 in sputum although the placebo group also had a reduced percentage of eosinophils (Biltagi et al., 2009). Antioxidants like Zn and vitamin C may also improve asthma and may add to the effectiveness of n-3 LCPUFA. Broughton et al. showed the importance of the formation of n-3 LCPUFA derived lipid metabolites in the protective effect of fish oil supplementation. In this study n=19 adult asthmatics were provided fish oil with an average of 3.3 g EPA and DHA daily, based on dietary analysis to acquire a n-3 over n-6 ratio of 0.5:1, for a period of 4 weeks (Broughton et al., 1997). Leukotrienes were measured in the urine as a measure of compliance. Subjects having enhanced levels of 5-series leukotrienes when supplemented fish oil (responders, N=9) were protected against methacholine induced drop in FEV_1 and several other parameters of airway function, whilst the non-responders were not (Broughton et al., 1997). Authors state that the obtained ratio of n-3 over n-6 PUFA may be critical for the necessary shift in leukotrienes production from the 4-series to the 5-series and when the latter ratio is more than one it may be protective against allergic airway dysfunction (Broughton et al., 1997). From these studies it can be concluded that except for exercise induced asthma, n-3 LCPUFA supplementation does not reduce challenge induced disturbances in lung function in asthma patients, however supplementation can suppress airway inflammation. A couple of studies have shown that n-3 LCPUFA supplementation can reduce inflammatory markers like eNO, plasma eosinophil numbers and TNFα and provide a beneficial pattern of lipid metabolites by lowering PGD_2 and cystLT (LTB_4, LTC_4, LTE_4) and enhancing LTB_5. Individual differences in capacity to produce 5-series leukotrienes from EPA or DHA was associated with a protective effect in human asthmatics and may therefore be a factor contributing to the success of the intervention.

5. Current understanding and future perspective

From these observational and intervention studies it becomes clear that high intake of n-3 LCPUFA and low intake of n-6 PUFA may reduce the allergy risk. Incorporation of n-3 LCPUFA at the expense of n-6 PUFA in immune cells may modulate immune polarization and the effector response. In mice, n-3 LCPUFA (fish oil) was found to compromise T-cell receptor signaling and reduce the T_H2 and T_H1 response while enhancing Treg frequency and reducing IgE. Also in human cells suppression of dendritic cell and T-cell activation was observed. In clinical studies supplementation with n-3 LC-PUFA has been shown to reduce sensitization or suppress allergy in children when given to mothers early in pregnancy and during lactation. Dosing and timing of the intervention may be of crucial importance in this respect. With regard to primary prevention of allergy, n-3 LCPUFA should be started early in life (week 20 of gestation) before the establishment of a T-cell response towards potential allergens in the infant. The study in which supplementation during pregnancy was continued during lactation showed strong allergy prevention in the offspring. This study needs confirmation taking into consideration that atopic constitution, the quantity and quality (high DHA over EPA may be preferable) of the marine oil, the allergen and gender could modify the effect size. Over time when n-3 LC-PUFA supplementation is abandoned, EPA and DHA membrane levels drop and the risk of developing allergies is re-established. In infants at risk that respond well to PUFA supplementation it may therefore be considered to prolong the period of supplementation in order to have continuation of its protective action. Also incorporation of PUFA in membranes and conversion of PUFA into 3 or 5 series of eicosanoids and protectins, resolvins and/or maresin is of importance (Lundstrom et al., 2013). The genetic constitution of the recipient in this regard may also be a determinant of whether n-3 LC-PUFA can suppress the risk on allergy. Furthermore other dietary components may add to the effect of n-3 LCPUFA in allergy prevention. For example retinoic acid, also known for its capacity to facilitate tolerogenic immune responses, may be such an agent. Furthermore, low levels of vitamin E, Zn and selenium or low/high levels of vitamin D or folic acid in pregnancy have been associated with increased risk of allergy development (De Giuseppe et al., 2014; Miles and Calder, 2015). In addition, vitamin B_6 levels may affect LCPUFA availability since low vitamin B_6 was associated with reduced plasma levels of AA, EPA and DHA (Zhao et al., 2012). Allergy prevention early in life or immunotherapy can be facilitated with adjusted allergens or allergen derived peptides aiming to induce immune tolerance against certain important allergens (Kostadinova et al., 2013). Combining these adjusted allergens or peptides with a dietary adjunct therapy including n-3 LCPUFA may help to improve immune maturation and further enhance tolerance induction in a preventive or therapeutic setting (Hayen et al., 2014). In mice n-3 LCPUFA improved airway function and suppressed airway inflammation and eosinophilia alike EPA and DHA derived resolvin E_1 and protectin D_1. N-3 LC-PUFA may also be beneficial in suppressing airway inflammation in human asthmatics. Although n-3 LCPUFA supplementation did not effectively suppress loss of lung function in asthma patients it did reduce markers for lung inflammation. Asthma patients are mostly treated with beta-agonists to improve lung function and corticosteroids to suppress airway inflammation. Since n-3 LCPUFA supplementation can reduce airway inflammation it may be capable of supporting corticosteroid treatment and allow a reduced medication dose. This food-pharma approach has not been performed yet but may be an interesting topic for future studies in asthma patients. Hence future studies may reveal whether dietary n-3 LCPUFA can support allergen specific oral tolerance induction, improve allergy prevention and contribute to the efficacy of drug therapy in asthma patients.

Conflict of interest

LW is employed at the Utrecht University and collaborates with Danone/Nutricia Research B. V. within a strategic alliance between the Utrecht Institute for Pharmaceutical Sciences of the Utrecht University and Danone/Nutricia Research B. V, Utrecht, The Netherlands.

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