# Effects of n-3 LCPUFA supplementation for pregnant and lactating women in preventing allergic diseases in early childhood

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Arachidonic acid |
| AAAAI | American Academy of Allergy Asthma and Immunology |
| ALA | α-Linoleic acid |
| APC | Antigen presenting cells |
| ASCIA | Australian Society of Clinical Immunology and Allergy |
| AUD | Australian dollars |
| BMI | Body mass index |
| CA | Corrected age |
| CD4+ | Cluster of differentiation 4 cells |
| CI | Confidential intervals |
| CRF | Case report form |
| DHA | Docosahexaenoic acid |
| DINO | DHA for the Improvement of Neurodevelopmental Outcomes in Preterm Infants |
| DMAC | Data Management and Analysis Centre |
| DOMInO | DHA to Optimise Mother Infant Outcome |
| DPA | Docosapentaenoic acid |
| EDD | Expecting Date of Delivery |
| EFSA | The European Food Safety Authority |
| EPA | Ecosapentaenoic acid |
| FMC | Flinders Medical Centre |
| GA | Gestational age |
| GA2LEN | Global Allergy and Asthma European Network |
| GEE | Generalised estimating equation |
| IFN-γ | Interferon gamma |

| | |
|---|---|
| IgE | Immunoglobulin E |
| IgG | Immunoglobulin G |
| IL-2 | Interleukin 2 |
| IL-4 | Interleukin 4 |
| IL-5 | Interleukin 5 |
| IL-10 | Interleukin 10 |
| IL-13 | Interleukin 13 |
| IL-17 | Interleukin 17 |
| ISAAC | International Study of Asthma and Allergies in Childhood |
| ITT | Intention to treat |
| LA | Linoleic acid |
| LCPUFA | Long chain poly unsaturated fatty acids |
| NF-κB | Nuclear Factor kappa B cells |
| NPV | Negative predictive value |
| OR | Odds ratio |
| PGE2 | Prostaglandin E2 |
| PPAR- ɤ | Peroxisome Proliferator Activated Receptors |
| PPV | Positive predictive value |
| PUFA | Polyunsaturated fatty acids |
| RAST | Radioallergosorbent test |
| RR | Risk ratio |
| SAP | Statistical analysis plan |
| SOP | Standard operation procedures |
| SPT | Skin prick test |
| Th1 | Type 1 T helper cells |

| | |
|---|---|
| Th2 | Type 2 T helper cells |
| Th17 | Type 17 T helper cells |
| Treg | Regulatory T cells |
| TNF | Tumor Necrosis Factor |
| TGF | Transforming Growth Factor |
| TGF-β | Transforming Growth Factor Beta |
| USD | American dollars |
| UK | United Kingdom |
| WAO | World Allergy Organization |
| WCH | Women's and Children's Hospital |
| WHO | World Health Organization |

# DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

A combination of Chapter 2 and 6 of this thesis (systematic review and meta-analysis) has been published in The Cochrane Library, Issue 7, 2015 and the protocol of the systematic review has been published in The Cochrane Library, Issue 9, 2012. I am responsible for conceiving, designing, developing, co-ordinating and writing the review, under the guidance of my supervisors Professor Maria Makrides and Dr Carmel T Collins.

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Anoja Wickrama Gunaratne

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# SUMMARY

It is postulated that maternal n-3 (omega 3) long chain polyunsaturated fatty acids (LCPUFA) supplementation may modulate a range of inflammatory and immune pathways involved in the development of allergic diseases in early childhood, potentially leading to a reduction of allergic diseases in children. Thus the focus of this thesis was to determine whether maternal n-3 LCPUFA supplementation during pregnancy or lactation could prevent allergies in children.

Two nested follow-up studies from two randomised controlled trials (RCTs) were performed, as well as a Cochrane systematic review to address this question. Of the two nested follow-up studies, one was a prenatal n-3 LCPUFA supplementation and the other a postnatal n-3 LCPUFA supplementation study. Parental reports of allergy outcomes were evaluated in children between birth to three years of age and birth to seven years of age in these studies. The Cochrane systematic review and meta-analysis was used to determine overall effects of maternal n-3 LCPUFA supplementation on allergy outcomes of the children involved. All relevant RCTs to date and the data from my two follow-up studies were included in the systematic review. Eight trials involving 3366 women and their 3175 children were included and in these trials, women were supplemented with n-3 LCPUFA during pregnancy (five trials), lactation (two trials) or both pregnancy and lactation (one trial). All trials randomly allocated women to either a n-3 LCPUFA supplement or a control group. The risk of bias varied across the eight included trials in this review with only two trials with a low risk of selection, performance and attrition bias. Overall, there is limited evidence to support maternal n-3 LCPUFA supplementation during pregnancy and/or lactation for reducing allergic disease in children. Few differences in childhood allergic disease were seen between women who were supplemented with n-3 LCPUFA and those who were not.

N-3 LCPUFA supplementation showed a clear reduction in the primary outcome of any allergy (medically diagnosed IgE mediated) in children aged 12 to 36 months (risk ratio (RR) 0.66,

95% confidence interval (CI) 0.44 to 0.98; two RCTs; 823 children), but not beyond 36 months (RR 0.86, 95% CI 0.61 to 1.20; one RCT, 706 children). For any allergy (medically diagnosed IgE mediated and/or parental report), no clear differences were seen in children either at 12 to 36 months (RR 0.89, 95% CI 0.71 to 1.11; two RCTs, 823 children) or beyond 36 months of age (RR 0.96, 95% CI 0.84 to 1.09; three RCTs, 1765 children).

For the secondary outcomes of specific allergies there were no clear differences for food allergies at 12 to 36 months and beyond 36 months, but a clear reduction was seen for children in their first 12 months with n-3 LCPUFA (both for medically diagnosed IgE mediated and medically diagnosed IgE mediated and/or parental report). There was a clear reduction in medically diagnosed IgE mediated eczema with n-3 LCPUFA for children 12 to 36 months of age, but not at any other time point for both medically diagnosed IgE mediated and medically diagnosed IgE mediated and/or parental report. No clear differences for allergic rhinitis or asthma/wheeze were seen at any time point for both medically diagnosed IgE mediated, and medically diagnosed IgE mediated and/or parental report. There was a clear reduction in children's sensitisation to egg and sensitisation to at least one allergen between 12 to 36 months of age when mothers were supplemented with n-3 LCPUFA. In terms of safety for the mother and child, n-3 LCPUFA supplementation during pregnancy did not show increased risk of postpartum haemorrhage or early childhood infections.

The data obtained in one of the nested follow-up studies in this thesis was used to compare the validity of parental reports of allergy outcome measures against medical diagnosis of allergies. This revealed that parental reports of doctor diagnosed eczema were the most reliable for the diagnosis of eczema in infants, but further studies are needed to validate other allergy outcomes before parent reports of allergy symptoms can be considered as a useful tool to evaluate early childhood allergies in large scale research.

# Chapter 1: Introduction

## 1.1 Overview

Allergy is a chronic disease with an immunological reaction to certain extrinsic substances (allergens) which has a significant impact on patients and their families [1]. When allergens enter the human body, they may trigger mild to severe reactions in prone individuals and, in rare cases, the severe life threatening anaphylaxis can occur [1]. Allergic diseases including food allergy, eczema, allergic rhinitis and asthma also have social impacts including increased health care costs, reduced productivity, the need for special care at school, and general poorer school attendance of affected children [2, 3]. Over the past few decades the prevalence of allergies has increased in Australia and other industrialised countries, from approximately 4% of the population to the current level of 20% [4, 5]. Australians have the highest global rate of allergic diseases among other developed countries with 19.6% of the population of Australians (4.1 million), having at least one allergy [6, 7]. If the current trend continues, 26.1% of Australians (7.7 million) will be affected with allergies by 2050 [7]. The total estimated annual cost of treating allergies is approximately AUD $9.4 billion in Australia and USD $20 billion in the United States of America [8]. Thus allergy is an important public health problem, placing a burden on individuals, society and the health care system [2, 3]. Consequently allergy prevention presents a major national and global challenge, with the World Allergy Organization (WAO) stating that allergy preventative strategies are urgently needed [9, 10].

While allergy predisposition has a genetic component, other factors such as socioeconomic conditions, hygiene standards, bacterial/viral infections, pollution and dietary changes also appear to have an influence on the increasing incidence of allergies [11, 12] (see section 1.2.3 below). Among the environmental factors, changes in the ratio of n-3 polyunsaturated fatty acids (PUFA) to n-6 PUFA in the Western diet may be a factor [13, 14]. The ratio of n-3

PUFA to n-6 PUFA consumption is now estimated as 1:20–30, compared with 1:1–2 for a traditional hunter and gatherer diet [15]. The timing of the substantial shift in dietary fatty acids, to favour n-6 over n-3 fatty acids, has coincided with the increased prevalence of childhood allergic disease and led to the suggestion that the two may be linked [13, 14]. The major source of n-3 long chain polyunsaturated fatty acids (LCPUFA) is in fish, especially oily fish (e.g. salmon, tuna, sardines and mackerel) and fish oil supplements and the major source of n-6 LCPUFA is meats, eggs and offal [16-18]. The current Western diet contains little fish and precautionary public health advice regarding the consumption of methyl mercury containing fish during pregnancy means that pregnant women tend to consume even less fish than other adults [19, 20].

When diets are high in n-3 LCPUFA, altered immunological effects such as $PGE_2$ synthesis and pro-inflammatory cytokine responses have been reported [21]. Thus, there are plausible mechanisms by which diets high in n-3 LCPUFA may modulate the development of allergic disease and regulate immune responses. There is also speculation that n-3 LCPUFA supplementation during pregnancy and/or lactation may benefit the developing immune system and reduce the development of allergies in the offspring [22-25]. In order to determine if there is sufficient evidence to support this hypothesis [22-25], I first conducted a systematic review and meta-analysis of randomised controlled trials (RCTs) of n-3 LCPUFA supplementation during pregnancy and/or lactation on allergy development in children, which is presented in Chapter 2. The results of the review revealed a need for larger studies with longer follow-up considering maternal populations with high or normal risk of allergy, supplementation during different 'windows of opportunity' and follow-up allergy assessment on children of different ages. Therefore, I evaluated allergy outcomes in children of different ages who had participated in two large, well-designed RCTs of n-3 LCPUFA supplementation: the DINO (DHA for the Improvement of Neurodevelopmental Outcomes in

Preterm Infants) trial [26] and the DOMInO (DHA to Optimise Mother Infant Outcome) trial [27] and the results are presented in Chapters 3 and 5. In addition, the initial systematic review highlighted that different studies used a range of allergy diagnosis methods which may have contributed to the heterogeneity in results. Therefore, I evaluated how well parental reports of allergy outcomes compared with medical diagnoses and these are presented in Chapter 4.

In Chapter 6, the contribution of my additional findings from the two studies presented in Chapters 3 and 5 to the question of whether maternal n-3 LCPUFA supplementation reduces allergies in offspring is assessed by updating the systematic review. This update is important, as it provides the most up-to-date synthesis of data for use in evidence based health care and includes the contribution of the core studies in this thesis.

## 1.2 Review of Allergy

### 1.2.1 What is allergy?

Allergy describes a medical condition, mediated by an immunological response that has reproducible physical symptoms to specific substances (allergens), with or without a hereditary tendency [1, 28]. Allergens are often proteins and are found in house dust mites, pets, pollen, insects, moulds, foods and some medicines [7, 28]. Initially allergens trigger an immune response (sensitisation), after which renewed encounters result in inflammatory responses that cause symptoms (hypersensitivity). Different types of immune reactions e.g. immunoglobulin E (IgE)-mediated or immunoglobulin G (IgG)-mediated have been associated with allergy [28]. Familial, personal and environmental factors are risk factors for allergy development [28] (see more at section 1.2.3). The term atopy refers to the genetic tendency to develop allergic diseases such as allergic rhinitis, asthma and atopic dermatitis (eczema). Atopy is typically associated with heightened immune responses (mediated by IgE), especially to inhaled allergens and food allergens [28]. Whilst most allergic reactions are mild to moderate, some people experience extreme irritation and discomfort and a small number of people may experience a severe allergic reaction, known as anaphylaxis, which is a serious condition requiring immediate lifesaving medications and actions [7, 28, 29].

In this thesis, the allergies of interest are food allergy, eczema, asthma and allergic rhinitis. Food allergy is characterised by hives (urticaria), swelling around the mouth, and vomiting after ingestion of a certain food, usually within 60 minutes. There may also be other symptoms such as a runny or blocked nose, abdominal pain, diarrhoea and even asthmatic symptoms when certain foods are ingested [29, 30].

Eczema, also known as atopic dermatitis, atopic eczema, or allergic eczema is characterised by recurrent skin lesions, dry, red, itchy inflamed skin and urtcaria, associated with elevated IgE antibodies [7, 28].

Asthma is a chronic inflammatory disease of the airways characterised by recurrent episodes of breathlessness and wheezing which occur due to inflammation in the epithelial cells and smooth muscles of the airways in response to allergen inhalation or food allergens [7]. Allergic rhinitis (hay fever) is triggered by allergen inhalation and may result in nasal and eye inflammation and associated symptoms of sneezing, itchy runny watery nose and itchy watery eyes [7].

### 1.2.2 The immune process of allergy

Allergic diseases are the result of the activation of the immune system in response to a foreign substance in the body. The allergic immune response begins when allergens (antigens) enter the body, (via an oral or a systemic route), triggering lymphocytes to release IgE antibodies, which then attach themselves to mast cells inducing the release of a series of bioactive compounds, such as histamine, $PGE_2$ and cytokines which then orchestrate the allergic reaction [7, 28]. Initial exposure to an allergen sensitises B lymphocytes such that on subsequent exposure to the same allergen a 'memory' response occurs, resulting in a faster IgE mediated reaction [31, 32]. The other important pathway in allergy development involves $CD4^+$ T lymphocytes, which secrete Th2 cytokines in response to activation by allergen-derived peptides [33]. Antigen presenting cells (APCs) present antigens to naïve T-cells which are then transformed into either type 1 T-helper (Th1) cells, type 2 T-helper (Th2) cells, type 17 T-helper (Th17) cells or T-regulatory cells (Treg cells) [33-35]. Th1 cells are characterised by the production of interferon (IFN)-gamma ($\gamma$), interleukin (IL)-2 and tumor necrosis factor (TNF)-beta ($\beta$) [33] and are mainly involved in cell-mediated immunity and inflammation. Th2 cells are charactersied by the production of cytokines such as interleukin (IL)-4, IL-5, IL-10 and IL-13. Th17 cells produce IL-17 which induces inflammatory responses through the release of pro-inflammatory mediators such as alpha-chemokines in human airways [36-38]. The development of Th17 cells is also down-regulated by IL-4

produced by Th2 cells and by IFN-ɤ produced by Th1 cells [39]. IL-4 is critical for mediating Th2 cell development and also antibody isotype switching to IgE synthesis and basophil activation [31, 32]. IL-13 promotes mucus secretion, airway hyper responsiveness and tissue remodelling and works similarly to IL-4 [40]. IL-5 mediates eosinophil activation and recruitment, which can be a pathological feature of chronic allergic disease, (asthma) [41]. During chronic allergic reactions release of pro-inflammatory mediators including chemokines, leukotrienes and cytokines also occurs [35, 39, 41]. This contrasts with acute allergic reactions which are mediated by IgE and short-lived secreted mediators such as histamine [35]. These multiple immune pathways mediated by a Th2 cytokine network promote allergic inflammation and ultimately the manifestation of clinical symptoms. Other cytokines, produced by APCs and lymphocytes, (transforming Growth Factor-beta (TGF-β) and IL-10 ), are then essential for the development of Treg cells, which mediate oral tolerance development and regulation of both allergic and inflammatory immune responses [42]. There is now increasing evidence that it is not only inappropriate immune activation to an allergen but also dysregulation of immune regulating processes and Treg cell function which leads to development of allergic disease [43].

### 1.2.3 Why is allergy increasing?

A number of factors have been linked to the incidence of allergy, including allergen exposure, gut microbiome, dietary fibre, dietary fats, vitamin D, smoking, pollutants, stress and environmental factors [11, 44-51]. Higher socioeconomic conditions, better hygienic standards, fewer respiratory infections, greater use of antibiotics early in life, fewer older siblings in the household, less contact with farm animals and general lack of microbial exposure are also reported to have contributed to the increased prevalence of allergic diseases [52, 53]. Early in postnatal life a lack of breastfeeding and changes in dietary patterns have been implicated [54-59]. Specifically, the duration or exclusivity of breastfeeding, low intake

of dietary n-3 and/or high intake of n-6 PUFA and insufficient antioxidants in the diet may influence childhood allergies [14, 60-62]. There is a growing interest in the role of n-3 LCPUFA in the modulation of the immune response in the fetus and early infancy and whether this may alter allergic disease and this is discussed in more detail in section 1.2.9. Although there is a genetic component in allergy predisposition, allergies are increasing too rapidly, for the changes to be entirely associated directly with genes, making it more likely that environmental influences on genes or epigenetic influences are involved [63-65]. Thus there is a possibility that some of the environmental factors may be modifying genes; specifically those which are associated with T helper cell differentiations and T regulatory cell production [63, 64, 66] and this is reviewed in more depth in section 1.2.9 below. Epigenetic modifications may be heritable across multiple generations from prenatal, parental or grandparental environmental exposures [64, 67].

It is important to consider also that not all of the reported increases in the prevalence of allergy may be due to allergy increasing per se, but perhaps also we have become better at recognizing/diagnosing/labelling allergic diseases with new diagnostic tools or diagnostic criteria [44, 68-70]. In addition care is needed when considering the types of evidence that contribute to prevalence data, especially with observational studies which may not be comparative due to differences in test/diagnostic criteria, methodology, source of population studied and sample sizes of interest [68-70].

### 1.2.4 Prevention strategies are needed for allergies

Allergic diseases are chronic, requiring treatment for long periods, which is costly to both patients and governments [8, 71]. The increasing prevalence of atopic diseases, particularly asthma, and treatment challenges from a public health point of view, has led researchers to focus on preventative measures [9, 10]. Although there is currently no known prevention for allergy, some preventative measures have been recommended by the Australian Society of

Clinical Immunology and Allergy (ASCIA) [7] and the American Academy of Allergy Asthma and Immunology (AAAAI) [72]. For the mother and young infant, these include cessation of smoking before and during pregnancy, breastfeeding for 4–6 months, avoiding environmental irritants, delaying introduction of solid foods until 4 months of age and once introduction of solids food has occurred, it is advised that new individual foods are introduced gradually every 2-3 days [7]. ASCIA does not recommend probiotics, fish oil supplementation and dietary restriction of allergenic food during pregnancy due to a lack of evidence from high quality RCTs and properly conducted systematic reviews [7]. Although information provided by cross-sectional studies is useful in the generation of hypotheses, which can be investigated by prospective longitudinal cohort studies, well-constructed RCTs and systematic reviews provide the highest level of evidence [73]. As a number of nutritional factors have been implicated in the increase in the incidence of allergy there is a growing body of research examining whether dietary modifications during pregnancy and early infancy can reduce the development of allergies during childhood [59, 74, 75]. The role of n-3 LCPUFA in the modulation of the immune response in the fetus and in early infancy and whether they may modify allergic disease in childhood are of particular interest. Prenatal and perinatal periods may be critical 'windows' for influencing immune development, during which n-3 LCPUFA exposure may be important in the etiology of allergic disease [13, 23, 45, 76]. As foods rich in n-3 LCPUFA during pregnancy may decrease allergic disease in the offspring and may influence fetal immune responses [77-80], n-3 LCPUFA have attracted interest as potentially cheap, readily available preventative measures for allergy. However, further investigations are warranted and effects of n-3 LCPUFA on allergies will be the focus of this thesis. Before discussing in detail the possible influences of dietary n-3 LCPUFA on the risk of allergic disease in childhood it is important to understand the diagnoses, treatment and natural history of allergic disease.

### 1.2.5 Diagnosis, symptoms and reporting of allergy

Most allergic responses are mediated by IgE antibodies, specific to the trigger allergen [41, 81, 82]. However, although the presence of IgE antibodies indicates a sensitised state, the most reliable diagnosis of allergic disease should take into account clinical history as well [81, 82]. Thus, diagnoses involving laboratory tests; radioallergosorbent test (RAST), skin prick test (SPT) and assessment of clinical symptoms are more reliable than clinical presentation or parental reports (using validated questionnaires), or laboratory reports alone.

#### *1.2.5.1 Medical diagnosis*

Medical diagnosis of allergies can be made by a pediatrician, allergist, clinical immunologist, general practitioner or specifically trained nurse practitioner [83]. Medical diagnosis is mainly based on clinical symptoms and response to specific medications, either with or without results of tests for sensitisation to allergens [7]. In this thesis, medical diagnoses of allergies and medical diagnosis of IgE mediated allergies were used.

*IgE mediated allergies*

Diagnosis of IgE mediated allergies is based on clinical symptoms and positive test results for sensitisation to allergens. Allergen-specific IgE can be detected by SPT results or by blood specific IgE testing (RAST).

*Skin prick test (SPT)*

The SPT is the most convenient and cost-effective method of allergen testing [7]. Consequently, SPT is the principal method of determining specific IgE sensitisation to an allergen extract [84]. A positive SPT to an allergen, along with clinical allergy symptoms, forms the basis for IgE mediated allergy diagnosis [84].

To perform a SPT, a drop of allergen extract is placed on the skin and a small prick is made through the drop. This allows a small amount of allergen to enter the skin. If allergic to the

tested allergen, a small lump (wheal) will appear at the site of testing over 15–20 minutes. A test is positive if there is a mean wheal diameter of 3mm or greater [84]. However, there are some disadvantages of SPT which include needing areas of normal healthy skin, patients need to discontinue antihistamine medication a number of days prior to the test and infrequently, severe allergic reactions may occur [7].

*Radioallergosorbent Test (RAST)*

The RAST is a well recognised blood test for allergy diagnosis [85]. Developed to assess allergen specific IgE antibodies, it is performed when the SPT is not readily available or advisable due to skin conditions such as severe eczema or when it is not possible to cease taking antihistamine medication [85]. The major disadvantages of RAST tests are expense, and they don't exist for every potential allergen. RAST tests are performed on a sample of blood taken from the patient and a positive RAST is identified when the blood IgE level is above 0.70 IU/ml (moderate atopy: IgE $\geq$ 0.70 IU/ml; moderate-high atopy: IgE $\geq$ 3.5 IU/ml).

These two tests are considered as effective for IgE antibody detection and are usually performed to confirm the allergen to which people are allergic. Oral food challenges can also be performed to confirm diagnosis of food allergies, if it is safe to do so [86, 87].

*Non-IgE mediated allergy*

Non-IgE mediated allergy – allergy reactions based on IgG antibodies and other allergens will not result in a positive SPT or RAST, despite presenting clinical symptoms being similar to IgE mediated allergies [28]. Enzyme immunoassay techniques are used on a sample of blood to detect raised IgG antibodies.

*Clinical Symptoms of Food allergy*

Food allergy develops as a result of interactions between food allergens (proteins or glycoproteins in food), the gastrointestinal tract and the immune system [88]. Food allergy is diagnosed by immediate (within 60 minutes) skin rash (hives, rash or swelling), with or without: respiratory symptoms (cough, wheeze or stridor), gastrointestinal symptoms (abdominal pain, vomiting or loose stools), and cardiovascular symptoms (collapse) following ingestion of certain foods [7]. The "gold standard" method of diagnosing food allergy is a double blind placebo-controlled food challenge, performed under medical supervision [87, 89].

*Clinical Symptoms of Eczema*

Eczema diagnosis still relies on the Hanifin and Rijka diagnostic criteria, which look for a history of an itchy rash distributed to the facial, flexural, or extensor surface of the skin which has followed a fluctuating or chronic course [90]. The UK refinement to the Hanifin and Rijka diagnostic criteria specified that an individual must have an itchy skin condition plus three or more of the following: history of flexural involvement, history of asthma/hay fever, history of generalised dry skin or visible flexural dermatitis [90, 91]. Severe eczema diagnosis is defined as being kept awake at night by this itchy rash for one or more nights per week [92].

*Clinical Symptoms of Asthma*

Asthma diagnosis is based on criteria defined by the International Consensus Guidelines and the National Asthma Council and includes the following diagnostic criteria: wheezing on exhalation, persistent irritable cough (especially at night), difficulty breathing and shortness of breath, tightness and heaviness in the chest, and wheezing or coughing with exercise (exercise induced asthma) [93].

Asthma can be classed as mild, moderate or severe. Mild asthma is defined as episodes of quiet wheezing, breathlessness on walking, slightly increased respiratory rate, and non-use of breathing accessory muscles, while still being able to speak sentences. Moderate asthma is defined as moderate to loud episodes of wheezing, breathlessness at rest, increased respiratory rate, and involvement of breathing accessory muscles, while only able to speak in phrases [94]. Severe asthma is defined as episodes of loud wheezing, breathlessness at rest and while sitting upright, highly increased respiratory rate, and considerable use of breathing accessory muscles, while only able to speak in words [94].

*Clinical Symptoms of Allergic Rhinitis*

Diagnosis of allergic rhinitis is based on a history of sneezing, or a runny or blocked nose, not associated with a cold or flu [95]. These symptoms can worsen with seasons, house dust sensitivity, mould sensitivity, pet exposure, foods and medications [5, 96]. When nasal signs are associated with eye signs – reddened, puffy, watering, itchy eyes – it is called rhino-conjunctivitis, which is known to be atopic [5, 96].

#### *1.2.5.2 Reporting of childhood allergy*

Although medical diagnoses of allergies are robust for assessing allergies in individuals presenting to their medical practitioner, they are expensive and time consuming for use in large scale clinical trials, especially when there is a need to re-assess participants frequently for research purposes. An alternative for use in studies of allergies in children is the parent reports of allergy, including both the parent reports of allergy symptoms and parent reports of doctor diagnosed allergy. Both validated and non-validated questionnaires have been used to collect parent reports of allergies, however as large variations in reporting of allergy outcomes were observed when these questionnaires were compared, which may be associated with interpretation, language and cultural issues [97, 98], this highlighted the need for uniform diagnostic criteria and reporting tools. To address this, the International Study of Asthma and

Allergy in Childhood (ISAAC) steering committee developed a questionnaire to streamline allergy diagnosis in large population based allergy studies [98, 99]. The questionnaire has since been recommended for epidemiological studies conducted in 56 countries worldwide, including Australia [5, 6, 100-103]. The questionnaire collects data on asthma, eczema and rhinitis symptoms and has been validated against medical diagnosis of the conditions from around the world [98]. The validated written ISAAC questionnaire is widely used to assess the prevalence of asthma, allergic rhinitis and eczema in children aged 6–7 years [98, 99] and in this age group it consists of 21 questions, divided into three modules – wheezing, allergic rhinitis and eczema. Of the 21 questions, 8 questions relate to wheezing, 6 to rhinitis and 7 to eczema. The ISAAC questionnaire (Appendix 5.1) collects information on the prevalence of allergy symptoms at any time during the child's life as well as in the last 12 months [98, 99].

Although the ISAAC written questionnaire is known to be a reliable, cost effective, widely used tool in epidemiological studies on children between 6–7 and 13–14 years of age [4-6, 100-103], it is not without drawbacks as it has been pointed out that the questions may not be specific enough to collect allergy symptoms/allergic diseases and it may not accurately catch the severity of allergic diseases [4-6, 100-103]. The ISAAC questions have also been used to assess allergies in other age groups; for example 4–5, 6–12 and 12–15 years of age [104, 105]. Despite many epidemiological studies on allergy prevalence being published, the number of studies that have used the ISAAC questionnaire on children below school age is limited [106, 107]. The reasons for this are unclear, but may be due to the ISAAC questionnaire not being validated or lacking suitability for this age group. In this thesis, both medical diagnoses of allergies and parental reports of allergies are investigated. Chapter 4 has a specific focus on investigating the use of ISAAC questions further in the diagnosis of allergies in infants and children below 3 years of age. Non-validated modified ISAAC questions and validated ISAAC questionnaires are used to collect parental reports of allergy outcomes from birth to 3

years of age and at 7 years of age respectively in children participating in two RCTs: the DOMInO and DINO trials [26, 27]. To the best of my knowledge, there has been no previous validation of the ISAAC questionnaire for 3 year olds.

### 1.2.6 Childhood allergies, development, prevalence and progression in childhood

All of the allergic diseases described above in section 1.2.5 - food allergies, allergic dermatitis (eczema), asthma, allergic rhinitis (hay fever) and rhino-conjunctivitis - are common in children [28]. As mentioned above, IgE mediated allergy may have a genetic predisposition [108]. Offspring face a 30% greater risk of allergy development if one first degree relative (parent or sibling) is atopic and 70% if both parents are atopic [108]. While it is known that atopy is a polygenic disorder, no specific genetic markers have been identified [28].

Allergy development in children differs depending on the allergy condition. Food allergy occurs in around 1 in 20 children and 1 in 100 adults in Australia [7]. The prevalence of food allergy differs with age worldwide; studies showed that food allergy affects 10% of infants up to 1 year of age (where food allergies had been confirmed by food challenges), between 4-8% of children aged up to 5 years of age and approximately 2% of adults [109-111].

The risk for anaphylaxis is significant in children with fish, seafood and nut allergies and a lesser risk for those with egg, milk and wheat allergies [29, 110]. This risk creates high anxiety for families over issues such as care at school, the need for emergency medication and risk of death [29]. The development of IgE mediated responses to a food begins with APCs in the gut epithelium [112]. Infants may develop IgE sensitisation to foods when they ingest several new foods at once, as this can overwhelm the immature gut [30]. Eggs, peanuts, milk, soy and wheat are the main allergenic foods for children aged below 5 years, while fish, shell fish, peanut and tree nut (e.g. cashew nut, almond, hazelnut and walnut) are the main allergenic foods for lifelong allergies [88].

Eczema occurs in around 1 in 5 infants and 1 in 10 children between 6-7 and 12-13 years of age in Australia [6, 113]. While most cases of eczema are IgE mediated, it can also occur without IgE mediation [7]. The epithelium of an atopic patient has significantly impaired antimicrobial defense, leading to inflammation in the skin [114]. Infantile eczema usually starts in the first 6 months of life and symptoms include a red rash and dry skin appearing on the cheeks. This may spread to the forehead and the backs of arms and legs. In severe cases it can involve the whole body. Due to scratching of the skin lesion, weepy heavy scaling can occur and become infected. Infantile eczema usually improves significantly between the ages of 3 to 5 years [7]. Childhood eczema may follow or can start for the first time between 2 to 4 years of age. The rash and dryness are usually found in the creases of the elbows, behind the knees, across the ankles and may also involve the face, ears and neck. This form of eczema usually improves significantly by the age of 10 years, but may continue into adult life with dry or sensitive skin [7].

Asthma is common, affecting around 1 in 4 children below 12 years of age, 1 in 7 teenagers and 1 in 10 adults in Australia [7]. Airway inflammation is caused by increased mast cells, eosinophils, lymphocytes, macrophages, dendritic cells and cell mediators such as cytokines, chemokines and leukotrienes [115-117]. People with asthma experience a narrowing of the airways in the lungs, which obstructs the flow of air into and out of the lungs [7]. This narrowing can be reversed using medications [7].

Symptoms of allergic rhinitis are more common in childhood and adolescence [118]. It also affects around 1 in 5 people (children and adults) in Australia and New Zealand [7]. Allergic rhinitis causes the release of cytokines, promoting IgE and mast cell production [119]. Mucosal mast cells produce cytokines which infiltrate nasal mucosa, leading to allergic rhinitis disease [119]. People with allergic rhinitis can also have an irritating cough and large amounts of mucus which may cause discomfort and lead to nausea at times [7]. Among self-

reported illnesses, asthma and allergic rhinitis are the most common allergies in people aged 12–24 years in Australia [7]. According to the Australian Bureau of Health Statistics National Health Surveys conducted in 1989–1990 and 2008–2009, 4.3% and 10% of all Australians respectively suffer asthma and 10.3% and 17% suffer allergic rhinitis, which represents approximately a doubling of the incidence of asthma and allergic rhinitis within two decades [120, 121]. This emphasizes the need to find strategies for preventing allergies in childhood, as some childhood allergies may progress towards adulthood.

#### *1.2.6.1 Allergic March*

Allergic disease symptoms typically start within certain age groups, persist over years and often disappear with age. This progression of allergic diseases is known as the "allergic march" [122]. For example, infantile eczema usually starts in the first 6 months of life and improves significantly between the ages of 3 to 5 years [7]. It can also begin between the ages of 2 to 4 years and if it starts later, it improves better than earlier onset [7].

The most common allergic diseases in the first year of life are food allergy and eczema [122]. Eczema prevalence remains high in 3 year olds, while asthma and allergic rhinitis may start in the first 2 years of childhood and progress through to adolescence [118, 122]. While the symptoms of eczema and food allergy generally decrease with age, some symptoms persist into adulthood, and some worsen. Children with eczema can also develop asthma and/or allergic rhinitis in adolescence through to adulthood [122]. If children under 2 years old have eczema and IgE sensitisation to foods, they are more likely to have asthma later in life [123]. Additionally, 50% of childhood asthma sufferers and 80% of hay fever sufferers continue their symptoms into adulthood [124, 125].

Age related sensitisation to allergens has also been reported with childhood allergic diseases [126-128]. Sensitisation to food allergens is common in infancy and very early childhood while sensitisation to inhalant allergens is common in later childhood [129]. It has also been

reported that infants or children who are sensitised to egg allergen in early life are more likely to have asthma and allergic rhinitis later in life [130]. The combination of egg allergy and eczema in infancy has also been reported to lead to increased risk of aero-allergen sensitisation and respiratory allergic diseases in early childhood (at 4 years of age) [130]. Poly allergen sensitisation in combination with an atopic phenotype is known to be associated with severe allergic diseases, where symptoms interfere significantly with children's life [129].

It is important to be aware of the allergic march and age related sensitisation when designing allergy studies and developing questionnaires to avoid erroneous results. Hence, in this thesis, care was taken to ensure that multiple allergic diseases and sensitisation status' were assessed not just at a single time-point in infancy/childhood.

Before going into detail regarding the postulated role of n-3 LCPUFA in relation to allergy, the basics of PUFAs and their metabolic pathways are outlined in the next section.

### 1.2.7 PUFAs and their metabolic pathways

Fatty acids are comprised of a hydrocarbon chain with a terminal carboxylic acid. Straight-chain molecules with no double bonds are classified as saturated fatty acids. Fatty acids with one double bond are classified as monounsaturated fatty acids whilst PUFAs contain two or more carbon double bonds in their hydrocarbon chain. There are both shorter chain and long chain PUFAs, which contain less or more than 20 carbon atoms, respectively. The two main families of PUFAs are referred to as the n-6 or omega 6 and the n-3 or omega 3 families [17]. The location of the first double bond from the methyl (omega) end of the hydrocarbon chain denotes whether the fatty acid is n-6 or n-3. For example, if the first double bond is on the sixth carbon from the methyl end, it is described as n-6 or omega 6. Similarly, if the first double bond is on carbon number 3 from the methyl end carbon it is described as n-3 or omega 3.

*Synthesis and competition between n-3 and n-6 PUFAs*

Linoleic acid (LA; 18:2n-6) and α linolenic acid (ALA; 18:3n-3) are essential fatty acids which cannot be synthesised in the human body and must be ingested through food [131, 132]. LA is found in abundance in plant oils such as corn, safflower and sunflower while ALA is found primarily in rapeseed, flaxseed and soybean oils [17]. Both LA and ALA are converted to their respective long-chained n-6 and n-3 PUFAs via processes of desaturation and elongation reactions (see Figure 1-1). The major end product of LA metabolism is arachidonic acid (AA; 20:4n-6) and the major end products of ALA metabolism are eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [17, 133].

As the metabolic pathways for LA and ALA both involve the same set of desaturase enzymes, the pathways are therefore connected and competing. As a result of this competition, the increase in n-6 fatty acids in the typical Western diet over recent years reduces the efficiency by which ALA is converted to EPA and DHA in the body [134-136]. The Δ6-desaturase is the rate-limiting step in humans [137, 138] and a number of factors are also known to influence the Δ6-desaturase activity for example low insulin, obesity, hormones, nutrition, smoking and alcohol [137, 139-141]. Competition also occurs at the level of incorporation into the cell membrane [21, 142] where increased dietary intake of n-6 PUFA limits the uptake of n-3 PUFA into cell membranes, leading to alterations in fatty acid composition and cell function [135].

Although plant ALA is the precursor of EPA, DPA and DHA, the synthesis of DHA is different in humans to the synthesis of EPA and DPA [135]. High dosage (up to 40 g/d) feeding with ALA in adults produced a small increase of EPA and DPA in blood and breast milk, but no increase of DHA [135, 136, 143]. The conversion of ALA to EPA and DHA in

human adults is limited (≈5% of ALA is converted to EPA while <0.5% is converted to DHA) [136, 143].

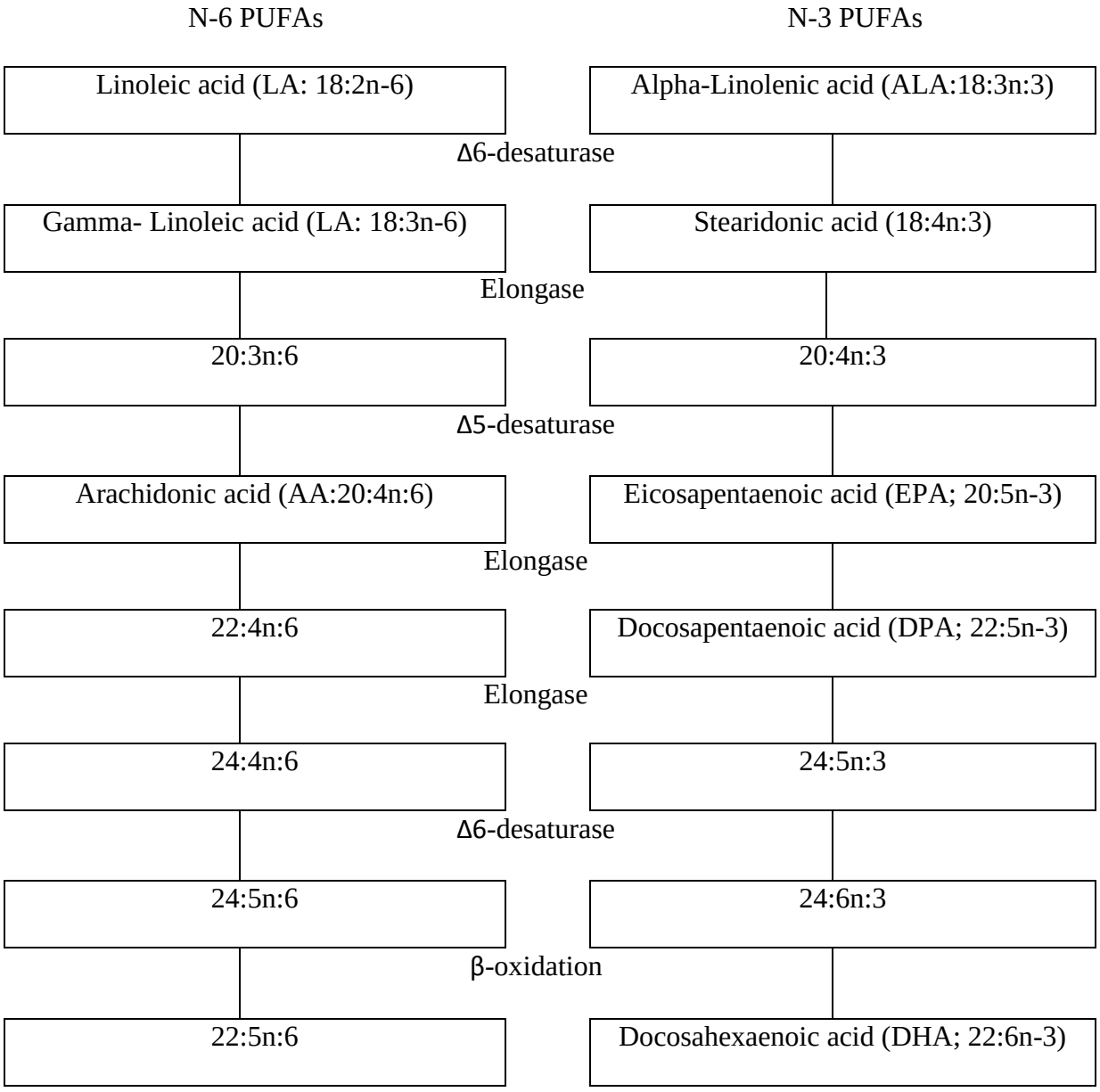


**Figure 1-1: The metabolic pathways for n-6 and n-3 poly unsaturated fatty acids - Linoleic acid and Alpha-Linolenic acid**

Due to this limited synthesis of DHA and EPA from ALA in humans, they are largely reliant on a DHA and EPA rich diet or supplementation [135, 136]. Marine foods such as fish, shrimps and crayfish are the main sources of the n-3 PUFAs (EPA, DPA, DHA), whilst the n-6 AA is found in egg and most types of meat. Lean red meat also has some EPA and DPA and eggs have some DHA. In Westernised countries, some supermarket foods e.g. bread, eggs, milk, yoghurt and orange juice are fortified with DHA [144].

*LCPUFA During pregnancy and lactation*

The developing fetus is thought to require around 70 mg of LCPUFA, mostly as DHA per day, to ensure optimal development of many organs and cells including the brain and retina [145-147]. As the biosynthesis pathways of the developing fetus are immature, this requirement cannot be met by the fetus, which relies instead on the maternal circulation to supply DHA [148, 149]. The n-3 LCPUFA levels of women, in particular DHA and EPA, vary with their fat stores and diets [150]. Consequently the mother's body nutrient levels and her diet are important for the developing fetus. A number of studies have investigated ingestion of n-3 LCPUFA by pregnant mothers, circulating DHA levels in the mother and relation to fetal blood DHA concentration, leading to the suggestion that maternal diet may be the main factor for raising the DHA content which is transported to the fetus via the placenta [150-154]. In addition, some increase in DHA synthesis from precursor fatty acids occurs during pregnancy, under the influence of hormones [136, 155]. Supply of n-3 LCPUFA to the developing fetus is also thought to be enhanced through the placenta by a biological magnification process whereby placental DHA (but not EPA) increases significantly [156]. Placental DHA levels are positively associated with maternal and cord DHA levels and higher DHA levels in fetal blood, than maternal blood have been reported [157-159]. After birth, infants' sources of DHA are breast milk and/or supplemented formulae [160-162]. The World Health Organization (WHO) recommends that infants should be exclusively breast fed for at

least 6 months and states that ‘breastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants’[161]. Although breast milk overall composition is not dramatically changed with the diet, the nutrient content in breast milk can vary with maternal diet [163-165]. Postnatal cohort studies have shown that maternal fish intake during lactation is associated with breast milk fatty acid composition [165-168]. When the lactating mother ingests n-3 LCPUFA, it raises the breast milk DHA and EPA content and the content of n-3 LCPUFA in breast milk is dependent on the maternal intake of these fatty acids [165-168]. For example, Harris et al supplemented lactating mothers with different doses of fish oil and observed dose dependent increases in their breast milk DHA levels [165]. However, they used very high doses of fish oil, between 5-47 g/day [165]. Helland et al reported that supplementing lactating mothers with 0, 2.5, 5 and 10 ml of cod liver oil per day increased the DHA concentration in breast milk significantly in all supplemented groups, but EPA increased significantly in only the 5 and 10 ml groups [167]. Makrides et al also showed that in lactating mothers, who were supplemented with 0, 0.2, 0.4, 0.9, 1.3 g DHA per day, there was a strong, specific and dose-dependent effect on breast milk DHA [168].

### 1.2.8 N-3 LCPUFA intake

There is no consistent recommendation on n-3 LCPUFA intake for humans. The World Association of Perinatal Medicine Dietary Guidelines Working Group recommends pregnant and lactating women should aim to achieve an average daily intake of at least 200 mg DHA [169]. In contrast, the European Food Safety Authority (EFSA) recently suggested that daily intake of n-3 LCPUFA should be > 250 mg/day for adults and an extra 100–200 mg/day DHA should be taken by pregnant and lactating women [170]. This recommendation was calculated to compensate for oxidative losses of maternal dietary DHA and accumulation of DHA in the fetus. Nutrient Reference Values for Australia and New Zealand recommended that the maternal n-3 LCPUFA dietary intake should be around 115 mg/day in pregnancy and around

145 mg/day in lactation [171]. Regardless, the average consumption of n-3 LCPUFA is 120 mg/day in Australian women and the average DHA consumption is 99 mg/day in Australian pregnant women [172, 173]. Thus Australian pregnant mothers may be lacking in n-3 LCPUFA [170] putting the fetus at risk of not receiving the required 70 mg/day. No outcome specific recommendations are available yet on whether higher daily doses of n-3 LCPUFA (>1 g up to 2 g) during pregnancy and lactation may be beneficial for the immune system of the infant, due to a lack of conclusive data [170]. The research in this thesis could give potential reference to the future policy recommendation for maternal n-3 LCPUFA intake on allergy reduction in the offspring.

### 1.2.9 What is the role of LCPUFA in inflammatory process?

LCPUFA are basic constituents of phospholipid membranes. Upon receiving an appropriate signal, phospholipase $A_2$ enzymes cleave the phospholipids, releasing AA (n-6 LCPUFA) and EPA (n-3 LCPUFA) which are converted by cyclooxygenase and lipoxygenase enzymes to the biologically active eicosanoids (prostaglandins, prostacyclins, thromboxanes and leukotrienes) involved in modulating the intensity and duration of inflammatory responses [133]. The mediators derived from the n-6 and n-3 pathways are thought to have contrasting effects [133]. When diets are rich in vegetable oils, meats and animal products, the predominance of AA in tissues gives rise to eicosanoids such as PGE2 that can enhance the synthesis of Th2 cell cytokines which enhance IgE synthesis (Figure 1-2) [174]. IgE antibodies can play an important role in responding to environmental allergens quickly [175].

In contrast, when the diet is high in n-3 LCPUFA, it becomes incorporated into cellular phospholipids and in the process displaces AA [133]. N-3 LCPUFA may further modify cellular membrane and alter the Th2 cell cytokines by inhibiting cytokine production which then inhibits IgE synthesis [21]. EPA is considered to behave as an antagonist to AA [18]. Following membrane cleavage, EPA acts a substrate for the production of biologically active

derivatives that are anti-inflammatory and could ultimately lead to the reduction of allergies [35]. Increased n-3 LCPUFA levels alter the phospholipid membrane makeup of immune cells, which impacts pro-inflammatory signalling pathways [176, 177] where dietary n-3 LCPUFA have marked influence on both specific and nonspecific immune responses in modifying eicosanoid production [178]. In particular, n-3 LCPUFA may influence immune cell function by modifying lipid rafts, inhibiting T cell responses, decreasing $PGE_2$, influencing lymphocyte proliferation, and activating natural killer T cell activity and Th1 cytokine production (Figure 1-2) [21, 176, 179]. N-3 LCPUFA may also alter gene expression and so down regulate the expression of inflammatory cytokines which interact with transcription factors such as nuclear factor-kB (NF-κB) and peroxisome proliferator activated receptors (PPAR-ɤ) [180, 181]. NF-kB plays a role in inflammation and immunity and is inhibited by n-3 LCPUFA [182]. Also EPA and DHA activate PPAR-ɤ in immune cells which inhibits production of pro-inflammatory cytokines and T-cell proliferation [182-184]. In addition, DHA has an anti-inflammatory role, by directly effecting CD40 and IL-4 signaling pathways to inhibit IgE production in human B cells (Figure 1-2) [182, 184].

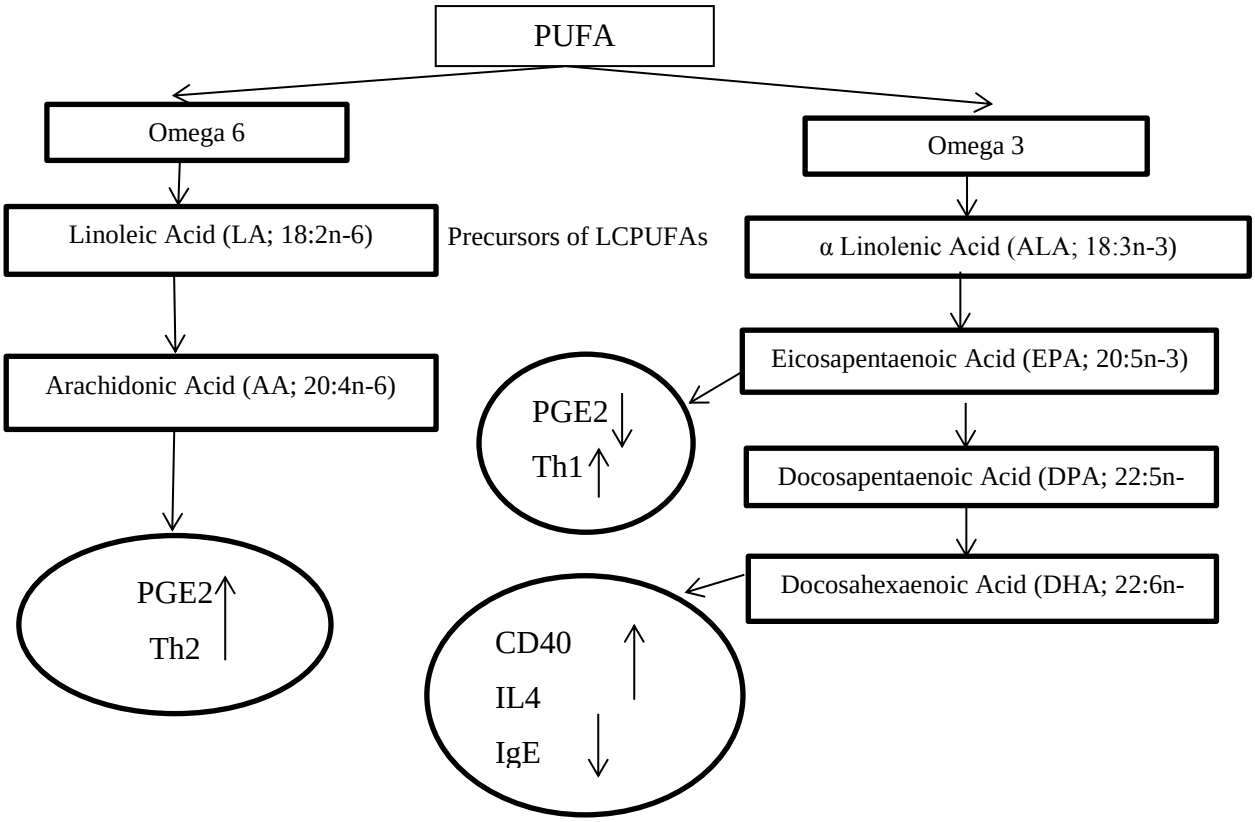


**Figure 1-2: n-3 and n-6 LCPUFA and inflammatory mediators**

Mechanistically, the known anti-inflammatory and immune-modulatory effects of n-3 LCPUFA have led to suggestions that maternal n-3 LCPUFA levels in pregnancy are an important factor that could influence the developing immune system of the infant [174, 185, 186]. The fetal immune responses to allergens may begin in the epithelial tissue where allergen proteins first encounter APC [185]. The pattern of cytokine production in the APC determines the pattern of T cell differentiation and B cell antibody production [39, 185]. In humans, n-3 LCPUFA may exert immune-modulatory effects increasing anti-inflammatory cytokines IL-4, IL-5, IL-10, and IL-13 [176, 187, 188] (see more details in section 1.2.2). N-3 fatty acids are responsible for increasing the IFN- ɤ and TGF-β production in the fetus [189]. In addition, n-3 LCPUFA may also inhibit IgE production and increase IgG production, helping to reduce allergic inflammation [142]. A well-regulated placental balance between

Th1 and Th2 responses is thought to be important for developing a robust immune system during pregnancy [190].

DHA may also regulate anti-inflammatory lipid mediators including resolvins and protectin [16, 191]. Maternal dietary n-3 LCPUFA have been linked to increased resolvin and protectin levels in the placenta, suggesting a pathway by which n-3 LCPUFA may improve pregnancy outcomes and inflammatory disease in children [192, 193]. These data all suggest that DHA-rich fish oil is useful to reduce allergy markers and indicate potential points in the inflammatory pathways where it may act to reduce allergy symptoms.

### 1.2.10 Dietary n-3 and n-6 LCPUFA and allergy development

Evidence from a number of observational studies worldwide points towards the involvement of LCPUFA in allergy development. In the early 1990s, researchers in Australia [194] and Europe [195] suggested that increased n-6 PUFA and decreased n-3 LCPUFA could be the culprits for the increasing incidence of allergic diseases in Western countries [195]. This suggestion was supported shortly afterwards by German researchers who observed that former Eastern Germans had an increased incidence of allergies post-unification, and suggested it was as a result of adopting a more Westernised diet, which included more margarines containing n-6 PUFAs than n-3 PUFAs [196]. Subsequent studies have also supported an association of increased allergy with increased margarine intake [195-198].

Additional dietary evidence from two studies conducted in Japan suggested that the low ratio of n-3 to n-6 in the diet impacted on the increased incidence of allergy [199, 200], which may be due to the fact that young Japanese tend to eat a more Westernised diet. Older people in Japan eat nearly 80 grams of fish and shell fish per day, providing about 60% of n-3 LCPUFA, whereas the younger generation only eat half of the adult consumption, 40 grams of fish and shell fish per day [200].

Regular fish consumption in infants before the age of 12 months also seems to be associated with a reduced risk of allergic diseases including asthma and allergic rhinitis and sensitisation to food and inhalant allergens during the first 4 years of life [201, 202]. In addition, low consumption of oily fish in children in the first two years has been found to be associated with a reduced risk of current asthma [203], however the addition of n-3 LCPUFA to the diet failed to improve asthma symptoms in three small studies (n=12-39) in children and adults with pre-existing asthma [204-206]. Although no clinical improvements were seen in these small three studies, some alterations were detected to eosinophils and neutrophils [204-206]. It may be that n-3 LCPUFA supplementation is not effective as a treatment regimen to cure established asthma or the small sample sizes may not have been sufficient to see clinical significance. However, the changes seen in the white blood cells may point towards n-3 LCPUFA being useful as a preventer of the disease.

*Associations of PUFA in children and childhood allergy*

A few studies have attempted to correlate n-3 and n-6 LCPUFA consumption or serum LCPUFA, in children with and without atopy [205, 207, 208]. Dunder et al reported on a population-based sample of 231 sex- and age-matched children with and without atopy in 1980 and 154 pairs in 1986. The n-3 fatty acids EPA and DHA were significantly lower in the children with atopic dermatitis and the children with atopic disease consumed more margarine and less butter, than the non-atopic children [208]. Fish consumption was not significantly different between atopic and non-atopic children [208].

Yu et al conducted an analysis of serum phospholipids in 22 Swedish 12-15 year old children with asthma and/or allergic dermatitis and 23 non-atopic controls of similar age [207]. They found that the DHA and total n-3 LCPUFA levels were lower in the allergic children than

controls and there was a correlation between high levels of n-6 LCPUFA and high levels of IgE in plasma [207].

Furthermore, high n-3 LCPUFA: low n-6 LCPUFA levels in children and adolescents was associated with a low prevalence of atopic diseases in Finland [209, 210]. Not all studies support the findings that consumption of n-3 LCPUFA or serum n-3 LCPUFA in childhood are associated with childhood allergies. For example, four studies have found no association [211-214]. The reasons for the discrepancies are unclear, but may relate to sample sizes of the studies (smallest n=45, largest n=616), methodologies and differences in characteristics of the cohorts studied.

In 2007, Blumer and Renz reviewed the consumption of n-3 fatty acids during perinatal life and reported that low dietary consumption of n-3 LCPUFA and higher consumption of n-6 LCPUFA in perinatal life increased the incidence of allergic disease [215].This review discussed the immuno-modulatory effects of n-3 LCPUFA supplementation in the perinatal life and found that n-3 LCPUFA affects T-cells and antigen presenting cells of the neonates and is likely to alter eicosanoid metabolism [215]. Because prenatal and perinatal life is a critical period during immune development, it has been suggested that intra uterine or perinatal exposure to n-3 LCPUFA may influence the developing fetal immune system and predisposition to childhood allergy [45, 186, 216, 217].

*Observational studies of Maternal fish consumption during pregnancy and childhood allergy*

Within the last decade, five large epidemiological studies have shown a promising association between maternal fish consumption in pregnancy and reduction of allergies in the offspring [79, 198, 218-220]. Although those 5 studies showed protective effects on allergy outcomes with maternal fish intake, wide variations were observed in the effect size. The variations may be due to differences in sample sizes (smallest n=279, largest n=2641), country, selection

criteria of the sample, assessments of the fish exposure, differences in outcome measures and confounding variables not accounted for in these types of studies. However, another two studies conducted in Japan, where fish consumption is high compared to the other parts of the world, found no association between maternal intake of fatty acids during pregnancy and allergy outcomes of the offspring [221, 222]. The reasons for these discrepancies are unclear. Of the 7 studies, only two conducted their follow-up to 6 years of age and reported that high fish intake (at least 2 fish meals per week) during pregnancy was associated with a reduction in atopic wheeze and a reduction in persistent wheeze [218, 220]. One study showed that consumption of foods rich in n-6 PUFA during pregnancy may increase the risk of allergies in the offspring [79].

A recent large Dutch study focused on different types of fish consumption in the first trimester of pregnancy (n=2976 mothers) and allergy outcomes of the children at 4 years of age, and had mixed results [223]. They observed maternal shellfish consumption was associated with overall increased risks of childhood wheezing and eczema and maternal fatty fish consumption was associated with increased overall risks of childhood eczema but not wheeze [223]. However, there were no associations between maternal total- or lean-fish consumption and wheeze or eczema [223]. Also one retrospective study (mothers with infants at risk of type 1 diabetes, n=2679) showed that lower maternal consumption of n-3 LCPUFA during the 8 month of pregnancy was associated with an increased asthma risk in children at 5 years of age, while a low intake of AA and high intake of total saturated fatty acids were associated with a decreased risk of asthma [224]. Overall however, no association between maternal consumption of fish and fish products and asthma risk was reported in these offspring [224]. These studies raise interesting questions on whether there is a window during pregnancy where increased fish consumption may be more beneficial than others and whether different fish types have an impact on allergy manifestations.

A number of studies have reported that the fatty acid composition of human milk is influenced by maternal lipid nutrition [225-228], leading to the suggestion that enhanced levels of n-3 LCPUFA in breast milk may benefit infant's immune development and may reduce childhood allergies. This is considered more in the next section.

*Breast milk LCPUFA and childhood allergy*

Observational studies have investigated associations between PUFAs in breast milk and childhood allergies. A study conducted in Sweden, found higher breast milk n-3 LCPUFA composition had a protective effect on early allergy development and atopic sensitisation at 12 months of age (n=120) than n-6 LCPUFA composition in breast milk [229, 230]. Hoppu et al conducted a small study in Finland (n=35 atopic mothers and their infants) and reported that lower EPA content in breast milk samples, taken 1 month postpartum, increased the risk of atopic dermatitis in infants at one year age [231]. They also reported that breast milk rich in saturated fatty acids and low in n-3 fatty acids was a risk factor for developing atopic dermatitis in infants and atopic sensitisation during the first year of life [231]. A larger study conducted in the Netherlands (mother-infant pairs n=310) found that higher levels of n-3 LCPUFA in breast milk at 1 month postpartum lowered the prevalence of parent reports of eczema, and clinically diagnosed atopic dermatitis at 2 years of age, as well as sensitisation to cow's milk, egg or peanut at 1 year of age [232].

However there is still controversy, as one study showed an increased association between breast milk n-3 LCPUFA and the risk of atopy in breastfed infants [233] and no association was found between breast milk fatty acids and atopic eczema at 1 year age in another study [234]. Both of these two studies examined the first milk-colostrum rather than mature milk. The differences between studies may be due to the timing of milk sample taken i.e. during different times of the day, different timing postpartum (first breast milk vs. later breast milk

sample), participants' risk of allergy, study analysis methods, age of mother, age of children at which sensitisation was measured, and definition of atopy used.

*Associations of cord blood PUFA and childhood allergy*

Maternal dietary intake of n-3 and n-6 PUFA during pregnancy has an impact on fatty acids in cord blood levels [235, 236] and some studies have related PUFAs in cord blood and development of allergies in children [237-241]. In one small (n=70) study conducted in Norway, lower levels of cord blood EPA and ALA were observed in children who developed allergic sensitisation and atopic dermatitis before the age of 3 years, compared to non-atopic children [240]. Sadeghnejad et al reported that elevated cord serum IgE is strongly associated with development of allergen sensitisation and asthma in children at 4 and 10 years of life [242]. Another small study (n=42) conducted in Kuwait, showed that increased levels of n-6 PUFA (LA) in umbilical cord serum were found in infants with high serum IgE compared with those with low or non-demonstrable serum IgE and that elevated cord serum IgE was strongly associated with development of atopic disease in these infants at one year of age [239]. Galli et al assessed levels of n-3 LCPUFA in cord blood serum and cord blood plasma in 57 new born infants who were at risk of atopy [241]. They found lower levels of n-3 LCPUFA in infants who subsequently developed atopic disease than in those with no atopic disease [241]. Another study examined the levels of LCPUFA in the umbilical cord blood of 50 healthy, full-term infants with a hereditary risk of atopic disease and compared with a control group of 50 infants from families without a history of atopic disease [237]. Significantly lower levels of LCPUFA levels were observed in the atopy group however these LCPUFA were not n-3 LCPUFA [237].

In contrast, other studies have failed to find an association [243, 244] or found increased levels of n-3 LCPUFA were associated with increased risk of allergy [154]. As per above,

reasons for the discrepancies are unknown. However, Newson et al have suggested that maternal supply, fetal demand, cord blood fatty acid levels of LCPUFA and transfer kinetics may all influence fetal exposure in late gestation [243] and thus prenatal n-3 LCPUFA supplementation dose may play a major role in this context.

*Conclusion from epidemiological studies*

Although a number of observational studies have pointed towards an association between fish intake or n-3 LCPUFA levels and reduction of allergies in children, not all studies agree. Kremmyda et al reviewed and synthesised results of epidemiological studies showing an association between maternal fish consumption in pregnancy and lactation on maternal LCPUFA levels and reduction of allergies in the offspring [245]. However, care is needed with extrapolation of all of these studies and assigning causality, because other constituents or contaminants of whole fish may contribute to or modify these associations e.g. vitamin D [50, 246, 247], vitamin E [248, 249 ], mercury [250, 251] and selenium [252, 253]. In addition, there are inherent difficulties with all types of cohort studies, such as dealing with confounders and biases occurring within the populations of the studies.

It is for these reasons that randomised controlled intervention studies are more robust and the most rigorous method of determining whether a cause-effect relationship exists between an intervention and outcome [254, 255].

### 1.2.11 Does n-3 LCPUFA supplementation in pregnancy or lactation work to prevent allergies in the offspring?

A well designed RCT provides the strongest evidence of effectiveness and safety of an intervention (provided it adequately controls for confounders, minimizes bias and is adequately powered to measure relevant disease incidence and other outcomes) [256-258]. Thus the RCT is believed to yield the highest level of evidence for causality [258]. Although the observational studies above have suggested links between fish intake and n-3 LCPUFA

levels with allergy development, RCTs are needed to establish whether increasing the intake of n-3 LCPUFA during fetal life and early infancy will alter the pattern of allergy development in childhood. A few trials have already attempted to address this question [189, 259, 260] and also assessed the immune mediators with postulated involvement.

There are two small prenatal fish oil intervention studies that support an immune programming hypothesis. Dunstan et al (n= 83 pregnant atopic mothers) [259] and Krauss et al (n=197 pregnant mothers) [189] both demonstrated down regulation of cord blood mononuclear cell cytokine responses (IL-5, IL-13) to allergens in response to fish oil treatment during pregnancy compared with placebo, as well as up-regulation of TGF-β in cord blood [189, 259]. On the postnatal side, Lauritzen et al studied mothers (n=122) who were supplemented with either 1.5 g of n-3 LCPUFA daily or placebo during first four months of lactation and investigated cytokine production in children at two and a half years of age [260]. This study showed that *in vitro* IFN-ɤ and IL-10 production were increased in the fish oil supplemented group compared to placebo, but the percentages of atopic children and plasma IgE were not different in the two groups [260]. However, the Lauritzen et al study was not specifically designed to look at atopy. Although the fish oil supplementation dosages were very different in these three studies, ranging from 0.65g/day [189] to 3.7g/day [259] in pregnancy and 4.5g/day in lactation [260], they collectively point towards a modulation of the neonatal immune responses towards a less allergic phenotype with maternal n-3 LCPUFA supplementation suggesting that these immune modulations during pregnancy and during early childhood may be accountable for the lessening of clinically relevant diseases such as asthma, allergic rhinitis, atopic dermatitis and allergic inflammation [189, 259, 260]. Damsgaard et al [261] also conducted a small postnatal feeding 2 x 2 RCT, supplementing cow's milk or formula with fish oil (5 ml/day) or placebo (no supplement) to healthy infants from 9-12 months of age (n=64) and observed production of TNF-α, IFN-ɤ and IL-10 in

whole blood cultures at the end of supplementation at 12 months of age. They demonstrated that fish oil supplementation significantly increased IFN-ɤ production (P=0.05) and tended to decrease IL-10 production (P=0.08) [261], however the study was not blinded.

Four trials have investigated the effect on n-3 LCPUFA supplementation during the postnatal period [26, 260, 262, 263] on clinical allergy outcomes in infancy and childhood. Two trials investigated the effect of n-3 LCPUFA supplementation via breastfeeding mothers [26, 260] and both of these trials are dealt with in detail in Chapters 2 and 6. Neither trial was designed with atopy as a primary outcome. Briefly, one trial [260] (n=122) supplemented mothers with either 1.5 g of n-3 LCPUFA, or olive oil daily during the first four months of lactation and found no reduction in parent reports of doctor diagnosed allergy in infants of the n-3 LCPUFA supplemented group. In the other breastfeeding supplementation trial [26], mothers of preterm infants (n=657) were supplemented with either 900 mg DHA and 195 mg EPA, or soy oil daily until they reached their expected due date and more details are provided in the Section 1.2.12 on preterm infants below.

In the remaining two relatively large postnatal trials, supplementation was directly to the infants [262, 263], with one trial beginning the intervention during the newborn period [262], and the other starting at 6 months of age, once infants commenced weaning [263]. D'Vaz et al [262] randomly allocated 420 newborn infants in Australia at high-atopic risk to a daily supplement of fish oil (about 400 mg n-3 LCPUFA per day) (n=218) or to an olive oil control (n=202) until they were 6 months of age. The fish oil capsules were given directly to the infants by piercing the capsule and squirting the oil into the infant's mouth [262]. Infants who were supplemented with fish oil from birth to 6 months of age, had significantly increased EPA and DHA levels in their blood at 6 months of age, together with a reduction of eczema, however they had no change in allergy outcomes including sensitisation, eczema, food allergy or asthma at 12 months of age [262].

The other study was the Childhood Asthma Prevention Study (CAPS) study conducted in Australia and included 616 high-risk infants who were randomly allocated to a fish oil supplement or a placebo [263]. Families in the active intervention group were provided with 500 mg tuna fish oil capsules containing approximately 184 mg n-3 fatty acid to add to the child's food once daily from the age of 6 months and received a supplement of tuna oil and canola oil-based oils and spreads for use in cooking, while the control group received a vegetable oil supplement and n-6 rich polyunsaturated oils and spreads for use in home prepared foods [263]. Atopy and symptoms of asthma were assessed at 18 months, 3 and 5 years of age. Although the intervention resulted in a reduction in the proportion of children with wheeze at 18 months [264] and cough at 3 years of age [265], there were no differences in the prevalence of atopy, asthma or wheeze at 5 years [213]. This may suggest that intervention was started too late for maximum benefit to be displayed or that some of the benefits may be transient in nature.

As mentioned earlier, prenatal and perinatal life may be critical periods during immune development and intra uterine and/or perinatal exposure of n-3 LCPUFA may influence the developing immune system in the fetus, perinate and infant [45, 186, 216, 217] and the contribution of this is hard to determine from direct postnatal supplementation studies alone. Thus, in this thesis my main focus will be on maternal n-3 LCPUFA supplementation (not direct infant postnatal supplementation) and clinical allergy outcomes in infants.

There are 5 trials examining the effect of prenatal n-3 LCPUFA supplementation [27, 259, 266-268] and 1 trial where the intervention was applied both prenatally and postnatally on allergy outcomes of infants/children [269].

The prenatal supplementation trials commenced supplementation between 18 to 20 weeks'[268], at 20 [26, 259, 267] and 30 [266] weeks of gestation and continued until delivery. The trial which supplemented in both the prenatal and postnatal period, commenced

supplementation from 25 weeks' gestation and continued until the infant reached four months of age [269].

In these trials, the allergy outcomes were determined by parental reports of allergy symptoms using a non-validated questionnaire [268], and medical diagnosis [27, 259, 266, 267, 269] with one trial [266] using data extracted from medical registries.

The age of assessments differed in the trials. Assessments were conducted at one month and three months [268], six months [27, 267-269], 12 months [27, 259, 269], 18 months [268], 24 months [269], 36 months [27], and 16 years of age [266]. Of the five trials that used medical diagnosis of allergies [27, 259, 266, 267, 269], four performed skin prick tests (SPT) [27, 259, 267, 269] and included children at high risk of allergy. Two trials [267, 269] reported SPT results for children under 12 months of age, three trials [27, 259, 269], reported SPT between 12 to 36 months of age, and one trial [27] reported SPT in children at 36 months of age. IgE mediated allergies were reported in Furuhjelm 2009 [269] and Makrides 2010 [27].

Briefly, these trials showed significant reductions with n-3 LCPUFA supplementation on some outcomes i.e. IgE mediated food allergy and sensitisation to any allergen at 6 months of age [269], IgE mediated eczema and sensitisation to egg at 12 months of age [27], asthma at 16 years of age [266] and less severe allergic disease in infants [259]. In order to synthesise the evidence available from these maternal supplementation trials, I undertook a systematic review and extensive details of these trials can be found in Chapters 2 and 6. At the beginning of this work, data from five of these trials was available and is included in the systematic review performed in Chapter 2 [27, 259, 266, 267, 269]. One study [268] did not publish allergy data in full at my first search date and was included under ongoing trials in Chapter 2. However, all 6 trials are included in the most up-to-date systematic review in Chapter 6.

### 1.2.12 Preterm infants, n-3 LCPUFA and allergy development

While term born infants can synthesise small amounts of n-3 LCPUFA from dietary ALA, preterm infants are deprived as they lack the normal intrauterine supply of n-3 LCPUFA that occurs during the last trimester of gestation [270, 271]. Preterm infants also have minimal fat reserves and rely mainly on the n-3 LCPUFA they receive through their diet. For preterm infants receiving human milk, the amount of n-3 LCPUFA, in particular DHA and EPA, varies with a mother's fat stores and diet. When lactating women ingest n-3 LCPUFA this raises the DHA and EPA content of the breast milk allowing a greater amount to be available to their infants [166]. For infants who receive formula, the DHA content is determined by manufacturers and varies widely [272].

The first six months of postnatal life are known to be critical for immune development [273]. Preterm infants are more Th2 polarized and have a slower rate of Th1 polarization than term infants [274, 275]. It is believed that normally peripheral T lymphocyte populations mature during the last trimester of gestation [276, 277]. Therefore, preterm infants may also have less mature immune systems in early life which could lead to greater incidences of allergic diseases and infections compared with term infants.

Disparities have been seen in observational studies focused on allergy and respiratory outcomes in infants and children who were born preterm and very low birth weight compared with term born and normal birth weight infants [278, 279]. One observational study (n=315) showed that infants who were born prematurely had a lower incidence of eczema at 1 year of age [278]. This contrasts with a larger observational study (n= 4,795) suggesting low birth weight and preterm male infants are at a higher risk of eczema at 18 years of age compared with normal birth weight and term born male infants [279]. The differences observed in these two studies may be due to differences in the assessment age, large differences in sample size or other reasons.

Some studies have also shown that children who were born prematurely or with a very low birth weight had a higher incidence of asthma [280, 281] and a recent systematic review on epidemiological studies investigating the association between preterm birth and asthma/wheezing disorders revealed that preterm birth increases the risk of asthma [282]. If this is indeed the case, there is an urgent need to identify preventative interventions, as preterm infants are experiencing greater survival rates. Preterm infants were excluded or not separately analysed in a number of the n-3 LCPUFA intervention studies [27, 259, 266, 267, 269] which will be discussed in depth in the next chapter and it is important to take account of trials with preterm infants. To date, only one study with 657 preterm infants, supplemented either via breast milk (mother supplemented) or formula with fish oil or placebo from birth until the expected date of delivery, followed–up for parental reported allergies at 12 and 18 months of age and showed a lower risk of hay fever at 12-18 months, especially among boys [283]. In a subset (n=143) of this study, parental reports of allergy outcomes at 3-5 years of age were assessed and showed a higher risk of eczema in the high-DHA group [284]. However, no longer term follow-up was conducted. In this thesis, I have followed-up infants from this study at school age, to add more evidence to longer term follow-ups on allergy outcomes in children and the results are presented in Chapter 5.

## 1.3 Scope of the thesis

The main focus of this thesis is to examine the effects of maternal n-3 LCPUFA supplementation during pregnancy and/or lactation on allergy outcomes in childhood. As there are a number of different RCTs in this field, it is important to bring them together in a systematic way to understand the evidence and the research gaps. For this reason, I performed a rigorous systematic review and meta-analysis of n-3 LCPUFA supplementation trials during pregnancy and/or lactation and allergy outcomes in the offspring and this is presented in Chapter 2. I developed a protocol for the systematic review, which underwent peer review and was published in the Cochrane Database Systematic Reviews, Issue 9, 2012 (Appendix 2.1). Strict inclusion and exclusion criteria, as well as assessment of bias and trial qualities were applied in my review. During the analysis of the review, presented in Chapter 2, some research gaps were identified which I then addressed using two follow-up studies of n-3 LCPUFA supplementation RCTs, DOMInO and DINO trials (one prenatal and one postnatal) and the results are presented in Chapters 3 and 5. In Chapter 6, I have updated the systematic review to include the contribution of the studies I undertook in Chapters 3 and 5. In the interim, there were further publications [285, 286] from an included study [27] and data from an ongoing study [268] in Chapter 2 became available. The updated review in Chapter 6, which underwent peer review and has been published in the Cochrane Database Systematic Reviews, Issue 7, 2015 thus provides the most up-to-date data synthesis, including the contribution of the core studies in this thesis, of optimal strategies for allergy prevention and the best times to apply them in order to tackle the allergy 'epidemic'.

Although, other reviews have been published on a similar topic [25, 245, 287], they have not included the four most recent trials in the area [26, 27, 267, 268]. The Anandan et al review was a systematic review with meta-analysis [287] and included only three maternal supplemented studies (n=562) [259, 260, 266]. They assessed trial qualities in their review;

however there was no clear evidence of a benefit of n-3 LCPUFA supplementation for reducing the risk of allergy outcomes or allergic sensitisation [287]. Kremmyda et al reviewed the effect of early exposure to fish, oily fish or n-3 LCUPFA supplementation during the perinatal period on allergies (irrespective of IgE status) in children, but did not conduct a meta-analysis or, assesses trial qualities and the review was not restricted to RCTs of n-3 LCPUFA supplementation [245]. The conclusion was that maternal n-3 LCPUFA supplementation during pregnancy may reduce some allergic disease, severity of allergic disease and sensitisation [245]. Klemens et al reported fish oil supplementation during pregnancy and lactation reduced the incidence of childhood asthma but had no effect on atopic dermatitis in their meta-analysis of 5 RCTs [189, 259, 260, 266, 269] with n=949 participants [25]. They assessed trial qualities and also commented on differences in pregnancy versus postnatal supplementation on allergic diseases [25].

Another review on a slightly different topic focused on prenatal, perinatal and postnatal n-3 LCPUFA supplementation and synergistic effects with probiotic supplementation on immune and allergy development in children and it was a narrative rather than a meta-analysis [22].

Although important, systematic reviews are prone to a number of bias' and a large number of decisions can impact the results with reviews performed by different research groups sometimes coming to conflicting conclusions [288-290]. None of the meta-analyses above attempted to separate the effects of n-3 LCPUFA supplementation on IgE mediated allergies and non IgE mediated allergies, subgroup analyses on timing of supplementation, familial allergy risk or fetal maturity. Thus there is a need to update the synthesised evidence available from all n-3 LCPUFA maternal supplementation trials during pregnancy and/or lactation which have allergy outcomes, so that the outcomes of the review can focus further research in the area and lead to suggestions of use in clinical practice. Thus, the inclusion of extra trials [26, 27, 267, 268] in the final updated Cochrane review (Chapter 6) which were not available

when the earlier meta-analyses were published, adds an additional 2504 participants to the analysis pool, and thus adds more weight to conclusions. In addition, the trials presented in the final updated Cochrane review cover different timing of supplementation: during pregnancy (n=5), during lactation (n=2) and both pregnancy and lactation (n=1), thus yielding the potential to gain new information regarding the timing of n-3 LCPUFA supplementation and effects on allergy outcomes.

The evidence obtained both from primary clinical research and systematically gathered information (systematic reviews and meta-analyses) allows clinicians and clinical policy makers to make best informed decisions on clinical care for patients [291]. Thus, properly conducted individual intervention studies, as well as systematic reviews with meta-analyses which combine all relevant RCTs to date will provide the best available evidence to determine the effects of maternal n-3 LCPUFA during pregnancy and/or lactation on allergy development in children.

This thesis also investigates the use of parent reports of allergy as a tool for allergy diagnosis in children participating in an RCT in Chapter 4.

# Chapter 2: Maternal n-3 LCPUFA supplementation for prevention of allergies in early childhood – a systematic review and meta-analysis

## 2.1 Introduction

This chapter discusses the systematic review that was performed to determine if maternal n-3 LCPUFA supplementation for pregnant and/or lactating women reduces allergies in their offspring. As discussed in Chapter 1, there is rising interest in prenatal and postnatal n-3 LCPUFA supplementation for the prevention of allergies. While observational and mechanistic studies have shown protective effects for the development of allergies, without clear evidence from well conducted RCTs, justification for practice change cannot be made. Although previous syntheses [25, 245] provided some evidence of allergy reduction in childhood, both reviews concluded that more large, appropriately powered RCTs were needed. In addition, they also suggested that supplementation timing should be clearly defined for maximal benefit for allergy prevention. However, these two reviews [25, 245], one with a meta-analysis and one without, were published prior to more recent randomised controlled trials [26, 27, 267] and there has been no Cochrane systematic review on maternal n-3 LCPUFA supplementation for prevention of allergies in early childhood. Cochrane reviews involve a clearly formulated question that uses a clear search strategy, as well as systematic and explicit methods to identify, select, critically appraise and analyse relevant research data from the studies that are included in the review [292]. Statistical methods (meta-analysis) may or may not be used to analyse and summarise the results of the included studies. The use of predefined, rigorous and explicit methodology avoids prejudices or commercial interests and is intended to assist health care providers or advisory bodies in making practical decisions [292]. A Cochrane review was therefore undertaken as part of this thesis. This review focused on RCTs involving n-3 LCPUFA supplementation to mothers during pregnancy and/or lactation, compared with placebo or no intervention, on allergy outcomes in their offspring.

Further details regarding Participants, Interventions, Comparisons, Outcomes, and Study design (PICOS characteristics), specified in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, are covered in section 2.2 below [293].

## 2.2 Protocol for the systematic review

The protocol for this review underwent international peer review and has been published in The Cochrane Database Systematic Reviews, Issue 9, 2012 (Appendix 2.1) [294].

### 2.2.1 Aims and objectives

The aim of the systematic review was to assess the evidence from RCTs on the effect of n-3 LCPUFA supplementation compared to no supplementation and/or placebo in women during pregnancy or lactation on allergy outcomes in their children.

## 2.3 Methods

Standard Cochrane Systematic Review methods were used, as described in the Cochrane Handbook for Systematic Reviews of Interventions -Version 5.1.0 [updated March 2011] [292].

### 2.3.1 Types of studies

Studies were included in this review if they were RCTs or quasi-randomised trials focusing on n-3 LCPUFA supplementation of pregnant or lactating women, compared with placebo or no treatment, and assessing allergy outcomes of the infants or children. Cluster-randomised trials were also eligible for inclusion. Trials with a cross-over design were excluded as direct effect of interventions cannot be made and were not appropriate for this review. All included trials in the results were published in full. Trials with only biochemical outcomes were excluded because this review was examining clinical outcomes rather than just biomarkers.

### 2.3.2 Types of participants

Women and their children with normal or high risk of allergies were included, to enable investigation into effects in relation to allergy risk status. A fetus or child with a first degree

relative with medically diagnosed allergies, or who had a positive SPT or positive RAST, was defined as being at high risk of allergies. For the purpose of this review, infants were also considered at high risk of allergies if their cord blood IgE level was above 0.70 IU/mL.

### 2.3.3 Types of interventions

All randomised comparisons of n-3 LCPUFA supplementation (either with or without arachidonic acid), with placebo or no supplementation, in pregnant or lactating women were considered for inclusion – regardless of dose regimens and durations of intervention. Studies in which fish was the intervention were considered if appropriately controlled - for example, with appropriate adjustments/matching of the protein contribution of fish.

### 2.3.4 Types of outcome measures

Primary outcome measures included children with food allergy, atopic dermatitis (eczema), asthma/wheeze, allergic rhinitis (hay fever), or any allergies (children with one or more allergy types). Outcomes were assessed as:

- short term (occurring at less than 12 months of age)
- medium term (occurring from 12 to 36 months of age)
- long term (36 months of age and older)
- and combining short term, medium term and long term (any age follow-up) to assess the cumulative incidence.

### 2.3.5 Primary outcome measures

Maternal n-3 LCPUFA supplementation may be beneficial in reducing both IgE mediated allergies and all allergies, therefore the primary outcomes included were:

- Medically diagnosed allergy with sensitisation, i.e. IgE mediated allergies that have both signs and symptoms of the allergic disease and a positive SPT or RAST, and

- Medical diagnosis or parental report (using validated questionnaire) of allergy with or without sensitisation.

### 2.3.6 Secondary outcome measures

Secondary outcomes were:

- Skin prick test results; parent-reported allergies using non-validated questionnaires; infant safety e.g. infections; maternal safety e.g. postpartum bleeding and sepsis.

### 2.3.7 Search methods for identification of the studies

The search for the systematic review in this Chapter was performed early in the thesis. Search strategies were developed with the support of the research librarian from the University of Adelaide to search PubMed (1966 to 21 September 2012) (Appendix 2.1- page 9), CINAHL via EBSCOhost (1984 to 21 September 2012) (Appendix 2.1- page 10), Scopus (1995 to 21 September 2012) (Appendix 2.1- page 10), Web of Knowledge (1900 to 21 September 2012) (Appendix 2.1- page 11) and ClinicalTrials.gov (21 September 2012) (Appendix 2.1- page 12). Language restrictions were not applied, however trials were restricted to humans. The Cochrane Pregnancy and Childbirth Group's Trials Register was searched up to 20 September 2012 (this was early in thesis) by the Trials Search Coordinator of the Cochrane Pregnancy and Childbirth Group. The Cochrane Pregnancy and Childbirth Group's Trials Register is maintained by the Trials Search Coordinator and contains trials identified from:

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE;
3. weekly searches of EMBASE;
4. hand searches of 30 journals and the proceedings of major conferences;
5. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Details of the search strategies for CENTRAL, MEDLINE and EMBASE, the list of hand-searched journals and conference proceedings, and the list of journals reviewed via the current awareness service can be found in the 'Specialised Register' section within the editorial information about the Cochrane Pregnancy and Childbirth Group [295].

Trials identified through the searches described above are each assigned to a review topic (or topics). The Trials Search Coordinator searches the register for each review using the topic list rather than keywords.

#### 2.3.8 Study selection and data extraction

Two reviewers independently assessed the eligibility by reading abstracts of studies for inclusion in the review (Anoja Gunaratne – AG and Carmel Collins – CC). Disagreements were resolved through discussion or, if required, by consultation with the third review author Maria Makrides (MM).

#### 2.3.9 Data extraction and management

A data extraction form was designed that adhered to the requirements of the Cochrane Pregnancy and Childbirth Group (Appendix 2.2). For eligible studies, two review authors (AG and CC) extracted the data independently using the form. Discrepancies were resolved through discussion or, if required, through consultation with the third author (MM). MM and CC were investigators on two trials, Makrides et al 2009 [26] and Makrides et al 2010 [27], that were included in the review. These trials were independently assessed for risk of bias and data extracted by AG and independent researcher Karen Best (KB) .When information regarding any of the above was unclear or incomplete, researchers attempted to contact authors of the original reports to provide further details. Contact authors of two original reports and one abstract report [259, 267, 296] were contacted via emails, two of them responded [267, 296]. One was a clarification regarding fish supplementation and appropriate adjustments/matching of the protein contribution of fish [267]. The other one was regarding

an abstract report and whether the trial had been published in full or data available. The contact author of the abstract replied that requested data could not be given because the study was an ongoing study and there would issues with blinding [268, 296] (see more details in Appendix 2.5 - Table 2-5). Available data were entered into Review Manager Software 5.2. and checked for accuracy [297].

### 2.3.10 Assessment of risk of bias

Eligible trials were assessed independently by AG and CC using the criteria outlined in the Cochrane Collaboration's tool for assessing risk of bias in randomised trials to assess the methodological quality of the trials [298]. The following criteria were used to assess the trial quality.

#### *2.3.10.1 Random sequence generation (checking for possible selection bias)*

The method used to generate the allocation sequence for each included study was described.

The method was assessed as:

- low risk of bias (any truly random process, e.g. random number table; computer random number generator);
- high risk of bias (any non-random process, e.g. odd or even date of birth; hospital or clinic record number), and
- unclear risk of bias where the allocation sequence for each included study was not described.

#### *2.3.10.2 Allocation concealment (checking for possible selection bias)*

The method used to conceal allocation of interventions prior to assignment and assess whether intervention allocation could have been foreseen before or during recruitment, or changed after assignment, was described.

The methods were assessed as:

- low risk of bias (e.g. telephone or central randomisation, consecutively numbered sealed opaque envelopes),
- high risk of bias (open random allocation, unsealed or non-opaque envelopes, alternation, date of birth), and
- unclear risk of bias where the method used to conceal allocation was not properly described.

#### *2.3.10.3 Blinding*

*Blinding of participants and personnel (checking for possible performance bias)*

The methods used for included studies to blind participants and personnel from knowing which intervention a participant received were described. The studies were considered as low risk of bias if they were blinded, or if the lack of blinding would be unlikely to affect results. Blinding was assessed separately for different outcomes or classes of outcomes.

The methods were assessed as:

- low, high or unclear risk of bias for participants;
- low, high or unclear risk of bias for personnel.

*Blinding of outcome assessment (checking for possible detection bias)*

The methods to blind outcome assessors from knowing which intervention a participant received were described for each included study. Blinding was assessed separately for different outcomes or classes of outcomes.

The methods used to blind outcome assessment were assessed as:

- low, high or unclear risk of bias.

#### *2.3.10.4 Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)*

For each included study the completeness of data, including attrition and exclusions from the analysis, was described. For each outcome or class of outcomes, it was noted whether attrition and exclusions were reported and the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion were reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported, or could be supplied by the trial authors, missing data were re-included in the analyses.

The methods were assessed as:

- low risk of bias (e.g. no missing outcome data; missing outcome data balanced across groups),
- high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups, 'as treated' analysis done with substantial departure of intervention received from that assigned at randomisation), and
- unclear risk of bias where the attrition and exclusions from the analysis were not described clearly for each outcome or class of outcomes.

#### *2.3.10.5 Selective reporting (checking for reporting bias)*

For each included study the possibility of selective outcome reporting bias was investigated and described.

The methods were assessed as:

- low risk of bias (where it was clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review were reported),

- high risk of bias (where not all of the study's pre-specified outcomes were reported, one or more reported primary outcomes were not pre-specified, outcomes of interest were reported incompletely and so could not be used; study failed to include results of a key outcome that would have been expected to have been reported), and
- unclear risk of bias where it was not clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review were reported.

*2.3.10.6 Other bias (checking for bias due to problems not covered by 2.3.10.1 to 2.3.10.5 above)*

For each included study any concerns about other possible sources of bias were described.

Whether each study was free of other problems that could put it at risk of bias was assessed as:

- low risk of other bias;
- high risk of other bias;
- unclear whether there was risk of other bias.

*2.3.10.7 Overall risk of bias*

A judgment about whether studies were at high risk of bias was made according to the criteria in the Cochrane Collaboration's tool for assessing risk of bias in randomised trials [298]. With reference to criteria 2.3.10.1 to 2.3.10.6 above, the likely magnitude and direction of the bias was assessed and whether it was likely to impact on the findings was considered.

### 2.3.11 Measures of treatment effect

*2.3.11.1 Dichotomous data*

For dichotomous data, results were presented as summary risk ratio (RR) with 95% confidence intervals.

*2.3.11.2 Unit of analysis issues*

2.3.11.2.1 Studies with more than two treatment groups

If studies in the review included one or more treatment groups (multi-arm studies), groups were combined to create a single pair-wise comparison, where appropriate. The methods described in the Cochrane Handbook -Version 5.1.0 [updated March 2011] were used to ensure that there was no double counting of participants [292].

2.3.11.2.2 Dealing with missing data

For included studies, levels of attrition were noted. The impact of including studies with high levels of missing data in the overall assessment of treatment effect was explored by using sensitivity analysis. Analyses were carried out, as far as possible, on an intention-to-treat basis. This means that all participants randomised to each group were included, and all participants were analysed in the group to which they were allocated, regardless of whether or not they received the allocated intervention. The denominator for each outcome in each trial was the number randomised minus any participants whose outcomes were known to be missing.

*2.3.11.3 Assessment of heterogeneity*

Statistical heterogeneity in each meta-analysis was assessed using $T^2$, $I^2$ and $Chi^2$ statistics. Heterogeneity was regarded as substantial if $I^2$ was greater than 30% and either $T^2$ was greater than zero, or P value less than 0.10 in the $Chi^2$ test.

### 2.3.12 Subgroup analysis

If substantial heterogeneity was identified, it was investigated using subgroup and sensitivity analyses. Whether an overall summary was meaningful was considered, and if it was, random-effects analysis was used to produce it. Subgroup analyses were restricted to the primary outcomes.

The following subgroup analyses were performed:

1. Timing of supplementation:
    - n-3 LCPUFA supplementation during pregnancy versus placebo or no supplementation during pregnancy;
    - n-3 LCPUFA supplementation during lactation versus placebo or no supplementation during lactation;
    - n-3 LCPUFA supplementation during pregnancy and lactation versus placebo or no supplementation during pregnancy and lactation;
2. Allergy risk:
    - maternal n-3 LCPUFA supplementation in infants at high risk of allergies versus placebo or no supplementation;
    - maternal n-3 LCPUFA supplementation in infants at normal risk of allergies versus placebo or no supplementation;
3. Infant maturity:
    - maternal n-3 LCPUFA supplementation in term born infants versus placebo or no supplementation;
    - maternal n-3 LCPUFA supplementation in pre-term born infants versus placebo or no supplementation;

Differences between subgroups were assessed by interaction tests available within Review Manager software 5.2 [297].

#### 2.3.13 Sensitivity analysis

It was planned to carry out sensitivity analyses for the review's primary outcomes to investigate the effect of trial quality by removing those trials rated as ‘high' or 'unclear' risk of selection, performance or attrition bias to establish whether it was likely to impact on the findings.

### 2.3.14 Data synthesis

Statistical analyses were carried out using the Review Manager software 5.2 [297]. For the pooled analysis, Mantel-Haenszel statistical method, fixed effects analysis model and risk ratio for the effect measure were performed. Fixed-effect meta-analysis for combining data was used where it was reasonable to assume that studies were estimating the same underlying treatment effect, i.e. where trials were examining the same intervention, and the trials' populations and methods were judged sufficiently similar. If heterogeneity was found between studies, the random effect model and risk ratio for the effect measure were used. If random-effects analyses were used, the results are presented as the average treatment effect with 95% confidence intervals, and the estimates of $T^2$ and $I^2$. If there was clinical heterogeneity sufficient to expect that the underlying treatment effects differed between trials, or if substantial statistical heterogeneity was detected, random-effects meta-analysis was used to produce an overall summary if an average treatment effect across trials was considered clinically meaningful.

## 2.4 Results of the systematic review

### 2.4.1 Results of the search

Searches in the databases yielded 1534 records. The Cochrane Pregnancy and Childbirth Trials Register search (to 20 September 2012) retrieved 110 reports. Other electronic databases, PubMed (1966 to 21 September 2012), CINAHL via EBSCOhost (1984 to 21 September 2012), Scopus (1995 to 21 September 2012), Web of Knowledge (1900 to 21 September 2012) and ClinicalTrials.gov (21 September 2012) retrieved 91, 12, 1214, 97 and 10 reports respectively.

After removing 282 duplicates, 1252 reports were screened. Another 1124 irrelevant reports were removed and 128 records were selected for application of the inclusion and exclusion criteria. From this, 27 trials with 59 reports were excluded leaving seven trials with 64 reports for inclusion in this review. There were three on-going trials with 5 reports (Figure 2-1) and the details of the three trials are described in the Appendix 2.5 –Table 2-5.

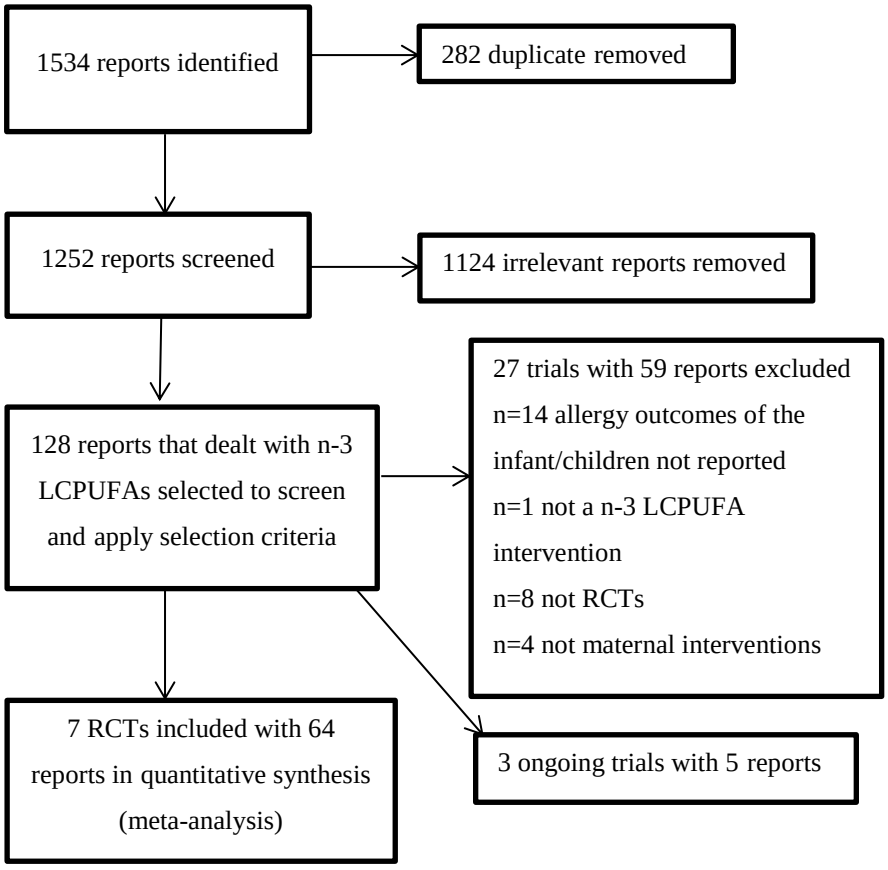


**Figure 2-1: Flow chart presenting process for the selection of included studies**

### 2.4.2 Description of the studies

#### *2.4.2.1 Included studies*

Seven RCTs were identified, with 2223 women (and 2330 children) supplemented with oily fish or fish oil in the pregnancy and/or lactation period and assessed for allergic outcomes of offspring.

### 2.4.3 Summary of study characteristics

#### *2.4.3.1 Study design*

All seven trials were parallel RCTs published in English. Five trials had two parallel groups [26, 27, 259, 267, 299], one trial included two parallel groups and a high fish intake non randomised reference group [260] and one trial had three parallel groups including intervention, placebo and no oil group [266]. A summary of the included trials is shown in Table 2-1 and full details of the included trials are provided in Table 2-2.

**Table 2-1: Summary description of included studies**

| Study | Intervention | Control | Supplementation timing for participants and allergy risk | Age assessed |
|---|---|---|---|---|
| **Dunstan; Australia 2003** | Fish oil = 3.7 g/day<br>DHA = 2.07 g/day<br>EPA = 1.03 g/day | Olive oil | Prenatal = High risk,<br>n = 98<br>20 weeks of gestation until delivery | 1 year |
| **Furuhjelm; Sweden 2009** | Fish oil = 2.7 g/day<br>DHA = 1.1 g/day<br>EPA = 1.6 g/day | Soy oil | Prenatal & postnatal = High risk<br>n = 145<br>25 weeks of gestation to 4 months of age | 6 months<br>1 year<br>2 years |
| **Lauritzen; Denmark 2005** | Fish oil = 4.5 g/day<br>DHA = 342 mg/day<br>EPA = 150 mg/day | Olive oil | Postnatal = Normal risk<br>n = 122<br>1 week after delivery to 4 months of age | 2.5 years |
| **Makrides; Australia 2009** | Fish oil = 3 g/day<br>DHA = 900 mg/day<br>EPA = 195 mg/day | Soy oil | Postnatal = Normal risk<br>n = 603<br>1 week after delivery until expected date of delivery | 1.5 years<br>3 years |
| **Makrides; Australia 2010** | Fish oil = 0.9 g/day<br>DHA = 800 mg/day<br>EPA = 100 mg/day | Vegetable oil blend | Prenatal = High risk<br>n = 706<br>20 weeks of gestation until delivery | 1 year |
| **Noakes; UK 2012** | Fish<br>DHA = 2.32 g/week<br>EPA = 1.14 g/week | No supplement | Prenatal = High risk<br>n =123<br>19 weeks of gestation until delivery | 6 months |
| **Olsen; Denmark 1992** | Fish oil = 2.7 g/day<br>DHA = 1.28 g/day<br>EPA = 0.92 g/day | Olive oil or No supplement | Prenatal = Normal risk<br>n = 533<br>30 weeks of gestation until delivery | 16 years |

**Table 2-2: Detailed description of included studies**

| **Description of Dunstan 2003 [259]** | |
|---|---|
| **Methods** | Randomised controlled trial<br>Dunstan 2003 was the main trial with 11 publications at different times with different outcomes. All 11 publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: Australia<br>98 pregnant atopic women whose fetus was considered to be at high risk of allergic disease. All women had a history of physician-diagnosed allergic rhinitis and/or asthma and one or more positive skin prick tests to common allergens.<br>Exclusions: Women who smoked, had other medical problems, complicated pregnancies, seafood allergy or if normal dietary intake exceeded two meals of fish per week. |
| **Interventions** | Intervention: 4 (1 gram) fish oil capsules per day comprising a total of 3.7 grams of n-3 LCPUFA with 56.0% DHA and 27.7% EPA to give 2.07 grams of DHA and 1.03 grams of EPA (n = 52).<br>Control: 4 (1 gram) capsules of olive oil per day containing 66.6% n-9 oleic acid and <1% n-3 LCPUFA (n = 46)<br>Duration of intervention: 20th week of gestation until delivery |
| **Outcomes** | Primary outcomes: Allergen-specific T-cell responses in cord blood<br>Secondary outcomes: Medically diagnosed allergies including incidence of asthma, atopic eczema, and food allergy at one year of age. The diagnosis of asthma was made in children with recurrent wheezing, i.e. three or more episodes with at least one episode confirmed by a paediatrician or general practitioner. Atopic eczema diagnosis was made in infants exhibiting typical skin lesions or physician-diagnosed eczema response to topical steroids. The severity was scored according to the modified assessment clinical tool called SCORing Atopic Dermatitis (SCORAD).<br>SPT was performed using a standardised technique and allergen extracts (egg, milk, peanut, house dust mite, cat), with histamine positive and glycerine negative controls, weal diameter of ≥ 2 mm was considered positive. |
| **Notes** | Contacted authors to determine if they diagnosed IgE mediated allergies – no response received to date.<br>Supported by grants from the National Health and Medical Research Council and Raine Medical Research Foundation, Australia. |

| Description of Furuhjelm 2009 [299] | |
|---|---|
| **Methods** | Randomised controlled trial<br><br>Furuhjelm 2009 was the main trial with six reports published. All six publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: Sweden<br><br>145 pregnant women at high risk of having a baby with atopy were recruited through antenatal care clinics during a two year period, 2003–2005. Women were considered at high risk if they, their husband or an older child had current or previous allergic symptoms, i.e. bronchial asthma diagnosed by a doctor, atopic eczema, allergic food reactions, itching and running eyes and nose on exposure to pollen, pets or other known allergies. |
| **Interventions** | Intervention: Women took nine capsules a day containing a total of 2.7 g of ω-3 LCPUFA, 35% EPA and 25% DHA, to provide 1.6 grams of EPA and 1.1 grams of DHA, n = 70.<br><br>Control: Nine soy oil capsules a day in total 2.8 g of soybean oil, containing 58% linoleic acid (LA) to provide 2.5 gram LA/day and 6% alpha-linolenic acid (ALA) to provide 0.28 grams ALA/day, n = 75.<br><br>Duration of intervention: 25th week of gestation to delivery with participants encouraged to continue during lactation (average 3 to 4 months). |
| **Outcomes** | 1. Furuhjelm 2009 reported [299]:<br><br>Medically diagnosed allergy outcomes at three, six and 12 months of age including IgE antibody analysis, food allergy and eczema.<br><br>Food allergy was defined as gastrointestinal symptoms, hives, aggravated eczema or wheeze following ingestion of egg or milk in the presence of detectable IgE antibodies or a positive SPT. Recovery from symptoms after elimination of the particular food from the diet and reoccurrence after ingestion of the food was required for the diagnosis. IgE-associated eczema was characterised as reoccurring and itching eczematous, lichenified or nummular dermatitis according to the criteria modified by Oranje 1995 [300] in the presence of detectable IgE antibodies or positive SPT towards egg, milk or wheat.<br><br>2. Furuhjelm 2011 reported [301]:<br><br>Medically diagnosed allergy outcomes at two years of age including: food allergy, eczema, allergic rhinitis, asthma |

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| | and any allergies with or without IgE association. |
| **Notes** | The trial was financially supported by the Medical Research Council of Southeast Sweden (FORSS), The Östergötland County Council, The Ekhaga Foundation, Swedish Asthma and Allergy Association, The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) and The Swedish Society of Medicine and Glaxo Smith Kline, Sweden. |
| **Description of Lauritzen 2005 [260]** | |
| **Methods** | Randomised controlled trial.<br>Lauritzen 2004 was the main trial with nine different publications [302]. All nine publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: Denmark<br>Recruited from women involved in the Danish National Birth Cohort study based on their intake of n-3 LCPUFA. Inclusion criteria included an uncomplicated pregnancy, pre-pregnancy body mass index < 30 kg/m², no metabolic disorders, and the intention to breast-feed for at least four months. Infants had to be healthy, term and singleton with normal weight for gestation and Apgar score > 7. Women with a fish intake below the population median (<0.4 grams n-3 LCPUFA/day) were recruited for the randomised intervention trial (n = 122) and women with a fish intake in the upper quartile (>0.8 grams n-3 LC-PUFA/day) as a high-fish-intake reference group. |
| **Interventions** | Intervention: Microencapsulated fish oil given in muesli bars. The fish oil supplement provided 1.5 gram/day of n-3 LCPUFAs (equivalent to 4.5 gram/day of fish oil) with 22.8% DHA and 10% EPA to provide 0.342 grams per day of DHA and 0.15 grams per day of EPA. As an alternative, the supplements were offered in homemade cookies or oil capsules (n = 62).<br>Control: Microencapsulated olive oil given in muesli bars. As an alternative, the olive oil supplements were offered in homemade cookies or oil capsules (n = 60).<br>Reference group: 64 women with high fish intake (>0.8 grams n-3 LC-PUFA/day).<br>Duration of intervention: 4 months postpartum |
| **Outcomes** | 1. Lauritzen 2004 reported [302]:<br>Primary outcomes: DHA content of breast milk and infant red blood cell membranes at two and four months of age, |

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| | and infant visual acuity at two and four months of age.<br>2. Lauritzen 2005 reported [260]:<br>Primary outcome: Immune function assessed by interferon gamma and IL-10 production and plasma IgE.<br>Secondary outcomes: Parent report of doctor diagnosed food allergy, wheeze or eczema at 2.5 years of age. |
| **Notes** | The high fish intake reference group were not included in our meta-analysis as this group was not randomised.<br>The trial was funded by FOTEK-The Danish Research and Development program for Food Technology and BASF Aktiengesellschaft. |
| **Description of Makrides 2009 [26]** | |
| **Methods** | Randomised controlled trial (the DINO trial).<br>Unpublished data from Makrides 2009 are included in this systematic review. There are 11 publications and one dissertation from this trial. All publications and the dissertation details are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: 5 Australian perinatal centres.<br>Infants born before 33 weeks gestation and within five days of any enteral feeds were eligible to participate (included n = 657 infants and 545 women).<br>Excluded were infants with major congenital or chromosomal abnormalities, from a multiple birth in which not all live-born infants were eligible, or were in other trials of fatty acid supplementation. Lactating women in whom tuna oil was contraindicated (for example, because of bleeding disorders or therapy with anticoagulants) were also excluded.<br>To meet the inclusion criteria for this systematic review only infants whose mothers were supplying breast milk at trial entry (n = 603 infants) were included. A follow-up of the first 143 infants who participated in the pilot phase was conducted at three to five years corrected age (Simmonds 2007 [284]), again only infants whose mothers were providing breast milk at trial entry are included in this systematic review (n = 125). |
| **Interventions** | Intervention: Lactating women assigned to the high-DHA group consumed six 500 mg DHA-rich tuna oil capsules per day which provided 900 mg DHA and 195 mg EPA. The intent was to achieve a breast milk DHA concentration that was ~1% of total fatty acids without altering the naturally occurring concentration of arachidonic acid (AA) in breast |

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| | milk. If supplementary formula was required, infants were given a high-DHA preterm formula (1% DHA and 0.6% AA).<br><br>Control: Lactating women assigned to the standard-DHA group consumed six 500 mg soy oil capsules with no n-3 LCPUFA. If supplementary formula was required in this group, a standard preterm infant formula was used (0.35% DHA and 0.6% AA).<br><br>Duration of the intervention: within five days from the infant receiving any enteral feeds until infants reached their expected date of delivery. |
| **Outcomes** | Primary outcome: Neurodevelopment at 18 months corrected age.<br><br>Secondary outcomes of interest to this systematic review: Parental reported food allergy, eczema, asthma and allergic rhinitis at 18 months corrected age and ISAAC questionnaire to collect parent report of allergy diagnosis and parent report of doctor diagnosed eczema, allergic rhinitis and asthma at three to five years corrected age. (The ISAAC questionnaire is not validated for this age group). |
| **Notes** | This study was supported by the Australian National Health and Medical Research Council and the Channel Seven Children's Research Foundation of South Australia Inc. Treatment and placebo capsules were donated by Clover Corp and infant formula was donated by Mead Johnson Nutritionals and Nutricia Australasia. |
| **Description of Makrides 2010 [27]** | |
| **Methods** | Randomised controlled trial.<br><br>Makrides et al 2010 was the main trial with four publications [27]. All four publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: Australian maternity hospitals.<br><br>Women with singleton pregnancies at less than 21 weeks' gestation were recruited to the primary 'DOMInO' trial (Makrides 2010). Women already taking a prenatal supplement with DHA, with a bleeding disorder in which tuna oil was contraindicated, taking anticoagulant therapy, had a documented history of drug or alcohol abuse, participating in another fatty acid trial, fetus had a known major abnormality, or were unable to give written informed consent or if English was not the main language spoken at home were excluded from the trial (n = 2399).<br><br>Pregnant women were approached to enter the allergy follow-up (Palmer 2012) after randomisation into the DOMInO trial [303]. Only Adelaide based women were eligible for the allergy follow-up. Women were eligible if the unborn |

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| | baby had a mother, father, or sibling with a history of any medically diagnosed allergic disease (asthma, allergic rhinitis, eczema) (n = 706). |
| **Interventions** | Intervention: Three 500 mg capsules daily of DHA rich fish oil concentrate, providing 800 mg of DHA and 100 mg of EPA per day.<br>Control: Three 500 mg vegetable oil blend capsules daily without DHA. |
| **Outcomes** | 1. Makrides 2010 reported [27]:<br>Primary outcome: Maternal postnatal depression at six weeks and six months; child neurodevelopment at 18 months of age.<br>Secondary outcomes: A range of clinical outcomes including post-partum haemorrhage.<br>2. Palmer 2012 reported [303]:<br>Primary outcome: IgE associated allergic diseases – food allergy, eczema, asthma, allergic rhinitis and any allergy with sensitisation at one year of age. The data were imputed for all IgE mediated allergies.<br>Reported medically diagnosed allergic diseases with or without IgE mediated allergic diseases – food allergy, eczema, asthma, allergic rhinitis and any allergy with or without sensitisation based on medically diagnosed allergy at the age of one year. The data were imputed for food allergy, eczema and any allergies but not for asthma (wheeze) and allergic rhinitis.<br>Secondary outcome: IgE sensitisation – SPT at one year of age. Imputed data were used for egg, cow's milk, peanut, any SPT sensitisation but not for wheat, fish, pollen, house dust mite and cat allergens. |
| **Notes** | Supported by grants from the Australian National Health and Medical Research council and Australian Egg corporation Limited. Treatment and placebo capsules were donated by Efamol, UK. |
| **Description of Noakes 2012 [267]** | |
| **Methods** | Randomised controlled trial<br>Miles 2011 was the main trial with 10 reports [304]. All 10 publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: United Kingdom |

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| | Women aged 18 to 40 years who were at 19 weeks' gestation with a healthy uncomplicated pregnancy, reported low habitual consumption of oily fish (2 portions/month excluding canned tuna and no use of fish oil supplements in previous 3 months) and had a family history of atopy (one or more first-degree relatives of the infant affected by atopy, asthma, or allergy) were recruited to the trial (n = 123). Excluded were those participating in another research study, or with diabetes, autoimmune disease, learning disability, terminal illness or mental health problems. |
| **Interventions** | Intervention: Women in the salmon group (n = 62) were provided with two portions of salmon (300 grams)/week which contained 7.12 grams total n-3 LCPUFA (1.14 grams EPA/week and 2.32 grams DHA/week) into their diet from week 20 (trial entry) until they gave birth. They also received a cookbook that provided recipes for preparing and cooking salmon. Each portion of salmon was to replace a serve of protein (e.g. white fish, chicken or red meat).<br>Control: Women in the control group were asked to continue their habitual diets; these women received the information sheet that described the possible health benefits of consuming oily fish during pregnancy and the government recommendation that pregnant women consume one or two oily fish meals/week. They also received a cookbook providing recipes for healthy eating during pregnancy. |
| **Outcomes** | 1. Miles 2011 reported [304]:<br>Primary outcome: determine the effect on maternal and umbilical cord plasma n-3 LCPUFA content<br>2. Noakes 2012 reported [267]:<br>Effect on neonatal immune responses and diagnosis, by research nurse, of eczema and wheeze and SPT at six months of age. |
| **Notes** | Farmed salmon were raised at Skretting Aquaculture Research Centre, Stavanger, Norway, with the use of dietary ingredients selected to contain low concentrations of contaminants. |
| **Description Olsen 1992** [266] | |
| **Methods** | Randomised controlled trial<br>Olsen 1992 was the main trial with 13 publications [266]. All 13 publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: Denmark<br>Pregnant women were recruited from the midwife clinic at 30 weeks' gestation. Women with a history of placental abruption in a previous pregnancy, serious bleeding episode in the present pregnancy, used prostaglandin inhibitors |

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|---|---|
| | regularly, multiple pregnancies, allergy to fish, and regular intake of fish oil were excluded (n = 533). |
| **Interventions** | Intervention: Four 1 gram fish oil capsules daily containing 32% EPA and 23% DHA to provide 1.28 grams EPA and 0.92 grams DHA.<br>Control group 1: Four 1 gram capsules of olive oil daily<br>Control group 2: No supplement<br>Duration of intervention: 30th week of gestation until delivery. |
| **Outcomes** | 1. Olsen 1992 reported [266]:<br>Primary outcome: pregnancy duration.<br>Secondary outcomes: side effects and complications including post-partum bleeding.<br>2. Olsen 2008 reported [305]:<br>Primary outcome: Asthma at 16 years of age.<br>Secondary outcomes: Atopic dermatitis and allergic rhinitis at 16 years of age, medically diagnosed (from a mandatory registry that recorded diagnoses from hospitals in Denmark) allergy outcomes. |
| **Notes** | For this systematic review control groups one & two were combined to compare with the intervention group. |

#### *2.4.3.2 Participants*

Of the 2223 women included in the review, 618 were supplemented during the postnatal period only [26, 260], 145 received supplementation in the prenatal and postnatal period [299] and the remaining 1460 women were supplemented in the prenatal period only [27, 259, 266, 267]. Women whose fetus was at a high risk of allergies were included in four of the trials [27, 259, 267, 299], with the other trials including only women with a fetus at normal risk of allergies [26, 260, 266]. Only one trial examined allergy outcomes of the offspring who were born prematurely and if it was as a result of a multiple birth, all infants were included as one randomisation unit [26]. The main exclusion and inclusion criteria of the individual trials are listed in Table 2-2.

#### *2.4.3.3 Sample sizes*

The number of mothers in the included trials ranged from 98 [259] to 706 [27]. Four trials had approximately 100-150 mothers [259, 260, 267, 269] and three trials had > 500 [26, 27, 266] participants.

#### *2.4.3.4 Study location*

All countries that hosted trials were high income, well developed and industrialised. Three trials, including the two largest as well as the smallest, were undertaken in Australia [26, 27, 259], two in Denmark [260, 266], one in Sweden [299] and one in the UK [267].

#### *2.4.3.5 Intervention*

Five trials used fish oil capsules [26, 27, 259, 266, 269], one used oily fish [267] and in the remaining trial muesli bars containing microencapsulated fish oil were used [260]. However in this trial, cookies containing the fish oil supplement were also used when the muesli bars ran out (or if the women did not like the muesli bars), so not all women received the supplements in the same form [260].

The actual dosage of n-3 LCPUFA in fish oil/oily fish used in the intervention groups varied between 492 mg – 3700 mg/day. Each portion of oily fish replaced a serve of protein (e.g. white fish, chicken or red meat) in the Noakes trial [267]. Control groups received olive oil in three trials [259, 260, 266], soy oil in two trials [26, 299] or a blend of vegetable oils (rapeseed, sunflower, and palm in equal proportions) in one trial [27]. A third randomised group in the Olsen 1992 trial, and the control group in Noakes 2012 did not receive any supplementation [266, 267].

The prenatal trials commenced supplementation at 20 [27, 259, 267] and 30 [266] weeks of gestation and continued until delivery. One trial supplemented in the prenatal and postnatal period, commencing from 25 weeks' gestation and continuing until the infant reached four months of age [299]. In the two postnatal supplementation trials, supplementation commenced within one week after delivery [26, 260]. The duration of postnatal supplementation was four months in Lauritzen 2005 [260], while in Makrides 2009 [26] supplementation continued until the pre-term infants reached 40 weeks postmenstrual age (a median duration of supplementation of 9.4 weeks).

#### *2.4.3.6 Outcome measures*

The allergy outcomes were determined by parental reports of doctor diagnosed allergy in one trial [260] and parental reports of allergy symptoms and parental reports of doctor diagnosed allergy in another trial [26]. Parent reports of allergy outcome data were collected using a non-validated questionnaire in Makrides 2009 [26], therefore these data were only included in the secondary outcomes. Allergy was diagnosed by a clinician in the remaining trials [27, 259, 266, 267, 269]. In the Olsen study [266], the medical diagnosis of allergy was obtained from the Danish Medical registries, while a research nurse made the allergy diagnosis in Noakes 2012 [267]. The age of assessments differed in the trials, with assessments conducted at 6

months [267, 299], 12 months [26, 27, 259, 299], 18 months corrected age [26], 24 months [299], 30 months [260], three to five years corrected age (subgroup) [26] and 16 years of age [266].

Of the five trials that used medical diagnosis of allergies [27, 259, 266, 267, 269], four performed SPT and these four trials included children at high risk of allergy [27, 259, 267, 269]. Two trials reported SPT results for children below 12 months of age [267, 299] and three trials reported SPT between 12 to 36 months of age [27, 259, 299]. Only two studies separated reporting of IgE mediated allergies from other allergies [27, 299]. Two trials used blood samples for IgE detection [267, 299], one trial reported the results at birth and six months of age [267], the other trial analysed the serum IgE levels at three and 12 months of age, but results were not reported [299].

The type of allergies reported in the trials differed. Five trials reported food allergy [26, 27, 259, 260, 269]. Six trials reported atopic dermatitis or eczema [26, 27, 259, 260, 267, 269]. Three trials reported allergic rhinitis [26, 27, 299] and four trials reported any allergy [26, 27, 266, 269]. All trials reported asthma or wheeze [26, 27, 259, 260, 266, 267, 269].

With regards to safety outcomes, two trials reported post-partum haemorrhage [27, 266]. Three trials reported early childhood infections [26, 27, 267]. Infection outcomes (in-hospital proven late onset sepsis) for Makrides 2009 [26] were taken from the whole sample (n=657).

### 2.4.4 Risk of bias in included studies

The included studies were assessed using the criteria in section **2.3.10**.

#### *2.4.4.1 Summary of the risk of bias in included studies*

Overall the seven included studies had various levels of risk of bias for methodological quality (Figure 2-2 and Figure 2-3). A full summary of study quality and risk of bias of the included studies is in Appendix 2.3 – Table 2-3.

**Table 2-3: Summary of study quality and risk of bias of the included studies (see in Appendix 2.3)**

#### *2.4.4.2 Allocation (selection bias)*

All seven trials were at low risk of bias for sequence generation. Six trials reported adequate allocation concealment methods [26, 27, 259, 260, 266, 269] and one trial [267] had an unclear risk of bias as the method of allocation concealment was not described.

#### *2.4.4.3 Blinding (performance bias and detection bias)*

Women, care providers and research personnel were blinded in five trials [26, 27, 259, 260, 269]. In two trials women who were randomised to the 'no supplement' group or fish eating group could not be blinded [266, 267]. Assessors measuring outcomes were blinded to the trial supplementations in all seven trials [26, 27, 259, 260, 266, 267, 269].

#### *2.4.4.4 Incomplete outcome data (attrition bias)*

All trials reported withdrawals and dropouts. Three trials [26, 27, 266] were assessed as having a low risk of attrition bias. Makrides 2009 reported 6% of participant losses to follow-up at 18 months, 8% at three to five years of age and 10.5% at seven years of age [26]. Olsen 2008 [305] reported 1% of participant losses to follow-up at 16 years of age. Makrides 2010 reported participant follow-up losses of 3.5% and 9.6% at one and three years of age respectively [27]. Dunstan 2003, Furuhjelm 2009, Lauritzen 2005 and Noakes 2012 were assessed as having a high risk of attrition bias [259, 260, 267, 269]. Dunstan 2003 reported a

loss of 15% to follow-up and participant exclusion after randomisation, which differed between intervention (n = 12, 23%) and control (n = 3, 6.5%). More women in the intervention group discontinued treatment because of nausea (n = 7, 13.5%) than in the control (n = 1, 2%) [259]. Furuhjelm 2009 reported a 19% loss of participants to follow-up (n = 16), with 23% in the treatment group (n = 9) and 12% in the control group excluded from the analysis because they did not complete the 15-week intervention [269]. Lauritzen 2005 had large follow-up losses of more than 47%, with no reasons reported for the withdrawals (treatment n = 25, 40%; control n = 32, 53%) [260]. Noakes 2012 also had large follow-up losses (30%) at six months with no reasons reported (treatment n = 16, 23%; control n = 23, 37%) [267].

#### *2.4.4.5 Selective reporting (reporting bias)*

Five trials [26, 259, 260, 266, 267] were assessed as having a high risk of reporting bias, as either all of the expected outcomes of interest to this review were not reported or outcomes of interest to the review were not reported completely.

#### *2.4.4.6 Other potential sources of bias*

There was no obvious other potential bias identified in three trials [266, 267, 269]. There was an unclear risk of bias in the two trials that included subgroups of their original trials [26, 27]. In Olsen 1992 [266], the placebo group and no oil group were combined in this review. We conducted analyses with the n-3 LCPUFA supplementation group compared to olive oil control group separately to n-3 LCPUFA supplementation group compared to no supplement control group, and found that although the direction of effect differed it made little difference to the meta-analysis therefore for this review the control groups were combined. Dunstan 2003 [259] was rated as having an unclear risk of bias because preterm infants were excluded from their analysis after randomisation.

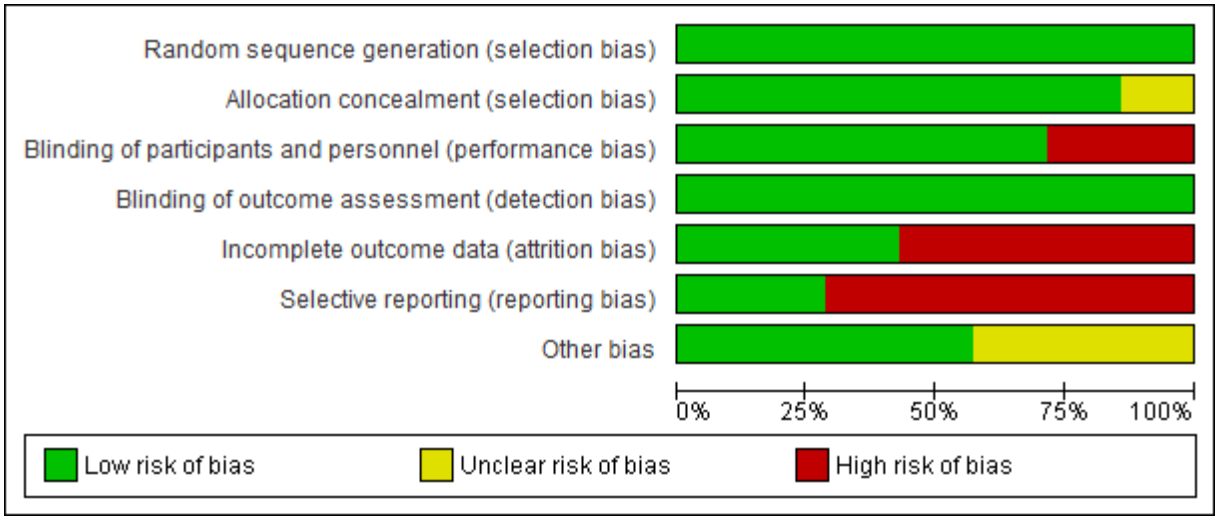


**Figure 2-2: Risk of bias graph: judgements about each risk of bias item presented as percentages across all included studies.**

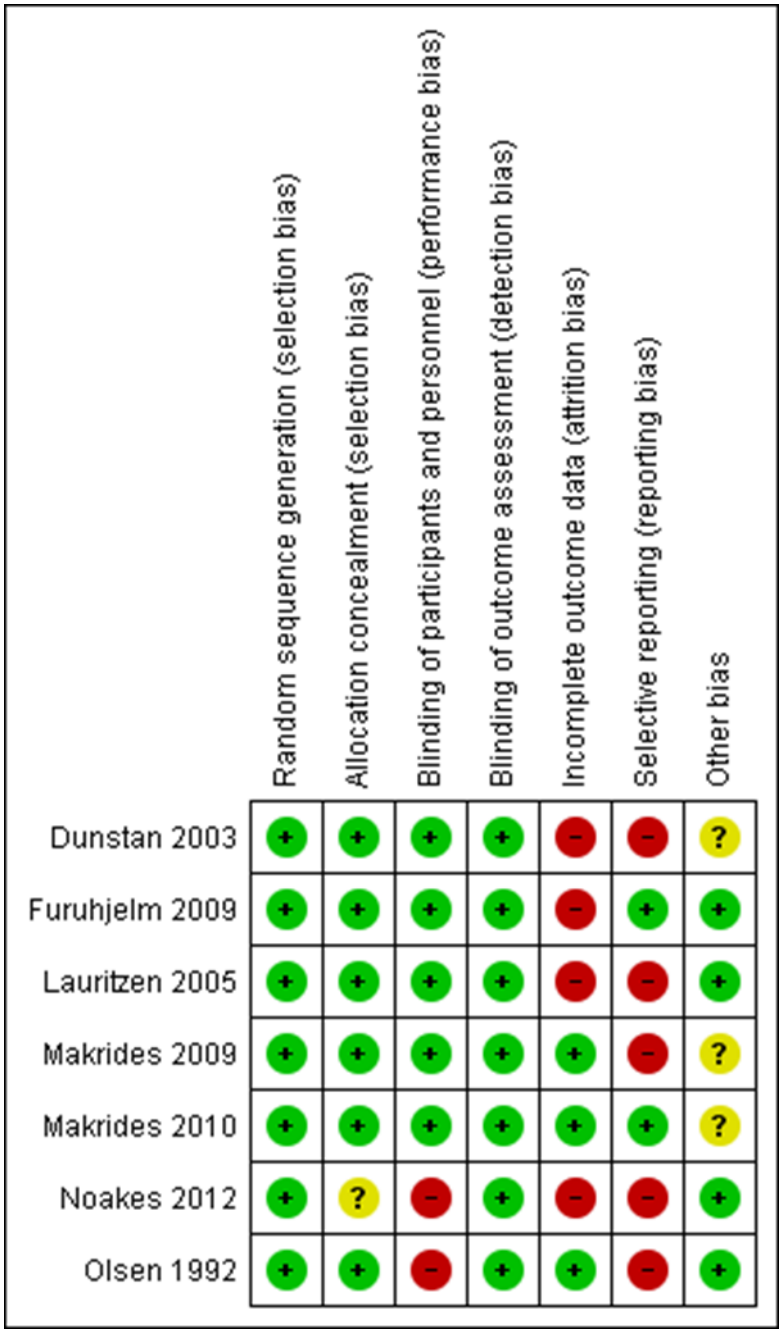


**Figure 2-3: Risk of bias summary: judgments about each risk of bias item for each included study.**

### 2.4.5 Excluded studies

Twenty seven trials were excluded (see characteristics of excluded studies table, Appendix 2.4 – Table 2-4). Fourteen trials were excluded as they did not report allergy outcomes of the infants or children [306-319], one trial [320] was excluded as it was not n-3 LCPUFA intervention, eight trials [79, 218, 219, 321-325] were not RCTs and four trials [261, 262, 326, 327] were not maternal interventions.

**Table 2-4: Characteristics of excluded studies**
**(see in Appendix 2.4)**

### 2.4.6 On-going studies

Three trials are on-going (see characteristics of on-going studies Appendix 2.5 – Table 2-5) [268, 328, 329]. One study [268] has been included under included studies in updated version of the review in Chapter 6 and other two studies will be included in future versions of this review.

**Table 2-5: Characteristics of on-going studies**
**(see in Appendix 2.5)**

### 2.4.7 Main results

**Primary outcome measures:**

#### *2.4.7.1 IgE mediated allergies (with sensitisation)*

One study conducted in Sweden (with pre- and postnatal n-3 LCPUFA supplementation) and one conducted in Australia (with prenatal supplementation only) reported IgE mediated allergies [27, 299] (Figure 2-4 - Figure 2-6).

##### 2.4.7.1.1 IgE mediated food allergy (Figure 2-4)

IgE mediated food allergy was significantly reduced in the first year of life in the n-3 LCPUFA supplemented group (1 trial, 117 participants, RR 0.13, 95% CI 0.02 to 0.95, P=0.04) (Table 2-6 and Figure 2-4). No significant difference between the n-3 LCPUFA group and control was found in children between 12–36 months of age and for any age follow-up (cumulative incidence; (Table 2-6)). A random effects model was used due to substantial heterogeneity between trials noticed for the cumulative incidence ($Chi^2 = 2.98$, df = 1; P = 0.08; $I^2 = 66\%$, Figure 2-4). The heterogeneity may be due to the duration of the intervention, the dose used and the difference in assessment ages. No studies reported this outcome for children over 36 months of age.

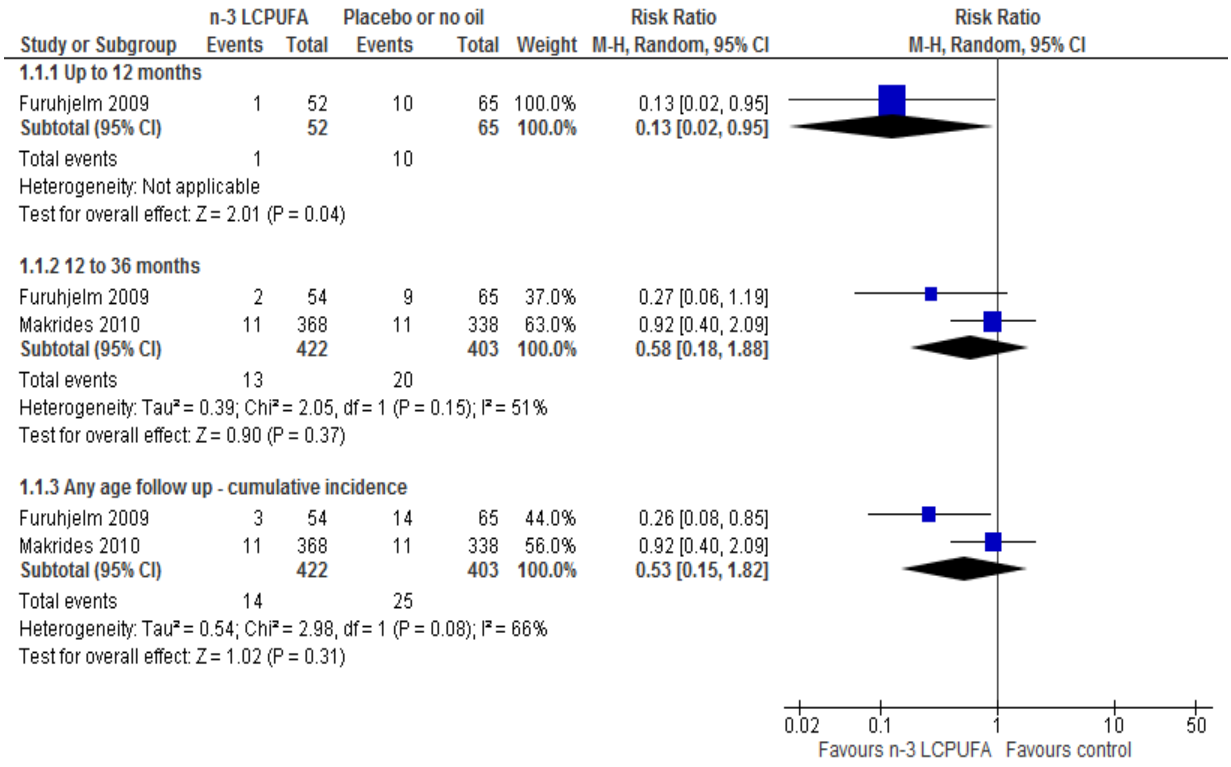


**Figure 2-4: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil) -Food allergies with IgE sensitisation**

2.4.7.1.2 IgE mediated eczema (Figure 2-5)

There was a non-significant reduction in eczema in infants below 12 months of age in the n-3 LCPUFA group (Table 2-6 and Figure 2-5). In children between 12–36 months of age the risk of IgE mediated eczema was significantly reduced (2 trials, 823 participants, RR 0.61, 95% CI 0.39 to 0.95, P = 0.03**)** (Table 2-6 and Figure 2-5). The cumulative incidence of eczema was significantly reduced in children assessed at any age (2 trials, 823 participants, RR 0.56, 95% CI 0.36 to 0.85, P = 0.006), however no studies reported this outcome for children over 36 months of age (Table 2-6 and Figure 2-5).

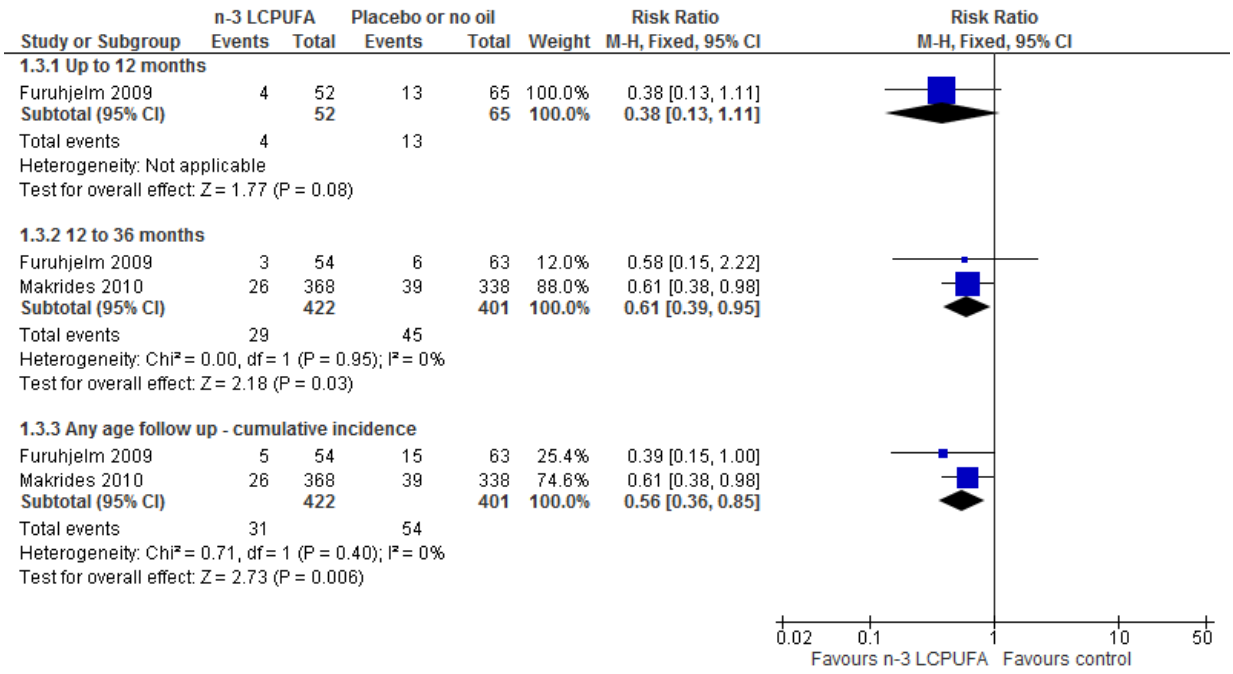


**Figure 2-5: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-Eczema with IgE sensitisation**

2.4.7.1.3 IgE mediated allergic rhinitis, asthma/wheeze

In children 12–36 months of age no significant difference was found between the n-3 LCPUFA and control groups in either IgE mediated allergic rhinitis or IgE mediated asthma/wheeze (Table 2-6). No studies reported these outcomes for children below 12 or over 36 months of age.

2.4.7.1.4 Any IgE mediated allergies

Risk of IgE mediated allergies were significantly reduced in children between 12–36 months of age (2 trials, 823 participants, RR 0.66, 95% CI 0.44 to 0.99, P = 0.04) and at any age follow-up (cumulative incidence) (2 trials, 823 participants, average RR 0.55, 95% CI 0.31 to 0.99, P = 0.05) in the n-3 LCPUFA supplemented group (Table 2-6 and Figure 2-6). A random effects model was used due to the substantial heterogeneity between trials reporting any allergies at any age follow-up ($Chi^2 = 1.72$, df = 1; P = 0.19; $I^2 = 42\%$, Figure 2-6).

It was not possible to estimate the proportion of children with IgE mediated any allergies below 12 or beyond 36 months of age because no included trials reported on combined IgE mediated allergies in infants under 12 months of age and no included trials reported on any IgE mediated allergies beyond 36 months of age (Table 2-6).

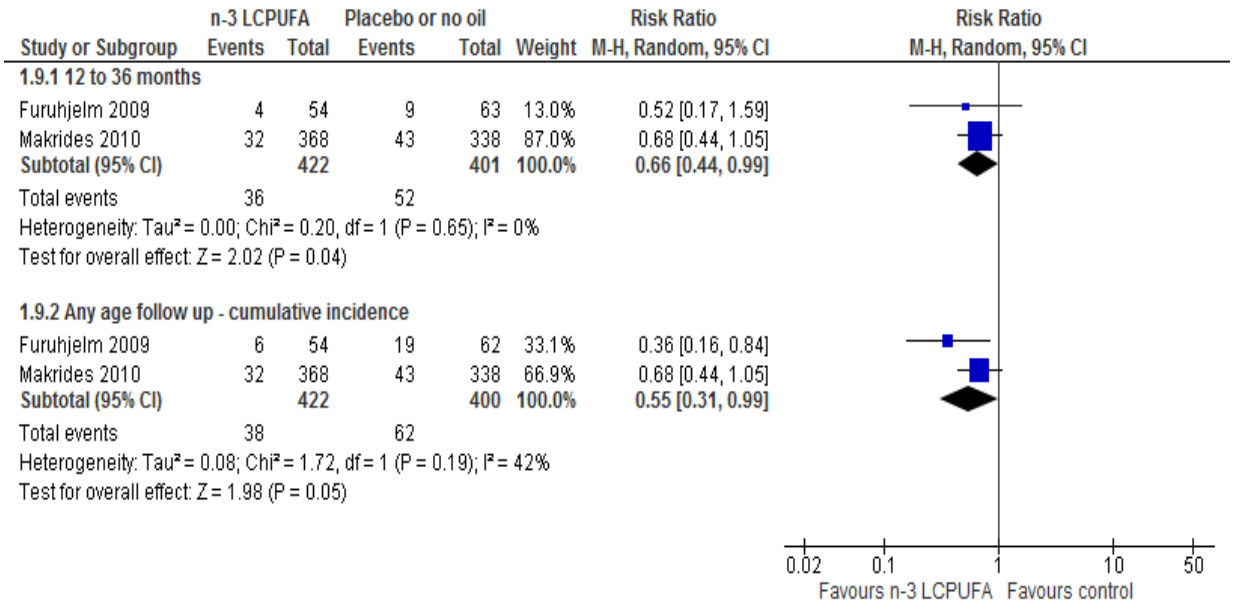


**Figure 2-6: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-One or more allergies with IgE sensitisation**

#### *2.4.7.2 All allergies (with or without sensitisation)*

In the meta-analyses, overall analysis of the effect of n-3 LCPUFA was performed with all data irrespective of the type of allergy assessment (with and without IgE sensitivity) and medical diagnosis or parental report (using validated questionnaire). Six studies reported allergies with/without sensitisation [27, 259, 260, 266, 267, 269].

##### 2.4.7.2.1 Food allergy – with and without IgE sensitivity

The incidence of food allergy (with and without IgE sensitivity) was significantly reduced in the first year of life (1 trial, 117 participants, RR 0.13, 95% CI 0.02 to 0.95, P = 0.04) in participants randomised to n-3 LCPUFA supplementation (Table 2-7). This was however due solely to the reduction in IgE mediated food allergies. No significant reduction was found in food allergy incidence (with and without IgE sensitivity) in children at any other time interval (4 trials, 973 participants, Table 2-7).

##### 2.4.7.2.2 Eczema – with and without IgE sensitivity

The risk of eczema (with and without IgE sensitivity) did not significantly differ in children of any age in the n-3 LCPUFA supplemented groups (Table 2-7 and Figure 2-7). No trials reported this outcome for children over 36 months of age. A random effects model was used due to the substantial heterogeneity between trials reporting eczema below 12 months of age ($Chi^2 = 3.43$, df = 1; P = 0.06; $I^2 = 71\%$, Figure 2-7). This heterogeneity may be due to differences in the intervention, the duration of the intervention or the way eczema was reported or a combination of these.

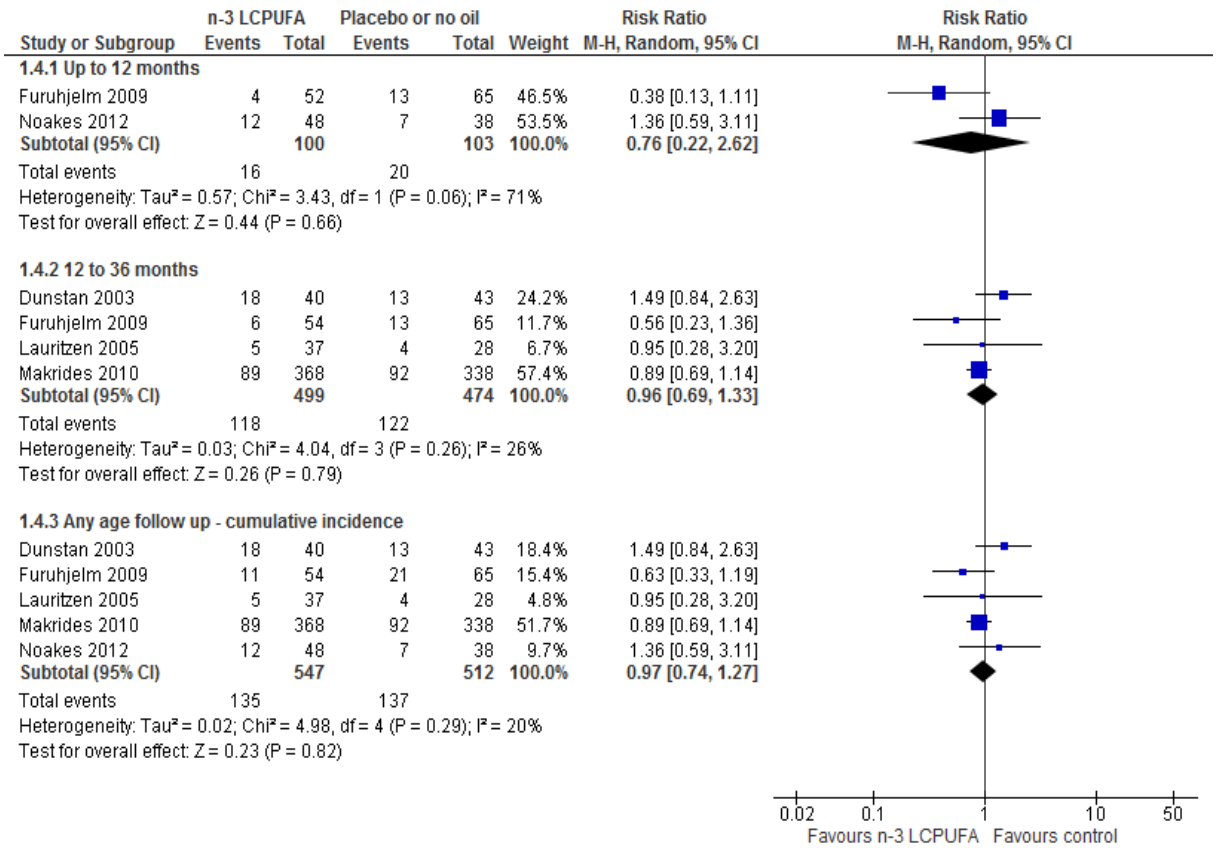


**Figure 2-7: Comparison: n-3 LCPUFA (fish or fish oil) supplementation versus control (placebo or no oil)-Eczema with/without IgE sensitisation**

2.4.7.2.3 Allergic rhinitis – with and without IgE sensitivity

There was no significant difference in allergic rhinitis (with and without IgE sensitivity) detected at any age group. No trials reported this outcome below 12 months (Table 2-7).

2.4.7.2.4 Asthma/wheeze – with and without IgE sensitivity

No significant differences for asthma in general (with and without IgE sensitivity) were detected between n-3 LCPUFA and control groups at any age group (Table 2-7).

2.4.7.2.5 Any allergies – with and without IgE sensitivity

At any age of follow-up, no significant differences on the proportions of children with at least one allergy (with and without IgE sensitivity) were observed between n-3 LCPUFA and control groups (Table 2-7 and Figure 2-8). No trials reported on combined allergy outcomes, below 12 months (Table 2-7).

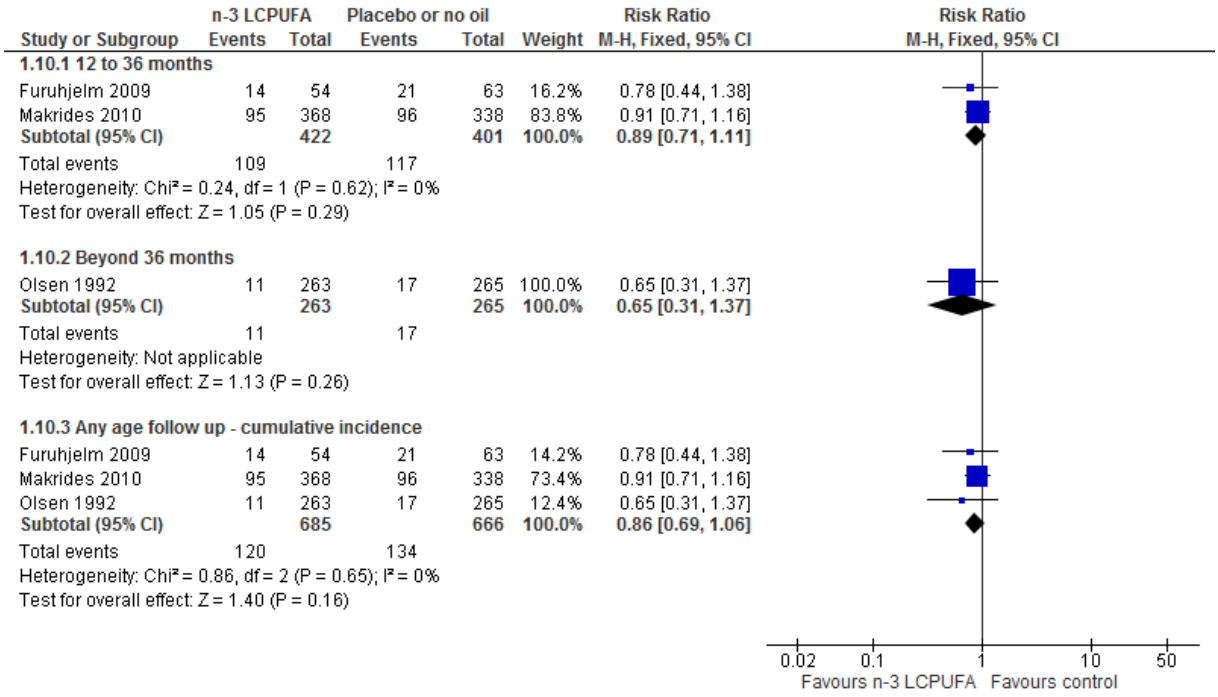


**Figure 2-8: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-One or more allergies with/without IgE sensitisation**

**Table 2-6: Summary of meta-analyses of n-3 LCPUFA supplementation on IgE mediated allergies using pooled analysis RR (M-H, Fixed/Random, 95% CI)**

| ***Allergy*** *(IgE mediated)* | ***Assessed age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Control*** | | ***Effect estimate RR (95% CI)*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Food allergy* | <12 months | 1 | 117 | 1 | 52 | 10 | 65 | **0.13 [0.02, 0.95]*** |
| | 12-36 months | 2 | 825 | 13 | 422 | 20 | 403 | 0.58 [0.18, 1.88]$^{R}$ |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 825 | 14 | 422 | 25 | 403 | 0.53 [0.15, 1.82] $^{R}$ |
| *Eczema* | <12 months | 1 | 117 | 4 | 52 | 13 | 65 | 0.38 [0.13, 1.11] |
| | 12-36 months | 2 | 823 | 29 | 422 | 45 | 401 | **0.61 [0.39, 0.95]*** |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 823 | 31 | 422 | 54 | 401 | **0.56 [0.36, 0.85]*** |
| *Allergic rhinitis* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 825 | 1 | 422 | 3 | 403 | 0.47 [0.07, 3.06] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 825 | 1 | 422 | 3 | 403 | 0.47 [0.07, 3.06] |
| *Asthma* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 824 | 3 | 422 | 4 | 402 | 0.86 [0.21, 3.49] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 824 | 3 | 422 | 4 | 402 | 0.86 [0.21, 3.49] |
| *Any allergies* | <12 months | | | | | | | |
| | 12-36 months | 2 | 823 | 36 | 422 | 52 | 401 | **0.66 [0.44, 0.99]*** $^{R}$ |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 822 | 38 | 422 | 62 | 400 | **0.55 [0.31, 0.99]*** $^{R}$ |

* Statistically significant $P \leq 0.05$, NE= Not estimable, $^{R=}$ Random effect estimate

**Table 2-7: Summary of meta-analyses of n-3 LCPUFA (fish or fish oil) supplementation on all allergies using pooled analysis RR (M-H, Fixed/Random, 95% CI)**

| ***Allergy*** (with and without IgE sensitivity) | ***Assessed age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Control*** | | ***Effect estimate RR (95% CI)*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Food allergy* | <12 months | 1 | 117 | 1 | 52 | 10 | 65 | **0.13 [0.02, 0.95]*** |
| | 12-36 months | 4 | 973 | 19 | 499 | 26 | 474 | 0.72 [0.40, 1.30] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 4 | 973 | 23 | 499 | 33 | 474 | 0.71 [0.42, 1.20] |
| *Eczema* | <12 months | 2 | 203 | 16 | 100 | 20 | 103 | 0.76 [0.22, 2.62]$^{R}$ |
| | 12-36 months | 4 | 973 | 118 | 499 | 122 | 474 | 0.92 [0.74, 1.14] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 5 | 1059 | 135 | 547 | 137 | 512 | 0.94 [0.76, 1.15] |
| *Allergic rhinitis* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 805 | 10 | 414 | 18 | 391 | 0.53 [0.25, 1.12] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 805 | 10 | 414 | 18 | 391 | 0.53 [0.25, 1.12] |
| *Asthma* | <12 months | 1 | 83 | 11 | 46 | 7 | 37 | 1.26 [0.54, 2.94] |
| | 12-36 months | 4 | 955 | 106 | 491 | 105 | 464 | 0.93 [0.73, 1.18] |
| | ≥36 months | 1 | 528 | 8 | 263 | 14 | 265 | 0.58 [0.25, 1.35] |
| | Any age follow-up | 5 | 1566 | 125 | 800 | 126 | 766 | 0.91 [0.73, 1.14] |
| *Any allergies* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 823 | 109 | 422 | 117 | 401 | 0.89 [0.71, 1.11] |
| | ≥36 months | 1 | 528 | 11 | 263 | 17 | 265 | 0.65 [0.31, 1.37] |
| | Any age follow-up | 3 | 1351 | 120 | 685 | 134 | 666 | 0.86 [0.69, 1.06] |

* Statistically significant $P \leq 0.05$, NE= Not estimable, $^{R}$ = Random effect estimate *(All allergies irrespective of IgE mediated or not and irrespective of type of assessment (doctor or parent reports))*

**Secondary outcomes measures:**

*2.4.7.3 Skin prick test results*

A positive SPT enables classification of an individual as atopic (hallmark of IgE mediated allergies). While a positive result may occur in the absence of clinical symptoms, it identifies the individual at risk for allergic symptoms and is a strong predictor of IgE mediated allergy [129]. No trials reported SPT results over 36 months of age.

2.4.7.3.1 Skin prick test results for food allergens

***Egg***

In children under 12 months of age a reduction in sensitisation to egg, approaching significance, was found in the n-3 LCPUFA group compared to control (2 trials, 203 participants, RR 0.44, 95% CI 0.19 to 1.04, P = 0.06) (Table 2-8 and Figure 2-9). In children between 12–36 months of age a significant reduction was found in the n-3 LCPUFA supplemented group for SPT sensitivity to egg (3 trials, 893 participants, RR 0.55, 95% CI 0.39 to 0.77, P = 0.0005) and at any age follow-up (cumulative incidence) 4 trials, 977 participants, RR 0.55, 95% CI 0.40 to 0.76, P = 0.0003) (Table 2-8 and Figure 2-9).

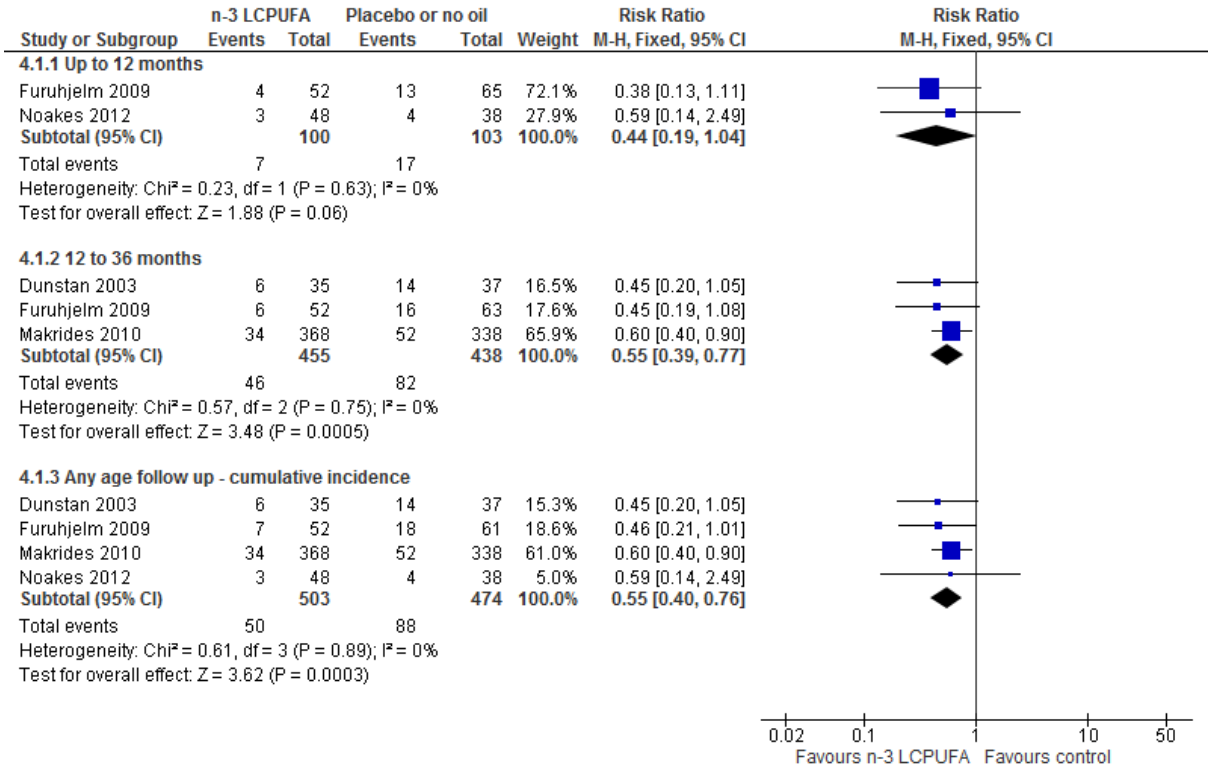


**Figure 2-9: Comparison: n-3 LCPUFA (fish or fish oil) supplementation versus control (placebo or no oil)-Skin prick sensitisation to egg**

### *Cow's milk, wheat, fish, peanut*

No significant differences in sensitisation to cow's milk, wheat, fish and peanut were found between children in the n-3 LCPUFA supplemented groups and controls (Table 2-8). Sensitisation to cows' milk and wheat was not significantly different between the treatment groups at any age of assessment, although no trials contained data for children aged 36 months or older. Similar results were seen with sensitisation to fish (no trials up to 12 months of age or above 36 months of age) and to peanut (children were only assessed for peanut allergy between 12 to 36 months of age) where no significant differences were detected between the n-3 LCPUFA supplemented groups and controls (Table 2-8). The number of children with positive SPT to cow's milk, wheat, fish and peanut were 31 out of 977; 5 out of 783; 3 out of 666 and 46 out of 778 respectively.

#### 2.4.7.3.2 Skin prick test results for inhalant allergens

The event rate was very low below 36 months of age for inhalant allergens (Table 2-8). For example only 7 out of 779, 7 out of 738 and 17 out of 824 children were sensitised to pollens, house dust mite and cat respectively. No significant differences in sensitisation to inhalant allergens was found in children up to 36 months of age in the n-3 LCPUFA supplemented group compared to the control group (Table 2-8).

#### 2.4.7.3.3 Skin prick test results for food and inhalant allergens

When sensitisation to one or more allergens was considered, there was no significant difference in sensitisation to any allergens in infants below 12 months of age in the n-3 LCPUFA supplemented group compared to control (2 trials, 201 participants) (Table 2-8 and Figure 2-10). However, a significant reduction in sensitisation to any allergens was found in the children between 12 to 36 months of age (3 trials, 892 participants, RR 0.70, 95% CI 0.53 to 0.94, P = 0.02) and at any age follow-up (cumulative incidence) up to 3 years of age (4 trials, 977 participants, RR 0.70, 95% CI 0.54 to 0.91, P = 0.007) who were supplemented with n-3 LCPUFA, which was largely driven by food allergens (Table 2-8 and Figure 2-10).

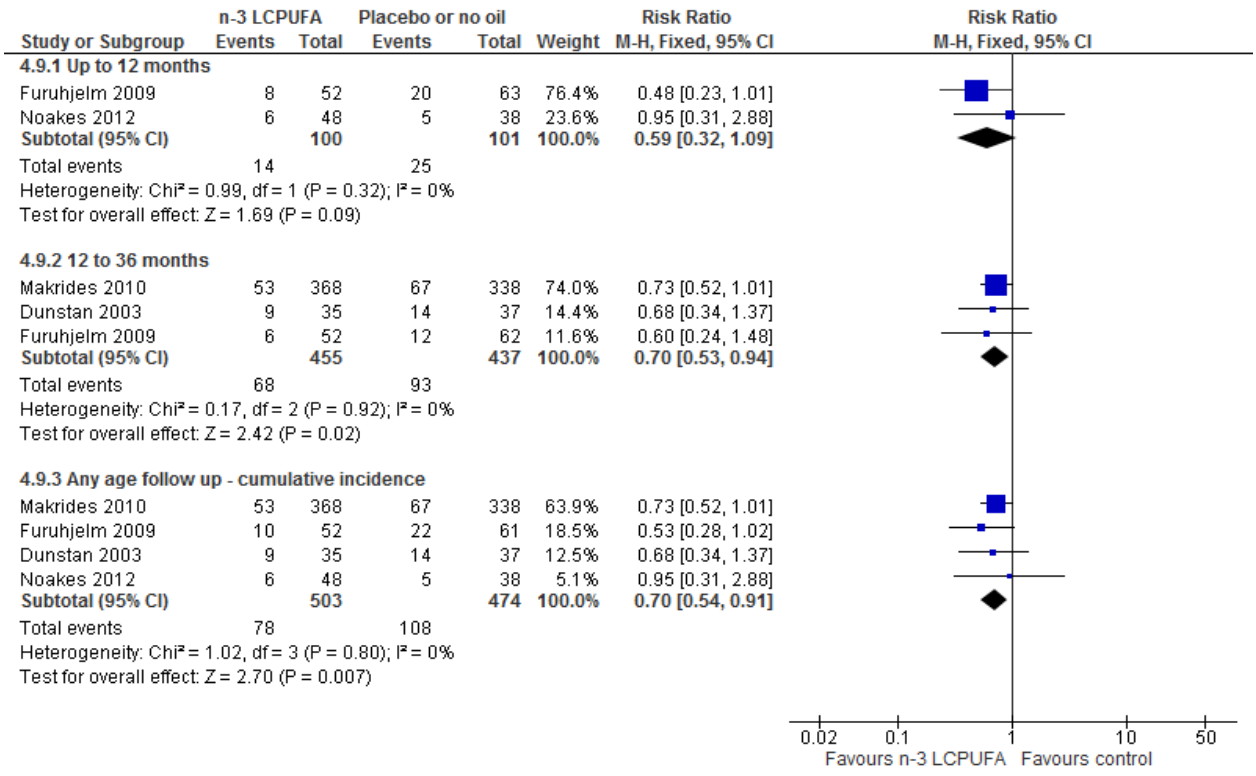


**Figure 2-10: Comparison: n-3 LCPUFA (fish or fish oil) supplementation versus control (placebo or no oil)-Skin prick sensitisation to one or more allergen**

**Table 2-8: Summary of meta-analyses of n-3 LCPUFA (fish or fish oil) supplementation on skin prick results for food allergens using pooled analysis RR (M-H, Fixed, 95% CI)**

| ***Food allergen*** | ***Assessed age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Control*** | | ***Fixed effect estimate RR (95% CI)*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Egg* | <12 months | 2 | 203 | 7 | 100 | 17 | 103 | 0.44 [0.19, 1.04] |
| | 12-36 months | **3** | **893** | **46** | **455** | **82** | **438** | **0.55 [0.39,0.77]**** |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | **4** | **977** | **50** | **503** | **88** | **474** | **0.55 [0.40, 0.76]**** |
| *Cow's milk* | <12 months | 2 | 205 | 5 | 102 | 7 | 103 | 0.75 [0.24, 2.29] |
| | 12-36 months | 3 | 897 | 9 | 457 | 13 | 440 | 0.72 [0.31, 1.68] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 4 | 977 | 13 | 503 | 18 | 474 | 0.73 [0.36, 1.48] |
| *Peanut* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 778 | 18 | 403 | 28 | 375 | 0.61 [0.34, 1.08] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 778 | 18 | 403 | 28 | 375 | 0.61 [0.34, 1.08] |
| *Wheat* | <12 months | 1 | 117 | 1 | 52 | 0 | 65 | 3.74 [0.16, 89.85] |
| | 12-36 months | 2 | 783 | 3 | 401 | 2 | 382 | 1.43 [0.24, 8.50] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 783 | 3 | 401 | 2 | 382 | 1.43 [0.24, 8.50] |
| *Fish* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 666 | 3 | 349 | 0 | 317 | 6.36 [0.33, 122.65] |
| | ≥36 months | 0 | 0 | | | | | NE |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Any age follow-up | 1 | 666 | 3 | 349 | 0 | 317 | 6.36 [0.33, 122.65] |
| ***Inhalant allergen*** | | | | | | | | |
| *Pollen allergens* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 779 | 2 | 400 | 5 | 379 | 0.44 [0.08, 2.30] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 779 | 2 | 400 | 5 | 379 | 0.44 [0.08, 2.30] |
| *House dust mite* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 738 | 3 | 384 | 4 | 354 | 0.76 [0.18, 3.28] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 2 | 738 | 3 | 384 | 4 | 354 | 0.76 [0.18, 3.28] |
| *Cat* | <12 months | 1 | 86 | 1 | 48 | 1 | 38 | 0.79 [0.05, 12.25] |
| | 12-36 months | 2 | 738 | 8 | 384 | 7 | 354 | 1.07 [0.39, 2.94] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 3 | 824 | 9 | 432 | 8 | 392 | 1.03 [0.40, 2.66] |
| ***Food/Inhalant allergen*** | | | | | | | | |
| *Any allergen* | <12 months | 2 | 201 | 14 | 100 | 25 | 101 | 0.59 [0.32, 1.09] |
| | 12-36 months | **3** | **892** | **68** | **455** | **93** | **437** | **0.70 [0.53, 0.94]*** |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | **4** | **977** | **78** | **503** | **108** | **474** | **0.70 [0.54, 0.91]*** |

***** Statistically significant P ≤ 0.05, ****** Statistically significant P < 0.005, NE= Not Estimable

#### *2.4.7.4 Maternal safety*

**Post-partum haemorrhage and infection/sepsis**

Trials in this review supplemented pregnant women with higher n-3 LCPUFA doses than currently recommended [166, 225], so it is valuable to consider any adverse effects. Post-partum bleeding was defined as blood loss of greater than 500 ml post-delivery. Two trials with 2,932 participants reported data for this outcome [27, 266]. No significant difference was found in the n-3 LCPUFA group compared to control (2 trials, 2,932 participants) (Table 2-9). A random effects model was used because heterogeneity between trials was substantial ($Chi^2$ = 2.49, df = 1; P = 0.11; $I^2$ = 60%, results not shown), most likely due to the large difference in sample size between the two trials (2399 vs. 533), different doses of n-3 LCPUFA (2.7 g/day vs. 0.9 g/day) and different durations of supplementation (10 weeks vs. 20 weeks) (more details in Table 2-1). No reports of post-partum sepsis were found in the included trials.

#### *2.4.7.5 Infant safety*

**Early childhood infection**

Three trials reported early childhood infections for 1446 participants [26, 27, 267]. No significant difference was detected between the n-3 LCPUFA and control groups for early childhood infections (3 trials, 1446 participants) (Table 2-9).

**Table 2-9: Summary of meta-analyses reporting the effects of n-3 LCPUFA (fish or fish oil) supplementation on post-partum haemorrhage and early childhood infections using pooled analysis RR (M-H, Fixed/Random, 95% CI)**

| ***Secondary outcomes*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Control*** | | ***Effect estimate RR (95% CI)*** |
|---|---|---|---|---|---|---|---|
| | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| Post-partum haemorrhage | 2 | 2932 | 91 | 1463 | 122 | 1469 | 0.73 (0.49, 1.10)[R] |
| Early childhood infection | 3 | 1446 | 119 | 736 | 117 | 710 | 0.98 (0.78, 1.24) |

[R]=Random effect estimate

#### *2.4.7.6 Parental reports of allergy outcomes*

In this systematic review, parent reported allergy outcomes collected using non-validated questionnaires were included as secondary outcomes. Only one trial used non-validated questionnaire to collect parental reports of allergy [26]. The results showed no significant differences in the parental reports of allergies in children assessed at any age up to 5 years of age between the n-3 LCPUFA supplemented and control groups (Table 2-10).

**Table 2-10: Summary of meta-analyses reporting the effects of n-3 LCPUFA supplementation on parental reports of allergy (non-validated questionnaires) using pooled analysis RR (M-H, Fixed, 95% CI)**

| *Outcomes* | *Assessed age* | *No of studies* | *No of participants* | *n-3 LCPUFA* | | *Control* | | *Fixed effect estimate* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Events* | *Total* | *Events* | *Total* | *RR (95% CI)* |
| *Food Allergy* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 565 | 10 | 274 | 13 | 291 | 0.82 [0.36, 1.83] |
| | ≥36 months | 0 | 0 | | | | | NE |
| | Any age follow-up | 1 | 565 | 10 | 274 | 13 | 291 | 0.82 [0.36, 1.83] |
| *Eczema* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 565 | 44 | 274 | 50 | 291 | 0.93 [0.65, 1.35] |
| | ≥36 months | 1 | 108 | 17 | 49 | 19 | 59 | 1.08 [0.63, 1.35] |
| | Any age follow-up | 1 | 578 | 58 | 281 | 69 | 287 | 0.89 [0.65, 1.21] |
| *Allergic rhinitis* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 565 | 6 | 274 | 9 | 291 | 0.71 [0.26, 1.96] |
| | ≥36 months | 1 | 109 | 14 | 49 | 11 | 60 | 1.56 [0.78, 3.12] |
| | Any age follow-up | 1 | 578 | 20 | 281 | 20 | 297 | 1.06 [0.58, 1.92] |
| *Asthma/wheeze* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 565 | 37 | 274 | 41 | 291 | 0.96 [0.63, 1.45] |
| | ≥36 months | 1 | 107 | 8 | 49 | 14 | 58 | 0.68 [0.31, 1.48] |
| | Any age follow-up | 1 | 578 | 44 | 281 | 54 | 297 | 0.86 [0.60, 1.24] |
| *Any allergies* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 565 | 82 | 274 | 92 | 291 | 0.95 [0.74, 1.21] |
| | ≥36 months | 1 | 110 | 25 | 49 | 34 | 61 | 0.92 [0.64, 1.30] |
| | Any age follow-up | 1 | 578 | 97 | 291 | 118 | 297 | 0.87 [0.70, 1.08] |

NE= Not estimable

#### *2.4.7.7 Summary of the results*

A summary highlighting the significant effects reported above of n-3 LCPUFA supplementation on childhood allergies is presented in Table 2-11.

Children up to 12 months of age had significant reduction of medically diagnosed IgE mediated food allergy with n-3 LCPUFA supplementation. Only 11 cases (9%) in both groups presented with IgE mediated food allergy out of 117 children assessed. After n-3 LCPUFA supplementation, significant reductions in the risk of children having IgE mediated eczema and IgE mediated any allergies when measured at 12-36 months of age and cumulatively were observed. Overall 85 cases (10%) presented with IgE mediated eczema out of 823 children and 100 cases (12%) presented with one or more IgE mediated allergies from 822 children in the both groups. No significant reduction was observed with maternal n-3 LCPUFA supplementation on IgE mediated allergic rhinitis and IgE mediated asthma or combined medically diagnosed or parental reported allergies which included IgE and non-IgE mediated allergic diseases (eczema, allergic rhinitis, asthma and any allergy) in children to and beyond three years of age except combined food allergy incidence which was significantly reduced in children less than 12 months with the reduction due to IgE mediated food allergy.

One of the secondary outcomes of the systematic review was skin prick sensitisation in children. The results show a reduction in egg sensitisation and sensitisation to any allergen when measured at 12-36 months of age and cumulatively when mothers were supplemented with n-3 LCPUFA. Out of 977 children, 138 children (14%) were sensitised to egg while 186 children (19%) were sensitised to one or more allergen when cumulative incidence were considered from birth to 3 years of age in combined n-3 LCPUFA and control groups.

**Table 2-11: Summary of the significant effects of n-3 LCPUFA (fish or fish oil) supplementation on childhood allergies/allergen sensitisation**

| *Assessment* | *Assessed Age* | *No of studies* | *No of participants* | *n-3 LCPUFA* | | *Control* | | *Effect Estimate* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Events* | *Total* | *Events* | *Total* | *RR(95% CI)* |
| ***Allergy (IgE mediated)*** | | | | | | | | |
| *Food allergy* | <12 months | 1 | 117 | 1 | 52 | 10 | 65 | 0.13 [0.02, 0.95] * |
| *Eczema* | 12-36 months | 2 | 823 | 29 | 422 | 45 | 401 | 0.61 [0.39, 0.95] * |
| *Eczema* | Any age follow-up | 2 | 823 | 31 | 422 | 54 | 401 | 0.56 [0.36, 0.85] * |
| *Any allergies* | 12-36 months | 2 | 823 | 36 | 422 | 52 | 401 | 0.66 [0.44, 0.99] * |
| *Any allergies* | Any age follow-up | 2 | 822 | 38 | 422 | 62 | 400 | 0.59 [0.40, 0.87] * |
| ***Allergy (with and without IgE sensitivity)*** | | | | | | | | |
| *Food allergy* | <12 months | 1 | 117 | 1 | 52 | 10 | 65 | 0.13 [0.02, 0.95] * |
| ***Skin prick results*** | | | | | | | | |
| *Egg* | 12-36 months | 3 | 893 | 46 | 455 | 82 | 438 | 0.55 [0.39, 0.77] ** |
| *Egg* | Any age follow-up | 4 | 977 | 50 | 503 | 88 | 474 | 0.55 [0.40, 0.76] ** |
| *Food and/or Inhalant allergen* | 12-36 months | 3 | 892 | 68 | 455 | 93 | 437 | 0.70 [0.53, 0.94] * |
| *Food and/or Inhalant allergen* | Any age follow-up | 4 | 977 | 78 | 503 | 108 | 474 | 0.70 [0.54, 0.91] * |

* Statistically significant $P \leq 0.05$, ** Statistically significant $P < 0.005$

**Subgroup analyses:**

*2.4.7.8 Subgroup analyses – timing of n-3 LCPUFA supplementation*

For the subgroup analyses, only the primary outcome (medically diagnosed IgE mediated allergy and allergies with or without sensitisation) were considered.

2.4.7.8.1 n-3 LCPUFA supplementation during pregnancy

Only one trial reported IgE mediated allergies in this subgroup [27]. This trial showed a significant reduction in IgE mediated eczema in children between 12–36 months of age whose mothers had received n-3 LCPUFA during pregnancy (706 participants, RR 0.61, 95% CI 0.38 to 0.98, P = 0.04) (Table 2-12 and Appendix 2.6-Figure 2-13). This trial showed no other significant effects for other IgE mediated allergies (Table 2-12, Appendix 2.6-Figures 2-11, 2-15, 2-17 and 2-19). Four trials reporting allergies with or without sensitisation, supplemented during pregnancy only [27, 259, 266, 267] and no difference between n-3 LCPUFA and control groups in the incidence of food allergies, eczema, allergic rhinitis, asthma or any allergy outcome at any age were detected (Table 2-12, Appendix 2.6- Figures 2-12, 2-14, 2-16, 2-18 and 2-20). No outcomes were reported for infants under 12 months in food allergy, allergic rhinitis and any allergies in these cases (Table 2-12).

2.4.7.8.2 n-3 LCPUFA supplementation during pregnancy and lactation

Only one trial intervened during both pregnancy and lactation [299]. A significant reduction in IgE mediated food allergy in children under 12 months of age (117 participants, RR 0.13, 95% CI 0.02 to 0.95, P = 0.04) with n-3 LCPUFA supplementation was found (Table 2-12). Cumulative analyses (any age follow-up) showed significant reductions of IgE mediated food allergy (119 participants, RR 0.26, 95% CI 0.08 to 0.85, P = 0.04), eczema (119 participants, RR 0.39, 95% CI 0.15 to 1.00, P = 0.05) and any allergy (119 participants, RR 0.36, 95% CI 0.16 to 0.84, P = 0.02) with n-3 LCPUFA supplementation (Table 2-12, Appendix 2.6-Figure

2-11, 2-13 and Figure 2-19). When all allergies (with and without IgE sensitivity) were considered, the results showed no significant reductions except for food allergy <12 months of age with n-3 LCPUFA supplementation (Table 2-12).

2.4.7.8.3 n-3 LCPUFA supplementation during lactation

Only one trial supplemented during lactation and no difference in allergy outcomes in infants of mothers supplemented with n-3 LCPUFA were detected (Table 2-12, Appendix 2.6 - Figures 2-12, 2-14 and 2-18) [260].

*2.4.7.9 Subgroup analyses – allergy risk*

Subgroup analyses were conducted according to whether children were at high risk of allergy or whether their risk was undetermined (i.e. mothers were not selected for participation in the trial on the basis of being a high risk of allergy) and presumed to be low/normal risk.

2.4.7.9.1 Children at high risk of allergy

Four trials [27, 259, 267, 299] provided n-3 LCPUFA supplements to women whose fetuses were at high risk of allergy development. Two [27, 299] of the four trials only reported IgE mediated allergies. The results are similar to those described in sections 2.4.7.1-5 and showed that n-3 LCPUFA supplementation significantly reduced IgE mediated food allergy in children under 12 months. In addition and importantly with larger sample sizes (n=825), n-3 LCPUFA supplementation significantly reduced IgE mediated eczema between 12–36 months of age and IgE mediated any allergies between 12–36 months of age, with the any age follow-ups being driven by the results of the 12-36 month data (Table 2-13). Four trials [27, 259, 267, 299] reporting allergies with or without sensitisation showed no significant risk reduction to any of the allergy outcomes at any age with n-3 LCPUFA supplementation (Table 2-13, Appendix 2.6 - Figures 2-21 to 2-25).

2.4.7.9.2 Children with unselected risk (low/normal risk) of allergy

Two trials reported data for this subgroup [260, 266], however low event rates (Appendix 2.6 - Figures 2-24 and 2-25) and small samples in some comparisons (Appendix 2.6 - Figures 2-21 and 2-22) were evident. No reduction in allergy outcomes including food allergies, eczema, asthma or any allergies were found in infants/children at low/normal risk of allergy (Table 2-13) with n-3 LCPUFA supplementation. None of these trials reported on infants up to 12 months of age or on IgE outcomes (Appendix 2.6 - Figures 2-21 to 2-25).

*2.4.7.10 Subgroup analyses – maturity*

No subgroup analyses based on infant maturity were conducted as planned in the systematic review. It was not possible to separate out children according to the maturity in the included trials [27, 260, 266, 267, 269] except one trial [259] which excluded preterm infants in their analysis after randomisation. The study that was conducted with preterm infants did not use a validated questionnaire and therefore can only be reported in secondary outcomes [26].

*2.4.7.11 Sensitivity analysis*

Sensitivity analyses were conducted for the primary outcome removing trials with high or unclear risk of selection, performance or attrition bias. Makrides 2009 and Makrides 2010 [26, 27] were the only trials with low risk of bias across these parameters. Removing trials with high or unclear risk of bias changed the direction for IgE mediated any allergy at the 12 to 36 months time point but did not change the direction for any other allergy outcomes at any other time points (data not shown).

**Table 2-12: Summary of meta-analyses reporting the effects of timing of n-3 LCPUFA (fish or fish oil) supplementation to mothers and allergy risk in infants using pooled analysis RR (M-H, Fixed, 95% CI)**

| Primary Outcome | | Pre-natal supplementation | | Post-natal supplementation | | Pre & Post-natal supplementation | |
|---|---|---|---|---|---|---|---|
| | | RR (95% CI) | | RR (95% CI) | | RR (95% CI) | |
| | | IgE mediated allergy (1 trial) | All allergies (IgE or not) (4 trials) | IgE mediated allergy | All allergies (IgE or not) (1 trial) | IgE mediated allergy (1 trial) | All allergies (IgE or not) (1 trial) |
| Food allergy | <12 months | NE | NE | NE | NE | **0.13 [0.02, 0.95]*** (n=117) | **0.13 [0.02, 0.95]*** (n=117) |
| | 12-36 months | 0.92 [0.40, 2.09] (n=706) | 0.84 [0.41, 1.69] (n=789) | NE | 2.27 [0.25, 20.68] (n=65) | 0.27 [0.06, 1.19] (n=119) | 0.27 [0.06, 1.19] (n=119) |
| | ≥36 months | NE | NE | NE | NE | NE | NE |
| | Any age follow-up | 0.92 [0.40, 2.09] (n=706) | 0.84 [0.41, 1.69] (n=789) | NE | 2.27 [0.25, 20.68] (n=65) | **0.26 [0.08, 0.85] *(n=119)** | 0.45 [0.19, 1.07] (n=119) |
| Eczema | <12 months | NE | 1.36 [0.59, 3.11] (n=86) | NE | NE | 0.38 [0.13, 1.11] (n=117) | 0.38 [0.13, 1.11] (n=117) |
| | 12-36 months | **0.61 [0.38, 0.98] *(n=706)** | 0.96 [0.76, 1.20] (n=789) | NE | 0.95 [0.28, 3.20] (n=65) | 0.58 [0.15, 2.22] (n=119) | 0.56 [0.23, 1.36] (n=119) |
| | ≥36 months | NE | NE | NE | NE | NE | NE |
| | Any age follow-up | **0.61 [0.38, 0.98] *(n=706)** | 0.98 [0.79, 1.23] (n=875) | NE | 0.95 [0.28, 3.20] (n=65) | **0.39 [0.15, 1.00] *(n=119)** | 0.63 [0.33, 1.19] (n=119) |
| Allergic rhinitis | <12 months | NE | NE | NE | NE | NE | NE |
| | 12-36 months | 0.31 [0.01, 7.49] (n=706) | 0.45 [0.20, 1.04] (n=686) | NE | NE | 0.60 [0.06, 6.46] (n=119) | 1.20 [0.18, 8.26] (n=119) |
| | ≥36 months | NE | NE | NE | NE | NE | NE |
| | Any age follow-up | 0.31 [0.01, 7.49] | 0.45 [0.20, 1.04] | NE | NE | 0.60 [0.06, 6.46] | 1.20 [0.18, 8.26] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | (n=706) | (n=686) | | | (n=119) | (n=119) |
| Asthma | <12 months | NE | 1.26 [0.54, 2.94] (n=83) | NE | NE | NE | NE |
| | 12-36 months | 2.76 [0.11, 67.43] (n=706) | 0.89 [0.69, 1.15] (n=771) | NE | 1.39 [0.58, 3.30] (n=65) | 0.59 [0.11, 3.11] (n=119) | 1.05 [0.41, 2.72] (n=119) |
| | ≥36 months | NE | 0.58 [0.25, 1.35] (n=528) | NE | NE | NE | NE |
| | Any age follow-up | 2.76 [0.11, 67.43] (n=706) | 0.87 [0.69, 1.10] (n=1387) | NE | 1.39 [0.58, 3.30] (n=65) | 0.59 [0.11, 3.11] (n=119) | 1.05 [0.41, 2.72] (n=119) |
| Any allergies | <12 months | NE | NE | NE | NE | NE | NE |
| | 12-36 months | 0.68 [0.44, 1.05] (n=706) | 0.91 [0.71, 1.16] (n=706) | NE | NE | 0.52 [0.17, 1.59] (n=119) | 0.78 [0.44, 1.38] (n=119) |
| | ≥36 months | NE | 0.65 [0.31, 1.37] (n=528) | NE | NE | NE | NE |
| | Any age follow-up | 0.68 [0.44, 1.05] (n=706) | 0.87 [0.69, 1.10] (n=1234) | NE | NE | **0.36 [0.16, 0.84] *(n=119)** | 0.78 [0.44, 1.38] (n=119) |

* Statistically significant $P \leq 0.05$, NE=Not estimable

**Table 2-13: Summary of meta-analyses of n-3 LCPUFA (fish or fish oil) supplementation and allergy risk in infants/children using pooled analysis RR (M-H, Fixed, 95% CI)**

| Primary Outcome | | Children with high risk of allergy | | Children with low/normal risk of allergy | |
|---|---|---|---|---|---|
| | | RR (95% CI) | | RR (95% CI) | |
| | | IgE mediated allergy 2 trials | All allergies (IgE or not) 4 trials | IgE mediated allergy | All allergies (IgE or not) 2 trails |
| Food allergy | <12 months | **0.13 [0.02, 0.95]* (n=117)** | **0.13 [0.02, 0.95]* (n=117)** | NE | NE |
| | 12-36 months | 0.58 [0.18, 1.88] (n=825) | 0.65 [0.35, 1.20] (n=908) | NE | 2.27 [0.25, 20.68] (n=65) |
| | ≥36 months | NE | NE | NE | NE |
| | Any age follow-up | 0.53 [0.15, 1.92] (n=825) | 0.66 [0.38, 1.12] (n=908) | NE | 2.27 [0.25, 20.68] (n=65) |
| Eczema | <12 months | 0.38 [0.13, 1.11] (n=117) | 0.76 [0.22, 2.62] (n=203) | NE | NE |
| | 12-36 months | **0.61 [0.39, 0.95]* (n=825)** | 0.96 [0.63, 1.47] (n=908) | NE | 0.95 [0.28, 3.20] (n=65) |
| | ≥36 months | NE | NE | NE | NE |
| | Any age follow-up | **0.56 [0.36, 0.85]* (n=825)** | 0.98 [0.71, 1.37] (n=908) | NE | 0.95 [0.28, 3.20] (n=65) |
| Allergic rhinitis | <12 months | NE | NE | NE | NE |
| | 12-36 months | 0.47 [0.07, 3.06] (n=825) | 0.53 [0.25, 1.12] (n=825) | NE | NE |
| | ≥36 months | NE | NE | NE | NE |
| | Any age follow-up | 0.47 [0.07, 3.06] (n=825) | 0.53 [0.25, 1.12] (n=825) | NE | NE |
| Asthma | <12 months | NE | 1.26 [0.54, 2.94] (n=83) | NE | NE |
| | 12-36 months | 0.86 [0.21, 3.49] (n=825) | 0.90 [0.70, 1.15] (n=908) | NE | 1.39 [0.58, 3.30] (n=65) |
| | ≥36 months | NE | NE | NE | 0.58 [0.25, 1.35] (n=528) |
| | Any age follow-up | 0.86 [0.21, 3.49] (n=825) | 0.93 [0.73, 1.17] (n=908) | NE | 0.84 [0.46, 1.53] (n=593) |
| Any allergies | <12 months | NE | NE | NE | NE |
| | 12-36 months | **0.66 [0.44, 0.99]* (n=825)** | 0.89 [0.71, 1.11] (n=825) | NE | NE |

| | | | | | |
|---|---|---|---|---|---|
| | ≥36 months | NE | NE | NE | 0.65 [0.31, 1.37] (n=528) |
| | Any age follow-up | **0.66 [0.44, 0.99]* (n=825)** | 0.89 [0.71, 1.11] (n=825) | NE | 0.65 [0.31, 1.37] (n=528) |

* Statistically significant P ≤ 0.05, NE=Not estimable

## 2.5 Discussion

Seven trials involving 2,223 women with 2,330 children were included in this review. Supplementation of n-3 LCPUFA occurred during pregnancy, lactation, or both pregnancy and lactation. The results show that IgE mediated food allergy (<12 months), IgE mediated eczema (12-36 months and any age) and IgE mediated any allergies (12-36 months and any age) were significantly reduced in children whose mothers received n-3 LCPUFA supplementation in pregnancy. Eczema and food allergy are the two main allergies present in infancy and children below 3 years of age [118] (see section 1.2.6 in Chapter 1). Only two studies contributed to IgE mediated allergies (including eczema and any allergies) and both of these included high risk populations for allergies [27, 299], however event rates for asthma and allergic rhinitis were very low in these studies and only one of the studies contributed IgE mediated food allergy below 12 months of age [299]. The 40% reduction in IgE mediated allergy in the n-3 LCPUFA supplemented group was driven by a reduction of the most frequent type of allergy, namely eczema.

Analysis of secondary outcomes showed a 30% reduced risk of having a positive SPT to one or more allergens (food or inhalant) at 12-36 months and at any age in the n-3 LCPUFA supplemented groups. This finding was driven by a 45% reduced risk of sensitisation to egg, which was the most frequent type of sensitisation observed. It has been suggested that sensitisation to egg allergen in early life appears to be a risk factor for late onset sensitivity to inhalant allergens and it acts as a predictor for asthma occurrence in later childhood [130]. In addition, sensitisation to more than one allergen has been associated with an atopic phenotype and may lead to severe allergic diseases [129]. The results found in this review may thus support a role for n-3 LCPUFA supplementation to reduce development of allergies in children. It should be noted however that the IgE mediated allergy and SPT results came from

the studies in which children were at higher risk of allergies [27, 259, 267, 299]. The medical diagnosis of IgE mediated allergies was consistent in the two studies included in the review. Overall, these findings may support a role for n-3 LCPUFA supplementation during pregnancy for women with a fetus at high hereditary risk of allergy [23, 108, 330].

When IgE mediated and non IgE mediated allergies were considered together, no effect of n-3 LCPUFA supplementation was seen. This may be due to the fact that n-3 LCPUFA acts by reducing IgE mediated allergies only or alternatively, the trials that reported all allergies in this systematic review used mixed allergy diagnosis methods (i.e. medical diagnosis, allergy diagnosis obtained from medical directories and parental reports of doctor diagnosis) of children at high or normal risk of allergies. Some of the heterogeneity seen in the meta-analyses may be due to these reasons, however no subgroup analysis was conducted with mixed allergy diagnosis methods. Only one study included children over 3 years of age with unselected (low/normal) allergy risk in the analysis and it reported asthma and any allergies only at 16 years in children [266]. In this trial, low event rates of allergies were observed, perhaps because medical directories were used to capture allergic disease and did not catch all the allergies [305].

*Subgroup analysis*

The evidence was found to be incomplete for all of the subgroup comparisons in each section below.

*Timing*

Of the four trials included in the review where women were supplemented women with n-3 LCPUFA in the prenatal period [27, 259, 266, 267], only one study [27] had a low-to-

moderate risk of bias and three were of moderate-to high risk of bias [259, 266, 267]. In addition, only one trial [27] reported IgE mediated allergies and no outcomes were available for infants under 12 months. Although the reduction in IgE mediated eczema in children aged 12-36 months came from the largest trial, more studies are needed. Only one trial [299] supplemented with n-3 LCPUFA supplementation during pregnancy and continued in lactation. However this trial was limited by small sample size and high risk of attrition bias [299] and consequently there is insufficient evidence to determine the effect of n-3 LCPUFA supplementation during lactation alone.

*Allergy risk*

Only two studies [27, 299] reported the effect of n-3 LCPUFA supplementation on IgE mediated allergies in children who were at high risk of allergies [27, 299] and one of these trials [299] was limited by small sample size and high risk of attrition and the other trial [27] had an unclear risk of bias due to the population of the sample (which was a subgroup of the main trial). There is a lack of trials in children with increased allergy risk evaluating allergies, IgE mediated or not after 3 years of age. Therefore although the findings are promising in that a reduction of IgE mediated eczema and food allergy was seen in high risk infants very early in childhood, further research is needed. Data were lacking on effects of n-3 LCPUFA supplementation on IgE mediated allergies in children at normal risk of allergies and little evidence was found to support n-3 LCPUFA supplementation of mothers with a fetus at normal risk of allergy development.

*Maturity*

Although preterm data were available in one trial [26], a non-validated questionnaire was used to assess the children. Therefore information is still lacking on preterm born infants.

A general consideration of the merits of sub-group analyses is discussed in the strength and weakness section below.

*Safety outcomes*

Among the studies included in this review, 2 trials [27, 266] contributed data on post-partum bleeding and 3 trials had data on early childhood infections [26, 27, 267], neither of which were increased with n-3 LCPUFA supplementation in the range of 492 mg – 3700 mg/day. Thus although these doses appear to be safe for both mother and fetus, further studies and investigation of additional safety factors which may affect maternal and infant wellbeing are advisable.

### 2.5.1 Overall strengths and weaknesses of evidence

The majority of the evidence assimilated came from offspring of women supplemented with n-3 LCPUFA during pregnancy and/or lactation and from women with a fetus at high risk of allergy (who were supplemented during pregnancy), with follow-up to 2 years of age. All of the trials analysed within this review were conducted in high income industrialised countries and the findings are therefore applicable to the most affluent societies where the burden of allergy is known to be high [2, 3, 8].

Allergy outcomes were used which were assessed by medical professionals and allergy diagnosis was reported by parents. All trials except one trial [260] used medical diagnosis of allergy for the analysis of primary outcomes.

The women in the included trials differed with respect to their fish intake, with three trials targeting women with a low fish intake [259, 260, 267], while fish intake was not related to inclusion criteria in other trials [26, 27, 266, 269]. One of the reasons for some of the mixed

results in this review may thus be due to mothers' baseline intake of n-3 LCPUFA not being considered in some studies.

Two systematic reviews [25, 245] have been published since 2010 on this topic. Kremmyda 2011 [245] aimed to determine the effect of n-3 LCPUFA supplementation during the perinatal period on allergies (irrespective of IgE status) in children, but did not conduct a meta-analysis. Klemens 2011 [25] conducted a meta-analysis and reported that n-3 LCPUFA supplementation during pregnancy reduced the incidence of childhood asthma, but had no effect on atopic dermatitis. The differences in findings to this review may be due to the most recent trials [26, 27, 267], not being included in the Klemens review [25] and the fact that the review in this chapter has separated IgE mediated allergies and allergies with or without IgE sensitivity. The IgE mediated allergies were assessed only by doctors in this review and doctor diagnosed allergies are considered robust and the most reliable diagnosis of allergic disease involves laboratory tests (RAST/SPT) and clinical presentation together [28, 81, 82].

The quality of the evidence presented in this review depends on the quality of the included trials. The risk of bias was noted to vary across the seven trials included in this review and a number of factors worthy of further consideration in relation to trial quality and consistency were identified and are considered below.

The doses of n-3 LCPUFA supplements varied, and women were supplemented with n-3 LCPUFA at a higher dose than is currently recommended [166, 225], which provided 331–2070 milligrams of DHA per day and 100–1600 milligrams of EPA per day, with most studies using about one gram of n-3 LCPUFA or greater. This review did not look at dosage dependent outcomes or whether the DHA to EPA ratio can act differently on allergy outcomes. This may be especially important in studies involving supplementation during

lactation as these studies tended to use lower doses. The use of fish, rather than fish oil as a supplement also needs consideration as it may contribute to the energy and protein content in the maternal diet and influence outcomes in a different way. In this review, one trial was included [267] which supplemented with fish, however this trial had been designed to overcome any additional effect on the diet by using it as a replacement for white fish, chicken and some red meat, thus minimising any additional energy and protein contributions (personal communication with author [267]). Women's adherence to the supplementation regime (compliance) may also impact on the outcomes. Blood analysis was used to check adherence to the supplementation in most trials [27, 259, 260, 266, 267, 269] and all reported a significant n-3 LCPUFA increase in the intervention group. Two trials performed analysis by intention to treat [26, 27], however Furuhjelm et al [299] performed analysis only on the compliant women – i.e. excluded women (n=25) that did not complete the requested 15 week intervention. The other 4 trials did not specify if intention to treat analysis was used [259, 260, 266, 267]. Also in two trials that included control groups (no placebo), the mothers in these groups may have had a high intake of dietary n-3 LCPUFA, because the benefits of intake of dietary n-3 LCPUFA in pregnancy was proclaimed in the countries that these two trials were conducted in [266, 267].

The Olsen trial [266] which was the only trial to assess teenagers (at 16 years of age) found maternal n-3 LCPUFA supplementation had an effect on asthma, but not on combined any allergies when compared to the placebo group. However, in this review maternal n-3 LCPUFA supplementation had no effect on asthma and any allergies. This may be due to the fact that placebo and no oil groups were combined this analysis. Alternatively, there may be a washout effect to consider with age or the current teenage diet may be more important than anything that happened with regards to n-3 LCPUFA supplemented during pregnancy. In

some respects, this trial may be considered more of an outlier as the children were much older [305].

Finally, in relation to diet and supplementation, some studies excluded women who were known to be allergic to fish [259, 266], as a safety precaution in case they may have reacted to fish oil capsules. Consequently, not many studies looked at fish allergy and hence data in this area may be limited by safety concerns [259, 266].

Three of the trials included in this review [259, 260, 267] had small sample sizes (< 50 per randomised group). Power calculations should be performed in well-designed RCTs to determine the appropriate sample size required to find a difference between the relevant groups, if one exists so that a type II error (falsely accepting the null hypothesis) is avoided. In this review only five trials reported power calculations [26, 27, 259, 267, 299] and two trials did not [260, 266]. Four trials had small sample sizes [259, 260, 267, 269] and clinical allergy outcomes were reported as secondary outcomes in four studies [26, 259, 260, 267]. Attrition and reduction of numbers at the completion of the trial was identified in four trials [259, 260, 267, 269]. Attrition is a particular problem when the numbers lost are not comparable between randomised groups [259, 260, 267, 269] and when for example remaining participants are identified as having different characteristics to those lost. Sample sizes that are too small in RCTs increases the risk of both type I and type II errors leading to a higher chance of making wrong conclusions. Type I error acceptance is usually assigned as 5% or less ($P \leq 0.05$). This means that with identical research conditions, I would accept the risk of concluding an ineffective intervention effective 5% of the time. In contrast, type II error is often reported as 0.1 or 0.2 (power 90% or 80%) which means that with identical research conditions there would be a 10% or 20% risk of concluding that an effective intervention is ineffective. The higher the power, the bigger the sample size required.

Observed event rates also need to be taken into consideration. For example, the observed event rates were too low for some allergies i.e. IgE mediated asthma and IgE mediated allergic rhinitis below 3 years of age [27, 299] and overall low event rates were observed for allergies reported in the Olsen trial [305].

Heterogeneity is to be expected in meta-analyses of RCTs performed by different groups, with different participants in different locations. The degree of heterogeneity observed in the results was quantified using the I-squared statistic [331], which reflects the percentage of variation observed between trials that is attributable to between-trial differences rather than to chance. Contributors to statistical heterogeneity include clinical heterogeneity, methodological heterogeneity, chance and bias. False conclusions about clinical heterogeneity, based on statistical heterogeneity can result in type I and type II errors. An exploration of some factors contributing to clinical heterogeneity was considered in the design stage of the review. When considering intervention trials in general, clinically relevant questions arise around questions relating to optimal timing for interventions and whether there are groups of participants that may be more susceptible to interventions than others and to whom interventions may be best targeted. In order to address these questions, I decided to undertake the sub-group analyses of the available data identified in the literature search. This sub-group analysis was pre-planned, identified in the review protocol which was published prior to undertaking the review [294] and was restricted to a small number of questions. It is important to be aware that sub-group analyses carry a higher risk of identifying results that appear statistically significant by chance (Type I or false positive error) and that increasing the number of sub-group analyses increases the risk of erroneously detecting a treatment effect. For this reason, only a small number of clearly focussed, clinically relevant questions were addressed in the sub-group analyses. The results of the sub-group analysis need treating

with caution, as formal effect modification treatment interaction effect tests were not undertaken due to a lack of studies identified, small sample sizes and high risk of attrition bias in the relevant trials [259, 260, 267, 269]. Although individual trials may be sufficiently powered to find a difference between treatment and control groups, statistical tests on smaller sub-groups will only have power to detect substantially larger effects for the same outcome and this increases the chance of a type II error whereby a real difference in subgroups may not be detected. In this thesis, it is intended for the sub-group analyses to be viewed as 'hypothesis generating' and useful to identify gaps in the knowledge for direction of future research using large scale, appropriately powered, well designed RCTs.

## 2.6 Conclusion and Rationale for Thesis

The findings of this current systematic review show that n-3 LCPUFA supplementation (492 mg-3700 mg per day) during pregnancy and lactation of women whose infant was at high risk for allergic disease, reduced the incidence of IgE mediated food allergy and eczema in their children as well as sensitisation to egg and one or more allergen. The pattern of allergy expression is known to differ with the age of the child. Eczema and food allergy are the early allergy manifestations [118] with up to 20% of children with eczema developing food allergy by the age of 3 years and 80% of these children remaining allergic for life [123, 124]. Children who are sensitised to egg allergen in early life are more likely to have asthma and allergic rhinitis later in life [130]. Hence it will be important for more trials to be conducted in both high allergy risk groups and normal risk groups, with longer term allergy outcome investigations of the children, to determine whether the early benefit is sustained during childhood.

There is a gap in knowledge on the effect on allergy development of supplementing mothers of premature infants with n-3 LCPUFA supplementation during the early postnatal period

which may be important as preterm infants are especially vulnerable to allergies and respiratory diseases [279, 280, 282].

To overcome the limitations of the findings in the review, particularly the lack of evidence from large RCTs and mixed methods of allergy diagnosis, I have performed a follow-up of children from two n-3 LCPUFA supplementation RCTs. One trial involves high risk allergy children born to mothers supplemented with n-3 LCPUFA during pregnancy [27] and the other involves preterm infants, with an unselected allergy risk, who were supplemented during lactation [26]. These studies and allergy assessments are discussed in Chapter 3 and Chapter 5. The systematic review is then updated with the additional outcomes obtained and presented with final discussion, conclusions and clinical implications in Chapter 6. In Chapter 4, some of the data collected during my thesis is used to evaluate the validity of parental reports of allergies in infants.

# Chapter 3: Effect of prenatal n-3 LCPUFA supplementation on parental reports of allergic outcomes in children up to 3 years of age

## 3.1 Introduction

The results of the systematic review reported in Chapter 2 revealed variance in the allergy assessment methods used in eligible studies and that there is a lack of larger studies evaluating maternal n-3 LCPUFA supplementation on longer term allergy outcomes of children, beyond 1 year of age. This chapter evaluates whether prenatal n-3 LCPUFA supplementation to women with a fetus at high risk of allergy alters the incidence of parentally reported allergic disease in children up to 3 years of age. The current study used the data available from participants enrolled in the allergy follow-up of the DHA to Optimise Mother Infant Outcome (DOMInO) trial that used medical diagnoses to assess the effects of n-3 LCPUFA on allergy outcomes in children [27, 303]. Although medical diagnoses of allergies are robust for assessing allergies in children, they are expensive and time consuming for large scale trials. In this allergy follow-up trial, which is the largest RCT to date of n-3 LCPUFA supplementation to pregnant women with a fetus at high risk of allergy [285, 303], parental reports of allergy were collected at 6 monthly intervals until the children reach 3 years of age. However, no previous evaluation or analyses were performed on the parent reported data. If parental reports offer similar results to medical diagnosis then they may be a more cost effective option for future studies. Thus previously unreported data from this trial presents a good opportunity to examine whether increasing the intake of n-3 LCPUFA during pregnancy alters the incidence of parent reported allergies in children from birth up to 3 years of age. Although a reduction of some medically diagnosed allergies in infants whose mothers were supplemented with n-3 LCPUFA during pregnancy has been reported [259, 303], no studies have examined the effect of n-3 LCPUFA supplementation during pregnancy on

parent reports of allergies in their offspring. This is important as different methods of allergy diagnosis may explain some of heterogeneity found in the systematic review (Chapter 2). For this chapter I undertook the 24, 30 and 36 months allergy parent report follow-up and used data previously collected at 6, 12 and 18 months of age.

## 3.2 Subjects and Methods

### 3.2.1 Study design

This is a study nested in the allergy follow-up of the DOMInO trial [27]. The DOMInO trial was designed to assess the effect of n-3 LCPUFA supplementation during the last half of pregnancy, from around 20 weeks' gestation until birth, on the incidence of postnatal depression in women and early childhood neurodevelopment outcomes [27]. The DOMInO trial was a double blind, multi-centre, RCT which was conducted in five perinatal centres around Australia. In it, 2,399 pregnant mothers were recruited from the Women's & Children's Hospital, Adelaide; Flinders Medical Centre & Flinders Private Hospital, Adelaide; Campbelltown Hospital, Sydney; Royal Brisbane and Women's Hospital, Brisbane and Sunshine Hospital, Victoria.

The DOMInO trial was registered as ACTRN12605000569606 in the Australian New Zealand Clinical Trials Registry.

### 3.2.2 Allergy follow-up study

Pregnant mothers recruited to the DOMInO trial were eligible for the allergy follow-up study if they were recruited in either of the two South Australian perinatal centres – based at the Women's & Children's Hospital and Flinders Medical Centre in Adelaide – from 20 March 2006 to 8 May 2008. Ethics approval was obtained prior to the study commencement from the Human Research Ethics Committees of the Women's & Children's Hospital and Flinders Medical Centre. This allergy follow-up study was registered as ACTRN12610000735055 in the Australian New Zealand Clinical Trials Registry.

#### *3.2.2.1 Inclusion and exclusion criteria for the DOMInO trial and the allergy follow-up*

Pregnant women were eligible for inclusion into the DOMInO trial if they had singleton pregnancies, were less than 21 weeks' gestation and able to give written informed consent. Women were excluded if they were already taking a prenatal supplement with DHA, their fetus had a known major abnormality, they had bleeding disorders in which tuna oil was contraindicated, were taking anticoagulant therapy, had a documented history of drug or alcohol abuse, were participating in another fatty acid trial, were unable to give written informed consent or if English was not the main language spoken at home. Pregnant women were eligible for the allergy follow-up study, if the fetus had a parent or sibling with a history of any medically diagnosed allergic disease.

There were 1,080 women who were eligible for inclusion in the allergy follow-up. Of these, 706 women consented and were recruited. All the above procedures were completed by research assistants between 2006–2008.

#### *3.2.2.2 Dietary intervention for the DOMInO and the allergy follow-up*

Dietary intervention for the allergy follow-up study was the same as the original DOMInO study. There were two groups, an n-3 LCPUFA intervention group and a control group. The intervention group was given three 500 mg fish oil capsules per day (900 mg n-3 LCPUFA/day providing 800 mg/day of DHA and 100 mg/day of EPA). The control group was given three 500 mg vegetable oil capsules per day, without DHA and EPA. The vegetable oil capsules contained a blend of 3 oils (rapeseed, sunflower, and palm) in equal proportions. This blend of oils was designed to match the polyunsaturated, monounsaturated, and saturated fatty acid profile of the average Australian diet [19]. Mothers were asked to take three capsules daily from trial entry (~ 20 weeks' gestation) until the birth of their child.

### 3.2.3 Allocation of the participants to the original study

Women were randomly assigned a unique study number and treatment group allocation through a computer driven telephone randomisation service according to an independently generated randomisation schedule, with balanced variable-sized blocks. Stratification was done by centre and parity (first birth vs. subsequent birth).

#### *3.2.3.1 Blinding*

Parents, outcome assessors, investigators and clinicians were blinded to the dietary allocation. To maintain the blind, both active and control capsules were identical in size, shape, and colour. Capsules were donated by Efamol, Surrey, England.

### 3.2.4 Hypotheses

For my study, the hypotheses was that prenatal supplementation of dietary n-3 LCPUFA will reduce the incidence of parental reports of allergies in offspring between birth and 36 months of age.

### 3.2.5 Objectives

- To determine whether dietary n-3 LCPUFA supplementation of pregnant women decreases parental reports of allergies at 6 month intervals from birth to 36 months of age
- To determine whether the dietary n-3 LCPUFA supplementation of pregnant women decreases the cumulative incidence of parental reports of allergies from birth to 12 months and birth to 36 months of age.

## 3.3 Outcomes

### 3.3.1 Primary outcomes – allergy follow-up study

The primary outcomes of the initial allergy follow-up study were medically diagnosed IgE associated allergic diseases – eczema and food allergy with sensitisation – at 1 year of age and eczema, asthma, allergic rhinitis and food allergy with sensitisation at 3 years of age [285,

303]. The case report form (CRF) developed at the beginning of the DOMInO trial, had a number of specific allergy questions which were asked every 6 months and were used in the current study to determine if the parental reports of allergic disease agreed with the findings of the medical diagnoses.

Primary outcomes of my study were therefore the 6 monthly parental reports of allergy (eczema, allergic rhinitis, asthma and food allergy) in treatment and control groups from birth up to 36 months of age. The parental reports of allergic diseases came from structured telephone interviews/CRF questions at 6, 18, 24 and 30 months of age and face to face interviews at the age of 12 and 36 months, which is when the medical examinations and diagnoses in the nested follow-up were performed.

#### *3.3.1.1 Data collection*

My involvement in the data collection in this study began when children were 24 months of age. I conducted phone interviews at 24 and 30 months of age and coordinated the 36 month medical assessment appointments, during which I collected parental reports of allergy symptoms and parental reports of local doctor diagnosed allergies. I was trained by the study supervisors and senior members of the study team to collect data from parents over the telephone according to the trial assessment criteria. For the quality assurance of the trial, all data were collected according to a set of standard operating procedures (SOPs). I was blinded to the trial supplementation until the three year data were analysed. The telephone interviews collected information relating to parental reports of allergy symptoms and parental reports of local doctor diagnosed allergic diseases (Appendix 3.1). These phone calls also provided an opportunity to update family contact details (to maximise follow-up rates). Telephone calls were made during the day, early evening and on Saturdays to those who were difficult to contact during normal business hours. If parents asked for an alternative to the telephone interview, questionnaires were sent through mail, email or via fax. Relocation interstate or

overseas did not prevent follow-up participation. At the medical assessment appointments, parental reports of allergy outcomes were collected before medical assessment (Appendix 3.2). I collected data for the study from August 2010 to July 2011.

#### *3.3.1.2 Baseline data*

Baseline characteristics including maternal age, maternal school education, maternal further education, paternal education, paternal further education, maternal smoking during pregnancy, parity and other possible confounding variables such as mode of delivery were available from the main DOMInO data set. Data on caesarean section, infant sex, gestational age at birth, birth weight, birth length and birth head circumference were also obtained from the DOMInO trial. Background information such as maternal, paternal and family history of allergic disease was collected at the beginning of the allergy follow-up.

#### *3.3.1.3 Allergy outcome data – Study instrument*

The standard questions for eczema, asthma and allergic rhinitis, outlined in Appendices 3.1 and 3.2, were based on the ISAAC questionnaire [98, 99] (see more details about ISAAC questionnaire development, validation and usage in section 1.2.5.2 and section 5.3.5.6). As there are no questions for food allergy in the ISAAC questionnaire, questions to obtain parental reports of allergic reactions to food and parental reports of doctor diagnosed food allergy were added because food allergy is an important early manifestation of allergic disease.

#### *3.3.1.4 Structure of the study instrument*

The questions used to collect parental reports of allergy symptoms and parental reports of local doctor diagnosis of allergies at 6 monthly intervals were similar in structure for eczema, asthma and allergic rhinitis (Appendix 3.1). Parent interview questions at 12 and 36 months asked for recall of allergy symptoms and local doctor diagnosis of allergic disease (except food allergy) over the whole time period since birth (Appendix 3.2). Thus cumulative

incidence for asthma, eczema and rhinitis was not an attempt to add up all responses obtained from the 6 monthly questions. Food allergy however was handled differently as at the start of the allergy follow-up study no direct question was asked to collect cumulative incidence from birth to 12 months of age and from birth to 36 months of age. Combined total incidence of food allergy symptoms reported in the results section below for the cumulative period to 12 months and the cumulative period to 36 months after birth were thus obtained by combining the children who had food allergy symptoms between birth to 6 and 6 to 12 months of age and combining children who had food allergy symptoms between 0–6, 6–12, 12–18, 18–24, 24–30 and 30–36 months of age, respectively.

#### *3.3.1.5 Parental reports of allergy symptoms- food allergy, eczema, asthma, allergic rhinitis*

Allergic reactions to specific foods were considered if a red skin rash on the face or body, hives, swelling of the face or body, wheeze or stridor, cough, vomiting, loose watery stools, blood stained stools and/or a floppy unresponsive baby/child occurred within 60 minutes of consumption of the food. Parental report of local doctor diagnosed food allergy required confirmation of all foods for which the baby/child had a clinical allergic reaction confirmed with a positive RAST or SPT. These questions were only asked in telephone interviews as SPTs were completed as part of the study medical assessment at 12 and 36 months where study doctors diagnosed children with food allergy by comparing food allergy symptoms with SPT results.

There was no question to collect parental reports of local doctor diagnosed food allergy between 6–12 months of age and 30–36 months of age at the face to face interview. So parental reports of local doctor diagnosed food allergy were only available between 0–6, 12–18, 18–24 and 24–30 months of age and overall cumulative assessment of parental report of

food allergy diagnosis by a local doctor was not available between birth to 12 months and birth to 36 months of age.

For eczema, rashes inside the fold of elbows, behind knees, front of ankles, behind ears, around eyes, around the neck, under the buttocks and flexural areas of arms and legs were classified as eczema, whereas rashes in the nappy area, entire body, under chin, shoulder, thigh and neck were not (see appendices 3.1 and 3.2). As very young children can't express whether a rash is itchy, this qualification was only asked at 12 and 36 months of age in the face to face interviews. Although still difficult to determine itch at these ages, asking at these two time points maintained consistency with the ISAAC questionnaire at medical diagnostic points.

For asthma, parental reports of wheezing or whistling in the chest and night time coughing when the child did not have a cold were considered as per appendices 3.1 and 3.2.

Parental reports of allergic rhinitis were indicated if the child experienced any problems with sneezing, or a runny or blocked nose when he/she did not have a cold (see appendices 3.1 and 3.2). There was no question at the face to face interviews to collect parental reports of local doctor diagnosed allergic rhinitis. Therefore parental reports of local doctor diagnosed allergic rhinitis or hay fever were only available between 0-6, 12-18, 18-24 and 24-30 months of age and overall cumulative assessment of parental reports between birth to 12 months and birth to 36 months of age was not available. At the time that the allergy follow-up began and prior to my involvement, it was decided that allergic rhinitis/hay fever was a secondary outcome of the allergy follow-up study, because allergic rhinitis is not an early manifestation of allergic disease.

#### *3.3.1.6 Parental reports of any allergy symptoms*

This study was designed to also investigate children with parental reports of one or more allergy symptoms (any allergy symptoms) or parental reports of local doctor diagnosed any

allergy disease, regardless of whether it was eczema, food allergy, asthma or allergic rhinitis. After individual allergy symptoms/diagnosed allergy were collected through questionnaires, children with one or more allergies were categorized accordingly. Although parental reports of combined any allergy symptoms were available from birth to 12 months and birth to 36 months of age, parental reports of local doctor diagnosed combined any allergies at these ages were not available as data were not available for food allergy and allergic rhinitis (as discussed in the section above 3.3.1.5).

#### *3.3.1.7 Data management*

Responses to questions and medical assessments were collected on paper based CRFs which were checked for completeness and logic prior to sending to the Data Management and Analysis Centre (DMAC) at the University of Adelaide for data entry into the database.

#### *3.3.1.8 Data synthesis*

Statistical analyses were performed using SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA) [332] on data that had been cleaned and checked for errors. The incidences of parent reported allergic symptoms and parent reported doctor diagnoses of allergies were compared across treatment groups using log binomial regression models, with the effects of treatment expressed as relative risks (RRs). Both unadjusted and adjusted analyses were performed, with adjustment for centre (WCH vs. FMC/private), parity (0 vs. ≥ 1), maternal history of allergic disease (yes vs. no) and infant sex (male vs. female). Statistical significance was set at $P \leq 0.05$.

## 3.4 Results

### 3.4.1 Flow of the participants

From 1,080 eligible families, 706 families consented and were recruited into this allergy follow-up. After randomisation, there were 368 infants whose mothers were assigned to the n-3 LCPUFA supplement group and 338 infants whose mothers were assigned to the control group. At each follow-up, more than 90% of children were assessed (Figure 3-1).

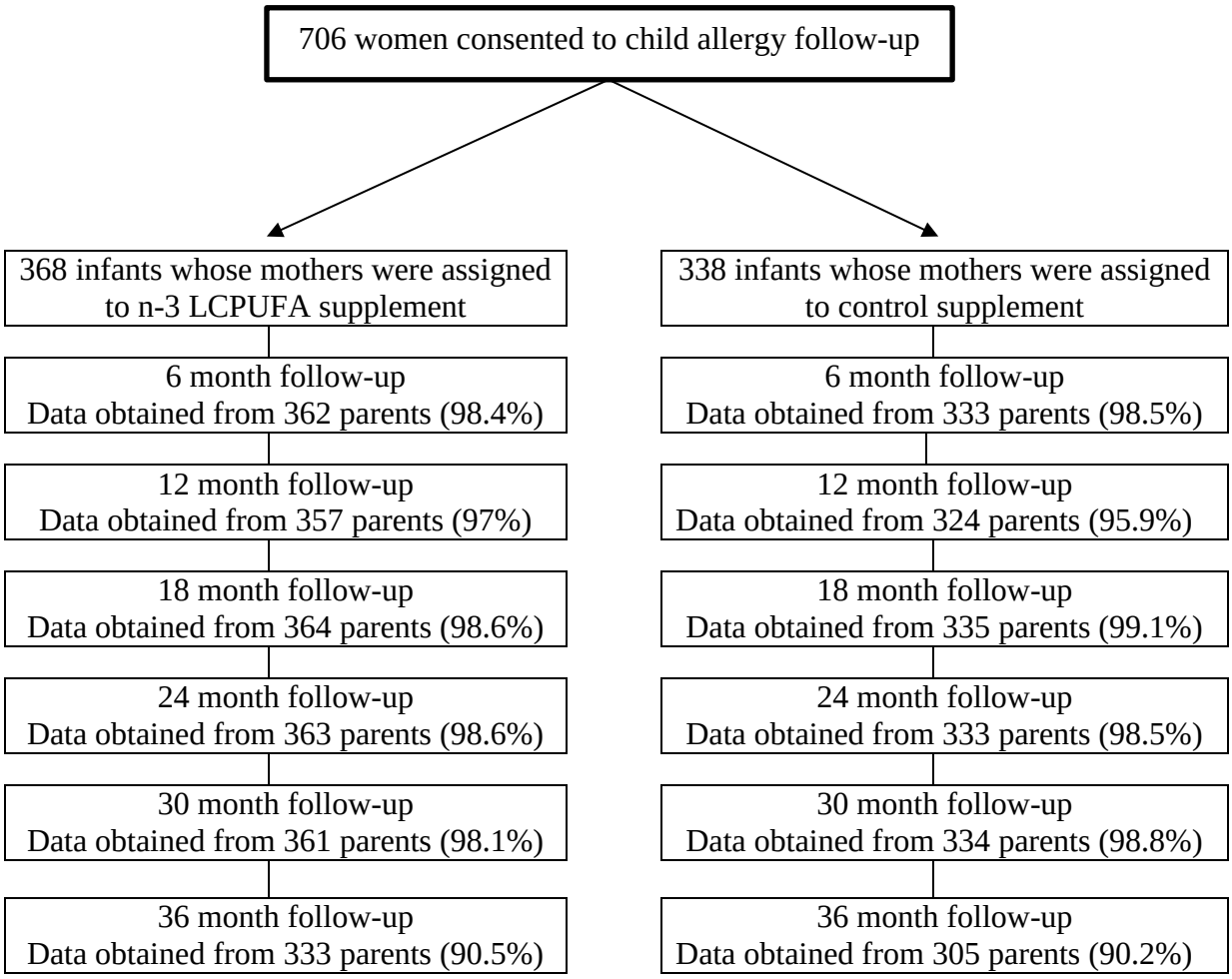


**Figure 3-1: Flow of the participants throughout follow-up periods**

### 3.4.2 Baseline characteristics

Baseline characteristics (Table 3-1) were mostly comparable between the n-3 LCPUFA group and the control group except for infant sex (n-3 LCPUFA 199 female versus control 170 female). As described above, adjusted analyses included infant sex.

**Table 3-1: Baseline characteristics at trial entry**

| Characteristic | Control (n = 338) | n-3 LCPUFA (n = 368) |
|---|---|---|
| **Centre: n (%)** | | |
| Flinders Medical Centre/Flinders Private Hospital<br>Women's & Children's Hospital | 122 (36.1)<br>216 (63.9) | 134 (36.4)<br>234 (63.6) |
| **Maternal age at consent (years): mean (SD)** | 29.5 (5.6) | 29.6 (5.7) |
| **Infant sex: n (%)** | | |
| Male<br>Female | 168 (49.7)<br>170 (50.3) | 169 (45.9)<br>199 (54.1) |
| **Mother completed secondary education: n (%)** | 222 (65.7) | 232 (63.0) |
| **Father completed secondary education: n (%)** | 182 (53.8) | 196 (53.3) |
| **Maternal smoking during pregnancy: n (%)** | 45 (13.3) | 47 (12.8) |
| **Parity: primiparous n (%)** | 131 (38.8) | 150 (40.8) |
| **Maternal history of allergic disease: n (%)** | 236 (69.8) | 257 (69.8) |
| **Paternal history of allergic disease: n (%)** | 178 (52.7) | 207 (56.3) |
| **Siblings history of allergic disease: sibling n (%)** | 21 (6.2) | 12 (3.3) |

### 3.4.3 Post randomisation characteristics

Most post randomisation characteristics were comparable between the treatment groups (Table 3-2). The infants in the n-3 LCPUFA group were heavier and longer than the control, although no significant difference was seen in birth head circumferences (Table 3-2). This result is consistent with the DOMInO RCT as a whole, except that no difference in the gestational age of the infants at birth or the incidence of caesarean sections between the intervention and control group was observed in the allergy follow-up participants [27].

**Table 3-2: Post randomisation characteristics**

| Characteristic | Control (n = 338) | n-3 LCPUFA (n = 368) | P-value |
|---|---|---|---|
| Birth weight (g): mean (SD) | 3415 (520) | 3495 (544) | **0.05*** |
| Birth length (cm): mean (SD) | 49.4 (3.0) | 50.0 (2.4) | **0.01*** |
| Birth head circumference (cm): mean (SD) | 34.8 (2.5) | 34.8 (1.6) | 0.87 |
| Gestational age at birth (weeks): mean (SD) | 39.2 (1.7) | 39.5 (1.5) | 0.17 |
| Caesarean Section: n (%) | 98 (29.0) | 104 (28.3) | 0.83 |

***Statistically significant (P ≤ 0.05)

**Primary outcomes - Parental reports of allergy:**

### 3.4.4 Eczema

*3.4.4.1 Incidence of parental reports of eczema*

Parental reports of eczema symptoms at 6 monthly intervals were compared between treatment groups and no differences were found in unadjusted and adjusted models. Eczema incidence was most prominent at 0–6 months of age (~46%) in both randomised groups and gradually decreased with age (Table 3-3).

*3.4.4.2 Cumulative incidence of parental reports of eczema*

There were no differences in the parental recall of eczema symptoms between n-3 LCPUFA and control groups over the longer term (between birth to 12 months,~15% vs.~ 13% and between birth to 36 months, ~ 25% vs.~ 27% respectively) in unadjusted and adjusted models (Table 3-3). The results from both randomised groups showed that the cumulative incidence of eczema symptoms was higher between birth to 36 months of age (~26%) than between birth to 12 months of age (~14%) (Table 3-3).

The variations between the 6 monthly reports and reports of eczema over longer time periods may be due to differences in recall every 6 months vs. over the longer period or the inclusion of 'itchy' in the question at the 12 and 36 month face to face assessments (itchy rash vs. any rash; appendices 3.1 and 3.2).

**Table 3-3: Effects of n-3 LCPUFA supplementation on parental reports of eczema symptoms between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported eczema symptoms over individual 6 month periods** | | | | | | |
| 0–6 months | 170/362 (46.96) | 152/333 (45.65) | 1.03 (0.88–1.21) | 0.73 | 1.04 (0.89–1.22) | 0.64 |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 156/364 (42.86) | 148/335 (44.18) | 0.97 (0.82–1.15) | 0.73 | 0.96 (0.82–1.14) | 0.66 |
| 18–24 months | 129/363 (35.54) | 126/333 (37.84) | 0.94 (0.77–1.14) | 0.53 | 0.95 (078–1.15) | 0.57 |
| 24–30 months | 116/361 (32.13) | 105/334 (31.44) | 1.02 (0.82–1.27) | 0.84 | 1.02 (0.82–1.27) | 0.86 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of eczema symptoms over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | 53/365 (14.52) | 42/333 (12.61) | 1.15 (0.79–1.68) | 0.46 | 1.17 (0.80–1.70) | 0.42 |
| 0–36 months | 84/331 (25.38) | 83/304 (27.30) | 0.93 (0.72–1.21) | 0.58 | 0.93 (0.72–1.21) | 0.61 |

*Statistically significant (P ≤ 0.05)
**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

#### *3.4.4.3 Incidence of parental reports of local doctor diagnosed eczema*

The proportion of parental reports of doctor diagnosed eczema did not differ between the n-3 LCPUFA supplemented group and the control group in unadjusted or adjusted models (Table 3-4). As with the parent reports of eczema above, parents reported fewer new doctor diagnoses of eczema at older ages (Table 3-4).

#### *3.4.4.4 Cumulative incidence of parental reports of local doctor diagnosed eczema*

No differences were found between the cumulative incidence of parental reports of doctor diagnosed eczema in the n-3 LCPUFA group compared to the control group in unadjusted or adjusted models (Table 3-4). As might be expected, the cumulative percentage of parental

reports of local doctor diagnosed eczema was higher between birth to 36 months of age (~31%) than between birth to 12 months of age (~24%) in both groups (Table 3-4).

Parental reports of eczema symptoms were very high (~46%) in the first six months of life – possibly due to parental concern for their new baby, or the lack of the 'itchy' criterion in the question at that age, as mentioned above. However it was interesting that parental reports of the cumulative incidence of local doctor diagnosed eczema were higher (~24%) than parental reports of cumulative incidence of eczema symptoms (~14%) between birth to 12 months of age. Possible reasons for this are outlined in the discussion section.

**Table 3-4: Effects of n-3 LCPUFA supplementation on parental reports of local doctor diagnosed eczema between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported doctor diagnosed eczema over individual 6 month periods** | | | | | | |
| 0–6 months | 57/362 (15.75) | 58/333 (17.42) | 0.90 (0.65–1.26) | 0.55 | 0.92 (0.66–1.29) | 0.63 |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 63/364 (17.31) | 53/335 (15.82) | 1.09 (0.78–1.53) | 0.60 | 1.10 (0.79–1.54) | 0.57 |
| 18–24 months | 48/363 (13.22) | 43/333 (12.91) | 1.02 (0.70–1.50) | 0.90 | 1.04 (0.71–1.53) | 0.84 |
| 24–30 months | 43/361 (11.91) | 36/334 (10.78) | 1.11 (0.73–1.68) | 0.64 | 1.11 (0.73–1.68) | 0.62 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of doctor diagnosed eczema over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | 83/365 (22.74) | 84/333 (25.23) | 0.90 (0.69–1.17) | 0.44 | 0.92 (0.70–1.19) | 0.51 |
| 0–36 months | 101/333 (30.33) | 99/305 (32.46) | 0.93 (0.74–1.18) | 0.56 | 0.93 (0.74–1.17) | 0.54 |

*Statistically significant ($P \leq 0.05$)

**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

### 3.4.5 Asthma

#### *3.4.5.1 Incidence of parental reports of asthma symptoms*

Nearly 30% of children were reported by their parents as having asthma symptoms of wheeze and night time cough in both treatment groups at 0–6, 12–18, 18–24 and 24–30 months of age. There were no differences between the n-3 LCPUFA and control randomised groups in parental reporting of asthma symptoms in unadjusted and adjusted models from birth to 36 months of age (Table 3-5).

#### *3.4.5.2 Cumulative incidence of parental reports of asthma symptoms*

When parents were asked to recall asthma symptoms between birth to 12 months of age and between birth to 36 months of age, the number of children with symptoms was similarly high (~33% between birth to 12 months and ~48% between birth to 36 months). No differences were found for parental reports of asthma symptoms, between 0–12 months of age and between 0–36 months of age, between n-3 LCPUFA supplemented and control groups in unadjusted and adjusted models (Table 3-5).

**Table 3-5: Effects of n-3 LCPUFA supplementation on parent reports of asthma symptoms between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported asthma symptoms over individual 6 month periods** | | | | | | |
| 0–6 months | 100/362 (27.62) | 93/333 (27.93) | 0.99 (0.78–1.26) | 0.93 | 0.99 (0.78–1.26) | 0.94 |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 106/364 (29.12) | 107/335 (31.94) | 0.91 (0.73–1.14) | 0.42 | 0.92 (0.74–1.15) | 0.48 |
| 18–24 months | 106/363 (29.20) | 99/333 (29.73) | 0.98 (0.78–1.24) | 0.88 | 0.99 (0.79–1.25) | 0.96 |
| 24–30 months | 98/361 (27.15) | 83/334 (24.85) | 1.09 (0.85–1.41) | 0.49 | 1.10 (0.86–1.42) | 0.46 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of asthma symptoms over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | 115/365 (31.51) | 116/333 (34.83) | 0.90 (0.73–1.12) | 0.35 | 0.91 (0.74–1.12) | 0.36 |
| 0–36 months | 160/332 (48.19) | 145/305 (47.54) | 1.01 (0.86–1.19) | 0.87 | 1.00 (0.86–1.18) | 0.97 |

*Statistically significant (P ≤ 0.05)
**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

*3.4.5.3 Incidence of parental reports of local doctor diagnosed asthma*

Medical diagnosis of asthma is difficult in early childhood and parental reports of local doctor diagnosed asthma were low compared with the parental reports of asthma symptoms in both treatment groups (Table 3-5 and Table 3-6). There were no differences in parental reports of doctor diagnosed asthma between treatment groups at any age (where analysis was permissible) although there was a tendency for more parents in the n-3 LCPUFA group to report doctor diagnosed asthma between 18–24 months of age (1.75, 95% CI; 0.97 to 3.15, P = 0.06) compared with control (Table 3-6, adjusted comparison). Incidence of parental reports of doctor diagnosed asthma increased with age but remained low as doctor diagnosis was not common before the age of 3 years (Table 3-6).

*3.4.5.4 Cumulative incidence of parental reports of local doctor diagnosed asthma*

The treatment groups did not differ in the cumulative incidence of parental reports of doctor diagnosed asthma between 0–12 and 0–36 months of age in unadjusted or adjusted comparisons (Table 3-6). There were large variations observed between cumulative parental reports of asthma incidence and cumulative parental reports of doctor diagnosed incidence (Table 3-5 and 3-6), with parental reports of asthma symptoms being much higher than reports of doctor diagnosed incidence.

**Table 3-6: Effects of n-3 LCPUFA supplementation on parental reports of local doctor diagnosed asthma between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported doctor diagnosed asthma over individual 6 month periods** | | | | | | |
| 0–6 months | 0/362 (0.00) | 1/333 (0.30) | ND | ND | ND | ND |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 21/364 (5.77) | 19/335 (5.67) | 1.02 (0.56–1.86) | 0.96 | 1.04 (0.57–1.90) | 0.90 |
| 18–24 months | 29/363 (7.99) | 16/333 (4.80) | 1.66 (0.92–3.01) | 0.09 | 1.75 (0.97–3.15) | 0.06 |
| 24–30 months | 29/361 (8.03) | 21/334 (6.29) | 1.28 (0.74–2.20) | 0.38 | 1.31 (0.76–2.24) | 0.33 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of doctor diagnosed asthma over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | 7/365 (1.92) | 7/333 (2.10) | 0.91 (0.32–2.57) | 0.86 | 0.92 (0.33–2.58) | 0.87 |
| 0–36 months | 48/333 (14.41) | 39/305 (12.79) | 1.13 (0.76–1.67) | 0.55 | 1.17 (0.79–1.73) | 0.44 |

ND - Not determined as log binomial model unable to produce sensible estimates because of very low event rates

*Statistically significant ($P \leq 0.05$)

**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

### 3.4.6 Allergic rhinitis

#### *3.4.6.1 Incidence of parental reports of allergic rhinitis*

The unadjusted relative risk for parental reports of the allergic rhinitis symptoms of sneezing, or a runny or blocked nose without having a cold were significantly lower in infants, between 0–6 months of age, in the n-3 LCPUFA supplemented group (0.80, 95% CI, 0.64–1.00, P = 0.05) although no significance was found in the adjusted analyses (0.81, 95% 0.65–1.01, P = 0.06) (Table 3-7). Parental reports of allergic rhinitis symptoms between 0–6 months were nearly double (~31%) those reported at other 6 month intervals. No significant differences were found for parental reports of allergic rhinitis symptoms between the n-3 LCPUFA supplemented group compared with the control at other ages (Table 3-7).

#### *3.4.6.2 Cumulative incidence of parental reports of allergic rhinitis*

No difference was found for parental reports of allergic rhinitis between birth to 12 months and birth to 36 months of age between n-3 LCPUFA and control groups in unadjusted or adjusted models (Table 3-7). The proportion of children with cumulative parental reports of allergic rhinitis was 3 times lower at 12 months of age (~10%) compared with birth to 6 months of age (~31%) despite similar survey questions (Table 3-7).

**Table 3-7: Effects of n-3 LCPUFA supplementation on parental reports of allergic rhinitis symptoms between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported rhinitis symptoms over individual 6 month periods** | | | | | | |
| 0–6 months | 99/362 (27.35) | 114/333 (34.23) | 0.80 (0.64–1.00) | 0.05* | 0.81 (0.65–1.01) | 0.06 |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 65/364 (17.86) | 53/335 (15.82) | 1.13 (0.81–1.57) | 0.47 | 1.15 (0.83–1.60) | 0.40 |
| 18–24 months | 64/363 (17.63) | 61/333 (18.32) | 0.96 (0.70–1.32) | 0.81 | 0.96 (0.70–1.32) | 0.82 |
| 24–30 months | 56/361 (15.51) | 60/334 (17.96) | 0.86 (0.62–1.20) | 0.39 | 0.87 (0.63–1.21) | 0.41 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of rhinitis symptoms over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | 34/365 (9.32) | 39/333 (11.71) | 0.80 (0.52–1.23) | 0.30 | 0.79 (0.51–1.22) | 0.29 |
| 0–36 months | 80/333 (24.02) | 65/305 (21.31) | 1.13 (0.85–1.50) | 0.42 | 1.12 (0.84–1.49) | 0.45 |

*Statistically significant ($P \leq 0.05$)
**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

*3.4.6.3 Incidence of parental reports of local doctor diagnosed allergic rhinitis*

A very low incidence of parental reports of local doctor diagnosed allergic rhinitis was exhibited in both n-3 LCPUFA supplemented and control groups at all of the follow-up periods and no difference was found between treatment groups at any age in unadjusted or adjusted models (Table 3-8). When parental reports of doctor diagnosed allergic rhinitis and parental reports of allergy symptoms were compared, parents reported more allergic rhinitis symptoms than reports of doctor diagnosed allergic rhinitis symptoms, which may be due to difficulty to recognising symptoms, however the low incidence of doctor diagnosed reporting makes further speculation difficult (Table 3-7 and Table 3-8).

*3.4.6.4 Cumulative incidence of parental reports of local doctor diagnosed allergic rhinitis*

Parental reports of local doctor diagnosed cumulative incidence of allergic rhinitis were not measured (Table 3-8) because the study specific doctors directly diagnosed allergic rhinitis at the face to face interview.

**Table 3-8: Effects of n-3 LCPUFA supplementation on parental reports of local doctor diagnosed allergic rhinitis between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported doctor diagnosed rhinitis over individual 6 month periods** | | | | | | |
| 0–6 months | 2/362 (0.55) | 0/333 (0.00) | ND | ND | ND | ND |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 4/364 (1.10) | 5/335 (1.49) | 0.74 (0.20–2.72) | 0.65 | 0.72 (0.20–2.65) | 0.62 |
| 18–24 months | 7/363 (1.93) | 3/333 (0.90) | 2.14 (0.56–8.21) | 0.27 | 2.15 (0.56–8.22) | 0.26 |
| 24–30 months | 8/361 (2.22) | 7/334 (2.10) | 1.06 (0.39–2.88) | 0.91 | 1.05 (0.39–2.85) | 0.92 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of doctor diagnosed rhinitis over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | - | - | - | - | - | - |
| 0–36 months | - | - | - | - | - | - |

ND - Not determined as log binomial model unable to produce sensible estimates because of very low event rates

*Statistically significant (P≤0.05)

**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

### 3.4.7 Food allergy

#### *3.4.7.1 Incidence of parental reports of food allergy symptoms*

There was no difference between the n-3 LCPUFA supplemented and control groups in parental reports of food allergy symptoms. Overall the highest incidence of parental reports of food allergy symptoms was reported between 12–18 months of age (~17%) which may be associated with an increasing variety of solid foods in the children's diet at this age (Table 3-9).

#### *3.4.7.2 Cumulative incidence of parental reports of food allergy*

Approximately 21% of children had parental reports of food allergy symptoms between birth to 12 months of age compared with 31% between birth to 36 months of age in both randomised groups. Parental reports of food allergy symptoms did not differ between the n-3 LCPUFA supplemented and control groups in unadjusted or adjusted analyses (Table 3-9).

**Table 3-9: Effects of n-3 LCPUFA supplementation on parental reports of food allergy symptoms between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported food allergy symptoms over individual 6 month periods** | | | | | | |
| 0–6 months | 37/362 (10.22) | 38/333 (11.41) | 0.90 (0.58–1.37) | 0.61 | 0.90 (0.59–1.37) | 0.62 |
| 6–12 months | 50/366 (13.66) | 40/334 (11.98) | 1.14 (0.77–1.68) | 0.51 | 1.14 (0.77–1.68) | 0.51 |
| 12–18 months | 65/364 (17.86) | 53/335 (15.82) | 1.13 (0.81– 1.57) | 0.31 | 1.15 (0.83–1.60) | 0.40 |
| 18–24 months | 25/363 (6.89) | 23/333 (6.91) | 1.00 (0.58–1.72) | 0.99 | 1.01 (0.59–1.75) | 0.97 |
| 24–30 months | 14/361 (3.88) | 11/334 (3.29) | 1.18 (0.54–2.56) | 0.68 | 1.15 (0.53–2.49) | 0.72 |
| 30–36 months | 12/344 (3.49) | 11/320 (3.44) | 1.02 (0.45–2.27) | 0.97 | 1.04 (0.47–2.33) | 0.92 |
| **Reported cumulative incidence of food allergy symptoms over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | 77/362 (21.27) | 70/331 (21.15) | 1.01 (0.76–1.34) | 0.97 | 1.01 (0.75–1.34) | 0.97 |
| 0–36 months | 106/346 (30.64) | 100/322 (31.06) | 0.99 (0.79–1.24) | 0.91 | 0.98 (0.79–1.23) | 0.89 |

*Statistically significant ($P \leq 0.05$)

**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

*3.4.7.3 Incidence of parental reports of local doctor diagnosed food allergy*

The proportion of children with parental reports of local doctor diagnosed food allergy with sensitisation was low at each follow-up and did not differ between treatment groups (Table 3-10). Study doctor diagnosed food allergy with sensitisation was similarly low and did not differ between the groups [285, 303].

*3.4.7.4 Cumulative incidence of parental reports of local doctor diagnosed food allergy*

Parental reports of local doctor diagnosed incidence of food allergy were not collected during the face to face interview because the study doctors diagnosed food allergies by using food allergy symptoms and SPT results at the face to face interview.

**Table 3-10: Effects of n-3 LCPUFA supplementation on parental reports of local doctor diagnosed food allergy between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported doctor diagnosed food allergy over individual 6 month periods** | | | | | | |
| 0–6 months | 3/362 (0.83) | 0/333 (0.00) | ND | ND | ND | ND |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 5/364 (1.37) | 2/335 (0.60) | 2.30 (0.45–11.78) | 0.32 | 2.20 (0.43–11.25) | 0.34 |
| 18–24 months | 5/363 (1.38) | 5/333 (1.50) | 0.92 (0.27–3.14) | 0.89 | 0.92 (0.27–3.16) | 0.90 |
| 24–30 months | 1/361 (0.28) | 3/334 (0.90) | 0.31 (0.03–2.95) | 0.31 | 0.31 (0.03–2.94) | 0.31 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of doctor diagnosed food allergy over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | - | - | - | - | - | - |
| 0–36 months | - | - | - | - | - | - |

ND - Not determined as log binomial model unable to produce sensible estimates because of very low event rates

*Statistically significant ($P \leq 0.05$)

**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

### 3.4.8 Any allergy

#### *3.4.8.1 Incidence of parental reports of any allergic symptoms*

The frequency of new reports of any allergic symptoms in the individual 6 month periods were also considered and defined as parental reports of one or more allergic symptoms including food allergy, eczema, asthma and rhinitis symptoms. Parental reports of any allergic symptoms were highest between 0–6 months of age (~72%) and lowest between 24–30 months of age (~54%). No difference were found for parental reports of any allergy symptoms at any age group between the n-3 LCPUFA vs. control group in unadjusted or adjusted models (Table 3-11).

#### *3.4.8.2 Cumulative incidence of parental reports of any allergy*

Parental reports of any allergy symptoms (which were based on number of children with at least one allergy symptom) were ~56% between 0–12 months of age and ~75% between 0–36 months of age in both randomised groups. The n-3 LCPUFA supplementation had no significant effect on parental reports of allergy symptoms in unadjusted and adjusted models (Table 3-11). However there was a non-significant increased risk of having parental reports of allergy symptoms in the n-3 LCPUFA group (1.09, 95% CI 1.00–1.18, P = 0.06) compared with control at 0-36 months of age (Table 3-11).

**Table 3-11: Effects of n-3 LCPUFA supplementation on parental reports of any allergy symptoms between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported any allergy symptoms over individual 6 month periods** | | | | | | |
| 0–6 months | 258/362 (71.27) | 243/333 (72.97) | 0.98 (0.89–1.07) | 0.62 | 0.99 (0.90–1.08) | 0.79 |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 232/364 (63.74) | 227/335 (67.76) | 0.94 (0.85–1.05) | 0.26 | 0.94 (0.84–1.04) | 0.23 |
| 18–24 months | 214/363 (58.95) | 203/333 (60.96) | 0.97 (0.86–1.09) | 0.59 | 0.97 (0.86–1.10) | 0.62 |
| 24–30 months | 194/361 (53.74) | 180/334 (53.89) | 1.00 (0.87–1.15) | 0.97 | 1.01 (0.88–1.16) | 0.90 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of any allergy over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | 200/361 (55.40) | 187/333 (56.16) | 0.99 (0.86–1.13) | 0.84 | 0.98 (0.86–1.12) | 0.76 |
| 0–36 months | 263/336 (78.27) | 226/312 (72.44) | 1.08 (0.99–1.18) | 0.09 | 1.09 (1.00–1.18) | 0.06 |

*Statistically significant ($P \leq 0.05$)
**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

*3.4.8.3 Incidence of parental reports of local doctor diagnosed any allergy*

Parental reports of doctor diagnosed any allergy were low compared to parental reports of any allergy symptoms at all age follow-ups (i.e. between 0-6 months of age -parental reports of any allergy symptoms (~72%) vs. parental reports of doctor diagnosed any allergy (~17%, Table 3-11 and Table 3-12). No effects of n-3 LCPUFA supplementation were found on parental reports of doctor diagnosed any allergic disease at any age group in unadjusted or adjusted models (Table 3-12).

*3.4.8.4 Cumulative incidence of parental reports of local doctor diagnosed any allergy*

It was not possible to estimate the parental reports of any doctor diagnosed allergic disease between birth to 12 months and birth to 36 months of age as these data were not collected and could not be derived from the available questions (Table 3-12).

**Table 3-12: Effects of n-3 LCPUFA supplementation on parental reports of doctor diagnosed any allergy between 0–36 months of age**

| Outcome | n-3 LCPUFA n/N (%) | Control n/N (%) | Unadjusted RR (95% CI) | P* | Adjusted** RR (95% CI) | P* |
|---|---|---|---|---|---|---|
| **Frequency of reported doctor diagnosed any allergy over individual 6 month periods** | | | | | | |
| 0–6 months | 58/362 (16.02) | 59/333 (17.72) | 0.90 (0.65–1.26) | 0.55 | 0.92 (0.67–1.28) | 0.64 |
| 6–12 months | - | - | - | - | - | - |
| 12–18 months | 83/364 (22.80) | 70/335 (20.90) | 1.09 (0.82–1.45) | 0.54 | 1.10 (0.83–1.45) | 0.52 |
| 18–24 months | 73/363 (20.11) | 63/333 (18.92) | 1.06 (0.79–1.44) | 0.69 | 1.09 (0.81–1.47) | 0.59 |
| 24–30 months | 70/361 (19.39) | 57/334 (17.07) | 1.14 (0.83–1.56) | 0.43 | 1.14 (0.831.56) | 0.43 |
| 30–36 months | - | - | - | - | - | - |
| **Reported cumulative incidence of doctor diagnosed any allergy over 0–12 and 0–36 month periods** | | | | | | |
| 0–12 months | - | - | - | - | - | - |
| 0–36 months | - | - | - | - | - | - |

*Statistically significant ($P \leq 0.05$)

**Treatment effect adjusted for centre, parity, maternal history of allergy and infant sex

## 3.5 Discussion

This study showed that n-3 LCPUFA supplementation of pregnant mothers had no effect on parental reported allergic disease of their offspring, including eczema, asthma, allergic rhinitis and food allergy, reported at 6 monthly intervals from birth to 36 months of age or on the cumulative incidence of parental reported allergic diseases assessed between birth to 12 months and birth to 36 months of age. The only significant finding was a reduction in the percentage of parental reports of allergic rhinitis symptoms from birth to 6 months of age in the unadjusted model, in children whose mothers received n-3 LCPUFA supplementation during pregnancy. However, after adjustment for confounders the effect was lost.

These parent reported allergy results are largely in agreement with the findings of Palmer et al [285] who reported that n-3 LCPUFA supplementation during pregnancy did not significantly reduce IgE associated allergic disease, diagnosed by study doctors, in the first 3 years of life in the same study cohort. In an earlier report, Palmer et al reported on the study doctor assessment of the infants at 12 months of age and noted that although n-3 LCPUFA supplementation in pregnancy did not reduce the overall incidence of IgE associated allergies, atopic eczema and egg sensitisation were reduced [303]. The reasons why the study doctor diagnosed reductions in IgE associated eczema seen at 12 months were not observed at 36 months in this cohort of children have not yet been elucidated, however Palmer et al [303] have speculated that it may relate to timing, duration and dosage of n-3 LCPUFA supplementation [285]. A postnatal study in which infants were supplemented with 400 mg n-3 LCPUFA daily until they were 6 months of age reported no effect on allergy outcomes at 12 months of age, but in infants with higher n-3 LCPUFA levels at 6 months of age a significant reduction in medically diagnosed eczema was observed [262]. In addition, Furuhjelm et al [299] who supplemented with n-3 LCPUFA during pregnancy and until 4 months of lactation showed a significant dose related reduction of IgE mediated allergies, associated with higher

maternal and infant DHA and EPA in plasma phospholipids at 2 years of age. Furuhjelm et al [299] also used a three times higher dosage than that used in this present study (and the Palmer study [303]). Further studies investigating n-3 LCPUFA supplementation timing, duration and dosage and effects on allergy outcomes would be worthwhile.

In the present chapter, parents reported only on clinical symptoms of allergic diseases and local doctor diagnosis of allergic diseases. As there was no way for parents to assess or report on skin prick tests and sensitisations it is not possible to comment solely on IgE mediated allergies. The incidences of the different parent reported allergy symptoms and their relation to medically diagnosed allergies within the same cohort, as well as to incidences reported in different cohorts are considered in detail below for each allergy in order to explore more whether parent reports could be reliable and cost effective for use in large scale clinical trials. The cumulative incidence of parental reports of eczema symptoms, parental reports of local doctor diagnosed eczema and study doctor medically diagnosed eczema in this study were ~14%,~24% and~26% at 12 months and ~26%,~31% and~29% at 36 months respectively. In contrast to the other allergic diseases investigated in this chapter, an under-reporting of the cumulative incidence of eczema symptoms by parents, compared to doctor diagnoses, was observed at both 12 and 36 months. The large variation in the parentally reported cumulative incidence of eczema symptoms compared to medical diagnosis at 12 months of age, may be due to the difficulty parents have in recognising and reporting eczema symptoms, particularly itch, in early infancy. Thus the 10% absolute difference between the two parental reports may relate to the itchy criterion wording differences in the questions asked at the face to face interviews, compared with the telephone interviews – with it being not possible for parents to decide whether the rash was itchy in very young children. In addition, difficulty with recall of symptoms over a longer period of time, or doctor's treatment of eczema being more memorable, may also contribute. At 36 months, the absolute difference

was 5% between parental reports of the cumulative incidence of eczema symptoms (26%) vs. parental reports of local doctor diagnosed (31%), supporting the possibility that the itchy criteria at very young ages may be difficult for parents to assess.

The inability of parents to report accurately the symptoms of eczema in early infancy, compared to doctor diagnosis of the condition, may contribute to the lack of finding of an effect of n-3 LCPUFA on parent reported symptoms of eczema up to 12 months of age in the same infants where a reduction in medically diagnosed atopic eczema was reported [303]. The power calculations of the original trial were based on medically diagnosed incidences and a reduction of power and hence ability to determine a difference may be expected if the true incidence of eczema is not captured by the study instruments. However, it is also noteworthy that there are two other small prenatal n-3 LCPUFA supplementation studies (one of which supplemented with fish [267]), on mothers whose fetuses were at high risk of allergies, which showed no significant reduction of atopic dermatitis with n-3 LCPUFA supplementation at 6 months and 12 months of age [259, 267]. These studies showed that 22% of 6 month old infants and 37% of 12 month old infants had atopic dermatitis compared to the 26% of medically diagnosed eczema in Palmer et al [259, 267, 303]. The differences in the results may be due to small sample sizes: n=83-124 [259, 267] compared to the Palmer et al study (n=706), [303] and different forms/dosage of n-3 LCPUFA supplementation (weekly fish vs. 2.7 g/day vs. 0.9 g/day) and age of assessment being 6 months in one study [267].

The diagnosis techniques used in this current study were parental report of allergies, so it is interesting to compare the incidence observed with other studies which have looked at parental reports of allergies in children in Australia. With regards to eczema, one population based study evaluated that parental reports of eczema prevalence was 20% in Australian children at normal risk of allergy between 0–36 months of age [113] compared to the ~31% in this study in a high risk group. Another population based study showed nearly 17% of

children had eczema symptoms in the last 12 months, compared to 32% of the children ever having an eczema diagnosis; the results were based on the ISAAC questionnaire at the age of seven years [70]. Differences in the incidence of eczema reported in this study to other publications may thus relate to differences in risk of populations assessed (high risk vs. normal risk), differences in the assessment age and validity of the allergy questions. In the present study non-validated questions were used and validation of allergy questions with the ISAAC questions has been recommended to overcome limitations of parental reported allergy outcomes in early childhood [104].

Asthma and other respiratory symptoms are difficult to recognise in young children. Whilst wheeze is the main symptom of asthma, it may also be caused by small airways and be seen after infections in young children [333, 334]. One study showed that 40% of parent reported asthma symptoms in children between 1–2 years of age, had no medical diagnosis of asthma [335]. Similarly in this present study,~33% of children had cumulative parental reports of asthma symptoms, compared with~2% of children that had parental reports of a clinical asthma diagnosis at 12 months of age. Although, these findings were on a high risk population, parental reports of asthma diagnosis in infants was similar to the data reported (2% - 4%) in the Australian Centre for Asthma Monitoring in 2008 [336].

The study doctor diagnosed IgE mediated asthma in the present study was <1% because the study doctor diagnosed asthma was defined as a history of 3 or more episodes of wheeze with the episodes less than 6 weeks apart and/or daily use of asthma medication, along with sensitisation to at least one of the aeroallergens tested and inhalant allergen sensitisation was not reported at the age of one in these children [303]. Even though the inhalant allergen sensitisation was reported at the assessment at 3 years of age in these children, IgE mediated asthma incidence was still low (~2%) at the age of 3 [285]. This needs to be further

investigated, as few inhalant allergens were tested in Palmer et al and the sensitivity of SPT tests were also questioned [285].

One Australian population based health survey showed that parental reports of asthma diagnosis at any stage (ever) were 16-26% in children [120]. However, this was in children between 0-15 years of age [120] and so, it is very difficult to compare with the current study which showed ~48% of children had cumulative parental reports of asthma symptoms, ~14% had parental reports of doctor diagnosed asthma and ~2% had diagnosed IgE mediated asthma at 36 months of age [285].

The findings with respect to reports of asthma in the present study are similar to those of two other n-3 LCPUFA studies in high allergy risk groups using similar medical diagnosis methods [259, 267]. Noakes reported that 24% of 6 month old infants had wheeze symptoms (obtained by research nurse), compared with the~28% of 6 month olds with parental reports of asthma symptoms in this present study [267]. Similarly, Dunstan reported that 27% of one year old children had recurrent wheezing and 10% of them had an asthma diagnosis by a doctor , compared to the 25% medical diagnosis of wheezing [303] and ~33% of parental reports of wheezing in the current study at one year of age . The variation in parent reported asthma symptoms, compared with medical diagnosis may reflect a reluctance of doctors to diagnose asthma prior to 3 years of age, due to its occurrence in tandem with other respiratory diseases which present as mixed wheezing [334]. Alternatively, a variability of the asthma disease spectrum may also explain the difficulty of assessing asthma symptoms in infancy by parents.

In this present study, the cumulative incidence of parental reports of allergic rhinitis was~11% in children between birth and 12 months and~23% in children between birth and 36 months of age. The cumulative incidence of study doctor diagnosed allergic rhinitis was much lower, with 4% at the age of one and 15% at the age of three respectively [285, 303]. Allergic rhinitis

is not common in infancy [122] and no other maternal intervention studies were found assessing the effect of n-3 LCPUFA supplementation on allergic rhinitis. The cumulative incidence of parental reports of allergic rhinitis symptoms may have been over reported in this study because of the difficulty for parents to differentiate allergic rhinitis symptoms from infections with similar symptoms (sneezing, nasal congestion and nasal discharge) [337] and parents may be more attentive to a new baby at 0–6 months of age [337].

ISAAC studies have reported variable incidences of allergic rhinitis, from very low to 50% of adolescents, with an average of over 30% worldwide [4, 96]. As these ISAAC studies were population based studies and were not in high risk groups [4, 96], there is little data available for comparison with the present findings.

The cumulative incidence of parental reports of food allergy symptoms between birth to 12 and birth to 36 months in this present study were˜21% and˜31% respectively. The study doctor diagnosis of IgE mediated food allergies with sensitisation to a particular food were <1% and 5% respectively in the same age ranges. The Dunstan study, in a high risk group reported that 10% of children had food allergies at 12 months of age [259] and that was similar to a challenge proven food allergy incidence of 10% in infants who were assessed in an Australian population based study on infants at normal risk of allergies [109]. The low rates of study doctor diagnosed IgE mediated food allergies in the present study suggests that most of the parental reports of food allergy reactions in this study were not due to children sensitised to the specific food [303]. Specific food challenge tests are known to be the gold standard for food allergy diagnosis [86, 87, 338]. The high rate of parental report of food allergy observed in this study may not be therefore food allergy per se, but probably reflects the fact that parents over interpret reactions and intolerance to foods as food allergy symptoms [339] as infants at weaning are more liable to spit out, refuse, regurgitate or vomit the new food.

### 3.5.1 Strengths and limitation of the study

This allergy study was a randomised, double blind, controlled trial of n-3 LCPUFA vs. vegetable oil supplementation in pregnancy and allergy outcomes of children up to 36 months of age, with high hereditary risk. The trial is the largest to date and was powered to detect an absolute reduction of 10% (relative reduction of 33%) in the cumulative incidence of IgE mediated allergies over the first 36 months of life [285]. Overall, although no significant reductions in the cumulative incidence of IgE associated allergies were detected over the first 3 years, the reductions of up to 22% reported by Palmer et al 2013 [285] may be significant in terms of reducing the burden of disease for affected families [2, 3, 8]. The follow-up rates were above 90% in each follow-up – a major strength of this study and participants, research staff and statisticians were blinded to treatment group allocation, reducing bias and increasing the internal validity of the results [340]. The balance of baseline characteristics between randomised groups was mostly comparable and the infants were healthy with mean birth weight, birth length, birth head circumference and gestational age at birth being within normal ranges. Caesarean sections comprised nearly 30% of the births, but were balanced between the two groups.

In this RCT, n-3 LCPUFA supplementation was started at < 21 weeks gestation. Immune-modulation properties in the fetal and neo-natal period are thought to be vital before allergies are established, however the optimal timing for modulation is unclear [341]. The results from this RCT, the largest to date, may contribute useful timing information for future meta-analysis to detect clinical implications of maternal n-3 LCPUFA supplementation below 12 months of age and until at 36 months of age.

In this chapter, no significant effect of n-3 LCPUFA supplementation on parental reports of allergy symptoms and parental reports of doctor diagnosed allergies were detected in the same

participants as Palmer et al [285, 303]. However, it is important to note that whilst Palmer et al report on IgE mediated allergies, in this chapter the parent reports of allergy symptoms would not specifically identify IgE mediated allergies. Parents were asked to recall allergy symptoms at 6 monthly intervals from birth to 36 months of age – a strength of this study. Parental reports of allergy symptoms were also collected from birth to 12 and birth to 36 months of age which required parents to recall symptoms over a much longer period. Variability between the recall periods at 6 monthly intervals and between birth to 12 months and birth to 36 months was apparent, suggesting that shorter intervals are better for parent recall studies/questions. With the exception of cumulative parent reported eczema at 12 and 36 months, the incidence of parent reported allergies was greater than the parent reports of doctor diagnosed allergies. This suggests that the questions may be permitting the false identification of infants with allergic diseases, perhaps because parents may be more attentive in first 6 months of the baby's life. Although the trial was adequately powered to evaluate the effect of n-3 LCPUFA on medically diagnosed IgE mediated allergy outcomes of the children [303] the results need treating with caution with regards to parent reported allergy outcomes, as it was evident that the questions did not accurately capture the incidence of clinical allergy outcomes, which would impact on the power calculations as discussed earlier. Although the reasons for this were not explored, there are a number of factors to be considered which may lead parents to erroneously report increased incidence of allergy symptoms, including: parent attentiveness in the first 6 months of the life, parental anxiety/responses to first born may be different to subsequent infants, parents of preterm infants may report differently due to more general complications/anxiety with preterm infants and maternal anxiety/depression may influence parental reporting. In addition, parent's education and domestic situation may also have influence on their reporting of allergy symptoms.

The questions used to collect parental reports of allergy symptoms were based on the ISAAC questionnaire. However this questionnaire is only validated to date for 6–7 and 12–14 year olds [98, 99] and therefore it may not be specific for infants and young children.

## 3.6 Conclusion

Of the 706 pregnant women with fetuses at high risk of atopic disease, 368 women received 900 mg/day n-3 LCPUFA, containing 800 mg/day DHA and 100 mg/day EPA. This supplementation had no significant overall effect on parental reports of childhood allergies, parent reports of local doctor diagnosed allergies or IgE-associated allergic disease in the first 3 years of life [285]. Although the results from the parent reports are in agreement with those from medical diagnoses caution is needed with the interpretation of the parent reported findings due to the fact that the parent reports did not appear to accurately capture the incidence of medically diagnosed allergies. While useful, the questions in their current form are unlikely to replace medical diagnosis of IgE and non IgE mediated allergies in clinical research. Based on the findings of this Chapter, a validation of parental reports of allergies in children 0-3 years of age was undertaken using Kappa statistics and diagnostic performance and the results are presented in Chapter 4.

# Chapter 4: Validation of parental reports of allergy symptoms or diagnosis in children who participated in a RCT

## 4.1 Introduction

This chapter will address the question of whether parental reports of allergy symptoms or diagnosis can be used as a valid tool to diagnose allergies in infancy and early childhood. Agreement, sensitivity, specificity, negative predictive value (NPV) and positive predictive values (PPV) were assessed for parental reports of allergy symptoms and parental reports of local doctor diagnosed allergy against study doctor diagnosed allergy at medical assessments in the same infants [342].

In the previous chapter, parental reports of allergy in children whose mothers were supplemented with n-3 LCPUFAs during pregnancy were investigated. The ISAAC questionnaire is being used as a tool to collect allergy outcomes worldwide [5]. The questions in the ISAAC questionnaire have been validated for reproducibility and reliability for 6–7 and 12–14 year old children [98]. Although there has been no validation for very early life, this study, as well as others, used modified versions of the ISAAC questions to collect allergy symptoms in toddlers and preschool children [104, 107]. Most of the questions that were used to collect parental reports of allergy outcomes between 0 to 3 years of age in this thesis were based on ISAAC questions. These questions were mainly used to collect prospective allergy history of the children with the answers supporting study doctors to diagnose allergies at 1 and 3 years of age. As a result, the study doctor's medical diagnoses of allergies were available within the study. This allowed an opportunity to compare parental reports of allergy symptoms and local doctor diagnoses of allergies with medical diagnosis of allergies by the study doctor for the same children.

As discussed in Chapter 3, medical diagnosis is the gold standard for allergy diagnosis, however it is very costly and time consuming for large scale research and alternatives, such as parent reports should be explored and evaluated further.

### 4.1.1 Objectives

The aim of this chapter was to evaluate the diagnostic use of parental reports of allergy symptoms and allergy diagnosis by a local doctor compared with medical diagnosis of allergies.

### 4.1.2 Hypotheses

Parental reports of allergy symptoms and parental reports of local doctor diagnosis of allergies can be used as an effective tool to evaluate allergies in infants and early childhood.

## 4.2 Methods

### 4.2.1 Participants

A total of 706 children who participated in the allergy follow-up of the DOMInO RCT with data available between 0–36 months were included in this analysis. Participants at each follow-up point are shown in Figure 3-1 (Chapter 3). Of the 706 participants, 681 and 638 had medical assessments at 12 months and 36 months of age respectively. Matching between medical diagnosis of allergy and parental reports of allergy was available only for those who had a medical diagnosis performed.

### 4.2.2 Parental reports of allergy

Parental reports of allergy were collected using structured telephone interviews at 6, 18, 24 and 30 months of age and face to face interviews 12 and 36 months of age. Full details of data collection of the study are described in Chapter 3. Within the parental reports of allergy outcomes, two separate outcomes were assessed – parental reports of allergy symptoms and parental reports of local doctor diagnosed allergy outcomes. These data were used to assess

the incidence of allergies at 6 month intervals and to assess cumulative incidence of allergies between 0–12 and 0–36 months of age.

### 4.2.3 Medical diagnoses of allergy

Medical diagnoses of allergies were available from the same children at 12 and 36 months of age. Specially trained medical practitioners (study doctors) made the diagnoses of allergic diseases at 12 and 36 months of age [285, 303].

The following criteria were used for medical diagnosis of allergies by study doctors:

- Eczema was defined as the presence of eczema criteria (according to [90]) on medical review or a history of an itchy rash distributed to the facial, flexural or extensor surface of the skin that had followed a fluctuating or chronic course [303].
- Asthma was defined as a history of 3 or more episodes of wheeze less than 6 weeks apart and/or daily use of asthma medication [303].
- Allergic rhinitis was defined as a history of sneezing or a runny or blocked nose accompanied by itchy-watery eyes when there were no symptoms to suggest an upper respiratory tract infection [303].
- Food allergy was defined as a history of immediate (within 60 min) skin rash (hives, rash or swelling) with or without respiratory symptoms (cough, wheeze, stridor), gastrointestinal symptoms (abdominal pain, vomiting, loose stools) or cardiovascular symptoms (collapse) following ingestion of a food and sensitisation to the implicated food [303].

### 4.2.4 Data analysis

Data analysis (parental reports vs. study doctor diagnoses) was performed using kappa statistics and diagnostic performance. Kappa statistics (or kappa coefficient) are the most commonly used and precise statistics for measuring agreement between two or more diagnostic methods or observers [342, 343]. Both the Bland Altman method and the Kappa

statistic assess agreement. Statistical advice was sought, after which it was decided that the Kappa statistic was the most appropriate method to use with the categorical/ordinal measures and comparisons (agreement between diagnostic methods) of this study [344]. The Bland Altman method is more appropriate for use with continuous measures [345].

Kappa statistics were performed only when the two different diagnostics methods were assessed at same time points. When the time periods differed for the parental reports and medical diagnosis of allergy, the kappa coefficient was not calculated e.g. parent reports of doctor diagnosed allergy between 0-12 months of age (data were available between 0-6 months, but no data on 6-12 months of age) vs. study doctor diagnosed allergy between 0-12 months of age, as agreement statistics are only relevant where the same underlying quantity is being measured by the two techniques [343]. The following guidelines were used for interpreting Kappa coefficients, with a kappa value of 0.0 (95%, CI -0.02, 0.00) indicating no agreement between the two groups and a value of 1.0 indicating very good agreement [342, 346] (Table 4-1).

**Table 4-1: Kappa rating table**

| Value of kappa | Strength of agreement |
|---|---|
| 0.00 | No |
| 0.01- 0.20 | Slight |
| 0.21–0.40 | Fair |
| 0.41–0.60 | Moderate |
| 0.61–0.80 | Good |
| 0.81–1.00 | Very good |

The validity of the diagnostic methods (parental report vs. medical diagnoses) were addressed using diagnostic performance statistics [342]. Validation involved calculating four measures of test performance: sensitivity, specificity, PPV and NPV. An ideal diagnostic test would correctly identify participants with and without allergies with 100% accuracy. The sensitivity of the parental reports quantifies the ability to correctly identify participants with allergies among those with medical diagnoses of allergies, whilst the specificity is the ability of

parental reports of allergic disease to correctly identify participants who do not have a medical diagnosis of allergic disease. The PPV and NPV are related to sensitivity and specificity through disease prevalence. The PPV indicates the probability that allergic disease is present when a parental report of allergic disease is given and the NPV indicates the probability that allergic disease is absent when a parental report of allergic disease is not given. As this study is simply interested in whether parental reports of allergy predict a measure of medical diagnosis, it is reasonable to measure diagnostic performances that provide the same underlying quantity using different assessment time periods.

## 4.3 Results

As outlined in the methods above, kappa statistics were not calculated where the time period for parental reports of symptoms/diagnosis and medical diagnosis differed. These are indicated throughout the results section below as not determined (ND), with additional reasons if applicable.

### 4.3.1 Eczema

The cumulative incidence of parental reports of eczema symptoms, parental reports of local doctor diagnosed eczema and study doctor medically diagnosed eczema in this study were ~14%, ~24% and ~26% at 12 months and ~26%, ~31% and ~29% at 36 months (see details in discussion section in Chapter 3).

#### *4.3.1.1 Parental reports of eczema symptoms vs. study doctor diagnosed eczema*

The questions used to collect parental reports of eczema symptoms and parental reports of local doctor diagnosed allergies are described in Chapter 3. The parent questions used to collect incidence were based on recall symptoms and diagnosis at 6 month intervals, while the cumulative incidences were based on recall over two longer intervals between 0–12 and 0–36 months of age. Study doctor diagnosed eczema was available between 0–12 and 24–36

months, but not 12–24 months of age in this study. Therefore kappa statistics were ND for 0-36 months of age.

#### *4.3.1.2 Kappa statistics – Parental reports of eczema symptoms and parental reports of local doctor diagnosed eczema versus study doctor diagnosed eczema*

The simple kappa statistics showed that the strength of agreement between parental reports of eczema symptoms versus study doctor diagnosed eczema were moderate 0.49 and parental reports of local doctor diagnosis of eczema versus study doctor diagnosed eczema were good 0.76, between 0–12 months of age (Table 4-2).

**Table 4-2: Agreement between parental reports of eczema outcomes and medically diagnosed eczema (by study doctor) at 12 months of age**

| Eczema | Age months | Kappa value | 95% CI |
|---|---|---|---|
| Parental reports of itchy rash in specific places* | 0–12 | 0.49 | 0.41–0.57 |
| Parental reports of local doctor diagnosed | 0–12 | 0.76 | 0.70–0.81 |

*inside fold of elbows, behind knees, front of ankles, behind ears, around eyes, under the buttocks, face, flexural areas of arms and legs

#### *4.3.1.3 Diagnostic performance – Parental reports of eczema symptoms versus study doctor diagnosed eczema*

Of the children with parental reports of eczema symptoms from 0–12 and 0–36 months, 84% and 93% had medically diagnosed eczema (by study doctor) from 0–12 and 0–12 or 24–36 months of age respectively (PPV). Of children without parental reports of eczema symptoms from 0–12 and 0–36 months, 83% and 82% did not have medically diagnosed eczema (by study doctor) from 0–12 and 0–12 or 24–36 months of age respectively (NPV). The specificity of the two methods of diagnosis was very high, 97% for both methods, from 0–12 and 0–36 months of age respectively, meaning that those without medically diagnosed eczema (by study doctor) also didn't have parental reports of eczema symptoms. The sensitivity of the questions was lower in children between 0–12 months of age than between 0–36 months of age. Of those children with medically diagnosed eczema (by study doctor), only 44% and

65% had parental reports of eczema symptoms from 0–12 and 0–36 months of age respectively (Table 4-3).

This suggests that the eczema questions asked as screening questions were more valid for 0–36 months of age than for 0-12 months of age, despite being the same questions.

**Table 4-3: Comparison of parental reports of eczema symptoms and medically diagnosed eczema (by study doctor)**

| Diagnostic performances | PPV | NPV | Specificity | Sensitivity |
|---|---|---|---|---|
| Parental reports of itchy rash in any of the recognised places* at 0–12 months versus medical diagnosis of eczema (by study doctor) at 0–12 months | 83.87 | 83.4 | 97.05 | 44.32 |
| Parental reports of itchy rash in any of the recognised places* at 0–36 months versus medical diagnosis of eczema (by study doctor) at 0–12 or 24–36 months | 92.73 | 82.32 | 96.95 | 65.11 |

*inside fold of elbows, behind knees, front of ankles, behind ears, around eyes, under the buttocks, face, flexural areas of arms and legs

#### *4.3.1.4 Diagnostic performance – Parental reports of local doctor diagnosed eczema versus study doctor diagnosed eczema*

Of those children with parental reports of local doctor diagnosed eczema from 0–12 and 0–36 months, 84% and 90% of them had a medical diagnosis of eczema (by study doctor) from 0–12 and 0–12 or 0–36 months of age respectively (PPV). Of those children without a medical diagnosis of eczema (by study doctor) from 0–12 and 0–12 or 0–36 months of age, 93% and 86% of them did not have parental reports of local doctor diagnosed eczema from 0–12 and 0–36 months of age respectively (NPV). The specificity where those without a study doctor medical diagnosis also didn't have parental reports of local doctor diagnosed eczema was very high (95%) between 0–12 and 0–36 months of age. The sensitivity was higher in children between 0–12 months of age than 0–36 months of age. The children with medically diagnosed eczema (by study doctor) had 80% and 75% parental reports of local doctor diagnosed eczema from 0–12 and 0–36 months of age respectively.

Diagnostic performance statistics showed that the questions used to collect parental reports of local doctor diagnosed eczema were more valid than parental reports of eczema symptoms (Table 4-3 and Table 4-4).

**Table 4-4: Comparison of parental reports of local doctor diagnosed eczema and medically diagnosed eczema (by study doctor)**

| Diagnostic performances | PPV | NPV | Specificity | Sensitivity |
|---|---|---|---|---|
| Parental reports of local doctor diagnosis of eczema at 0–12 months versus medical diagnosis of eczema (by study doctor) at 0–12 months | 84.34 | 93.05 | 94.88 | 79.55 |
| Parent reports of local doctor diagnosis of eczema at 0–36 months versus medical diagnosis of eczema (by study doctor) at 0–12 or 24–36 months | 89.50 | 86.34 | 94.67 | 75.21 |

### 4.3.2 Asthma

In this study~33% of children had cumulative parental reports of asthma symptoms, compared with~2% of children that had parental reports of doctor diagnosed asthma diagnosis at 12 months of age. At 36 months of age,~48% of children had cumulative parental reports of asthma symptoms and~14% of parental reports of doctor diagnosed asthma. Study doctor diagnosed asthma incidence was very low (<1%) at 12 months of age and~2% at 36 months of age [285, 303].

#### *4.3.2.1 Kappa statistics – Parental reports of asthma symptoms and parental reports of local doctor diagnosed asthma versus study doctor diagnosed asthma*

Agreement between parental reports of asthma symptoms (wheezing or whistling in the chest or night time cough without having a cold) and parental reports of local study doctor diagnosis versus study doctor diagnosed asthma couldn't be performed because the medical diagnosis of asthma incidence was very low between 0–12 months of age. In addition, kappa

statistics were ND between 0-36 months due to different timing of assessments as described above.

#### *4.3.2.2 Diagnostic performance – Parental reports of asthma symptoms versus study doctor diagnosed asthma*

Diagnostic performance was not assessed between 0–12 months of age because of the very low incidence of medical diagnosis by study doctors. At 0-36 months of age although the sensitivity and NPV were 100%, the PPV and specificity were low (Table 4-5) suggesting that the asthma screening questions were sensitive, but diagnosis of asthma below 3 years of age was difficult.

**Table 4-5: Comparison of parental reports of asthma symptoms and medically diagnosed asthma (by study doctor)**

| Diagnostic performances | PPV | NPV | Specificity | Sensitivity |
|---|---|---|---|---|
| Parental reports of wheezing and whistling in the chest and/or night time coughing without cold or flu at 0–12 months versus medical diagnosis (by study doctor) of asthma at 0–12 months | ND | ND | ND | ND |
| Parental reports of wheezing and whistling in the chest and/or night time coughing without cold or flu at 0–36 months versus medical diagnosis (by study doctor) of asthma at 24–36 months | 13.44 | 100.00 | 55.71 | 100.00 |

ND – Not determined

#### *4.3.2.3 Diagnostic performance – Parental reports of local doctor diagnosed asthma versus study doctor diagnosed of asthma*

As above, diagnostic performance was not assessed between 0–12 months of age because of the very low incidence of medical diagnosis of asthma by study doctors. However, between 0-36 months the sensitivity and specificity were 78% and 91% respectively (Table 4-6). The NPV was 98% and PPV was 37% (Table 4-6) suggesting that the questions used to collect parental reports of doctor diagnosed asthma were sensitive, although true diagnosis of asthma by medical professionals is difficult in this age group.

**Table 4-6: Comparison of parental reports of local doctor diagnosed asthma and medically diagnosed asthma (by study doctor)**

| Diagnostic performances | PPV | NPV | Specificity | Sensitivity |
|---|---|---|---|---|
| Parental reports of local doctor diagnosis of asthma at 0–-12 months versus medical diagnosis of asthma (by study doctor) at 0–12 months | ND | ND | ND | ND |
| Parental reports of local doctor diagnosis of asthma at 0–36 months versus medical diagnosis of asthma (by study doctor) at 24–36 months | 36.78 | 98.37 | 90.79 | 78.05 |

ND – Not determined

### 4.3.3 Allergic rhinitis

Cumulative incidence of parental reports of allergic rhinitis was˜11% in children between birth and 12 months and˜23% in children between birth and 36 months of age. The cumulative incidence of study doctor diagnosed allergic rhinitis was ˜4% and ˜15% at the age of one and at the age of three, respectively. As stated in section 3.4.6.4 no data on parental report doctor diagnosed allergic rhinitis was available at either time points.

#### *4.3.3.1 Kappa statistics – Parental reports of allergic rhinitis symptoms and parental reports of local doctor diagnosed allergic rhinitis versus study doctor diagnosed allergic rhinitis*

The very low incidence of study doctor diagnosed allergic rhinitis prevented investigations of agreement between parental reports of allergic rhinitis symptoms and parental reports of local doctor diagnosed allergic rhinitis versus study doctor diagnosed allergic rhinitis between 0–12 months of age. In addition kappa statistics were ND between 0-36 months due to different timing of assessments as described above.

#### *4.3.3.2 Diagnostic performance – Parental reports of allergic rhinitis symptoms versus study doctor diagnosed allergic rhinitis*

Diagnostic performance was not assessed between 0–12 months of age because the incidence of medical diagnosis of allergic rhinitis was very low. Between 0-36 months of age, the sensitivity and NPV were 100%, specificity was 91% and PPV was 67% (Table 4-7). This

shows that the allergic rhinitis screening questions were sensitive and specific, but the low PPV may reflect difficulty in true diagnosis.

**Table 4-7: Comparison of parental reports of allergic rhinitis symptoms and medically diagnosed allergic rhinitis (by study doctor)**

| Diagnostic performances | PPV | NPV | Specificity | Sensitivity |
|---|---|---|---|---|
| Parental reports of runny blocked nose without cold or flu at 0–12 months versus medical diagnosis of allergic rhinitis at 0–12 months (by study doctor) | ND | ND | ND | ND |
| Parental reports of runny blocked nose without cold or flu at 0–36 months versus medical diagnosis of allergic rhinitis at 24–36 months (by study doctor) | 66.90 | 100.00 | 91.13 | 100.00 |

ND – Not determined

### 4.3.4 Food allergy

Parental reports of food allergy symptoms between birth to 12 and birth to 36 months in this study were˜21% and˜31% respectively. The study doctor diagnosis of IgE mediated food allergies with sensitisation to a particular food were <1% and˜5% respectively in the same age ranges. No data on parental reports of doctor diagnosed food allergy was in either time points (see section 3.4.8.4).

#### *4.3.4.1 Kappa statistics – Parental reports of food allergy symptoms versus study doctor diagnosed food allergy*

Diagnosis of food allergy by study doctors was based on parental reports of reactions to foods and SPT sensitisation to the implicated food. Therefore, only the medical diagnosis of food allergy with sensitisation was available for comparison with parent report of food allergy symptoms. Slight agreement was noticed for parental reports of food allergy symptoms versus study doctor diagnosed food allergy with sensitisation between 0–12 month old children (Table 4-8).

Kappa statistics were ND between 0-36 months due to different timing of assessments as described above.

**Table 4-8: Agreement between parental reports of food allergy symptoms and medically diagnosed food allergy with sensitisation (by study doctor)**

| Food allergy symptoms | Age months | Kappa value | 95% CI |
|---|---|---|---|
| Reactions to specific foods* | 0-12 | 0.20 | 0.12–0.28 |

*cow's milk, egg, wheat, fish, nuts

*4.3.4.2 Kappa statistics – Parental reports of local doctor diagnosed food allergy versus study doctor diagnosed of food allergy*

The agreement between these two diagnostic methods for cumulative incidence of food allergy could not be performed because parental reports of local doctor diagnosis of food allergy was not collected between 0–12 or 0–36 months of age.

*4.3.4.3 Diagnostic performance – Parental reports of food allergy symptoms and medical diagnosis of food allergy with sensitisation (by study doctor)*

Of children with a medical diagnosis of food allergy with sensitisation (by the study doctor), 95% had parental reports of food allergy symptoms (sensitivity) between 0–12 months. In children without a medical diagnosis of food allergy with sensitisation (by study doctor), 82% did not have parental reports of food allergy symptoms (specificity) between 0–12 months. Also, in children with parental reports of food allergy symptoms between 0–12 months, 14% had a medical diagnosis (by study doctor) of food allergy with sensitisation (PPV) between 0–12 months and children without parental reports of food allergy symptoms between 0–12 months, 100% did not have a medical diagnosis (by study doctor) of food allergy with sensitisation (NPV).

In children with a medical diagnosis of food allergy with sensitisation (by study doctor) between 0–12 or 24–36 months of age, 93% had parental reports of food allergy symptoms (sensitivity) between 0–36 months. In children without a medical diagnosis of food allergy with sensitisation (by study doctor) between 0–12 or 24–36 months, 73% did not have parental reports of food allergy symptoms (specificity) between 0–36 months. Also, in children with parental reports of food allergy symptoms between 0–36 months, 16% had a

medical diagnosis (by study doctor) of food allergy with sensitisation (PPV) between 0–12 or 24–36 months and in children without parental reports of food allergy symptoms between 0–36 months, 100% did not have a medical diagnosis (by study doctor) of food allergy with sensitisation (NPV) between 0–12 or 24–36 months.

These results show that the questions used to collect parental reports of food allergy symptoms were sensitive and specific. However, when parental reports of food allergy symptoms were compared with medical diagnosis of food allergies with sensitisation, the PPV was very low (Table 4-9).

**Table 4-9: Comparison of parental reports of food allergy symptoms and medically diagnosed food allergy with sensitisation (by study doctor)**

| Diagnostic performances | PPV | NPV | Specificity | Sensitivity |
|---|---|---|---|---|
| Parental reports of reactions to specific foods at 0–12 months versus medical diagnosis of food allergy with sensitisation (by study doctor) at 0–12 months | 13.89 | 100 | 82 | 95 |
| Parental reports of reactions to specific foods at 0–36 months versus medical diagnosis of food allergy with sensitisation (by study doctor) at 0–12 or 24–36 months | 15.90 | 100 | 73 | 93 |

## 4.4 Discussion

This study was designed to compare the validity of parental reports of allergy outcomes with medical diagnosis of allergies. To achieve this, level of agreement and diagnostic performance were assessed between parental reports of allergy symptoms versus study doctor diagnosed allergies and parental reports of local doctor diagnosed allergy outcomes versus study doctor diagnosed allergies within children at higher risk of allergic disease.

### 4.4.1 Validity of questions on eczema

A moderate-good agreement between diagnosis of eczema by parent report of symptoms or by parent report of local doctor diagnosis vs. study doctor diagnosed eczema was observed. The questions on parental report eczema symptoms had low sensitivity (44%), but high specificity (97%) between 0–12 months of age. The quite low sensitivity predicts the 'underdiagnosis' of eczema by parents (14%) compared to local doctor diagnosis (~24%) and study doctor diagnosed (~26%). This may be due to the way the questions were asked "has your child had an itchy rash" and "was this itchy rash located in the following places" or because the two questions were combined in this analysis. The same questions offered higher sensitivity (65%) for parental reports of eczema symptoms between 0–36 months of age. This suggests that the "itchy" criteria question may not be a good predictor for eczema symptom diagnosis in early childhood. The questions on parental report of local doctor diagnosed eczema had high sensitivity (80%), high specificity (93%) and good agreement with study doctor diagnosed eczema between 0–12 months of age.

### 4.4.2 Validity of questions on asthma and allergic rhinitis

Although in this study ~33% and ~10% of infants had parental reports of asthma and rhinitis symptoms respectively, between 0–12 months of age, rates of asthma and rhinitis diagnosis by study doctors was very low in the infants. Therefore it was not possible to estimate agreement and diagnostic performances between 0–12 months of age for asthma and rhinitis.

However, the very low incidence of study doctor diagnosed asthma and allergic rhinitis compared to the parent report incidence may suggest that the parents report more incidences of asthma and allergic rhinitis than medical diagnosis. So, the questions that collected parent reports of asthma and allergic rhinitis may not be valid or may not be specific enough to collect asthma and allergic rhinitis symptoms/diagnoses or may not accurately catch the severity of the symptoms of asthma and allergic rhinitis diseases. Parent reports may differ due to a number of reasons as discussed in Chapter 3 and symptoms such as wheeze and cough are non-descript in early infancy. Interpretation issues due to language and cultural differences [5, 99, 347-349] have been reported, however in this trial mothers were recruited if they spoke English as the main language at home [27].

### 4.4.3 Validity of questions on food allergy

Food allergy was diagnosed between 0–12 months of age by the study doctor, however generally only two foods are given to infants below 6 months of age – breast milk and formula. Subsequently, when new foods are being introduced to baby, they are more likely to spat out, regurgitated or rejected which parents could misinterpret as food allergy symptoms. This study showed slight agreement of kappa and very low rates of PPV between parental reports of food allergy reactions and study doctor diagnosed food allergy with sensitisation between 0–12 months of age. The low PPV may be due to medical diagnosis (by study doctor) of IgE mediated food allergy using a combination of the questions that were collected in parental reports of food allergy reactions and the sensitisation to implicated food or because there was no way for parents to predict sensitisation in children between 0–12 or 0–36 months age.

The questions in the present study were based on the ISAAC questionnaire, which has been validated in older children and is considered to be a reliable and cost effective tool in children between 6–7 and 13–14 years of age [98, 99].

Asthma questions in the ISAAC have been validated against anti-asthmatic medication in 5 year old children in Finland and the questions were found to be highly valid [348]. However, eczema diagnosis using ISAAC questions and UK criteria [90], validated in Brazilian children between 4 - 12 years of age [349] suggested that an itchy criterion was not a good predictor for eczema diagnosis. The same type of validation study was conducted in Ethiopia where ISAAC eczema questions and UK criteria [90] were used to assess children between 1-5 years of age and it was reported that both the ISAAC and UK criteria were not good predictors for eczema diagnosis in those children [350], however this population was in a developing country where English is not the first language. However, other reports have also highlighted problems associated with the ISAAC questions that were asked [4-6, 100-104, 107]. It has been suggested that the questions may not be specific enough to collect allergy symptoms or allergic diseases, they may not accurately catch the severity of the allergic diseases and there may be interpretation issues due to both language and cultural differences [4-6, 100-103]. However, the questionnaire is employed in many epidemiological surveys.

### 4.4.4 Strengths and limitation of the study

This study had a good sample size and involved participants with a high hereditary risk of allergies. The major strength of this study was the high parent response rate (above 90%) at each follow-up time point. Medical diagnosis of allergies (by the study doctor) was available for 96% of children at 1 year of age and 90% of 3 year olds. There are some limitations of this validation study, worthy of consideration also. One of the main limitations of the study was that the time period for the parental reports of allergy and the medical diagnosis of allergy by study doctors in children did not fully coincide. Unfortunately as the timing of the medical

diagnosis was part of the protocol for the nested allergy follow-up of the DOMInO RCT which commenced before my thesis, there was no option to change the timing of the assessments. Also there were no comparisons made for parental reported doctor diagnosed allergic rhinitis and local doctor diagnosed food allergy between 0–12 and 0–36 months of age because the existing nested allergy follow-up study did not collect data on these two outcomes. In addition, even though a high risk population was used, the incidences of some allergies were very low i.e. medical diagnosis of asthma and allergic rhinitis. So, it was not possible to assess overall agreement for these two diseases.

Cohen's kappa statistic was used as a measure of agreement beyond chance for the different diagnostic methods for allergy in this chapter. However the kappa characteristic does have some limitations in that both prevalence and bias can impact on the magnitude of kappa. When the prevalence of a disease is either very low or very high, kappa can be decreased substantially and one study [351] reports that kappa is highest when disease prevalence is around 50%. However, none of the medically diagnosed allergies in this thesis had such high incidences, even in the high risk allergy population studied. In addition, it is possible that if the study had been performed in a population unselected on the basis of allergy risk that different kappa values may have been observed [342, 343, 351]. The magnitude of kappa is also influenced by bias which reflects the levels of disagreement between observers, or in the case of this study, between parents and doctors or between study doctors and local doctors on the diagnosis of or non-diagnosis of allergy/allergy symptoms. When there is a large bias, the kappa value is higher than when there is low or no bias [352] and the effects of bias are greater when kappa is small than large. Bias arising between different study doctors medical diagnoses of allergy in this study should be minimized however as they were all specifically trained and adhered to strict study SOPs. Although there are some methods for attempting to deal with the effects of prevalence and bias on kappa, there is no universally agreed method.

In addition, the assignment of kappa into categories between poor-very good was based on Landis and Koch 1977 [346]. These categories, although useful in medical diagnostics, are arbitrary and not defined based on statistical criteria and they have been criticized by some as being too positive [345, 353, 354]. Thus the moderate-good categorisation of the agreement observed for eczema may be questionable if different categorisations were applied.
In the diagnostic performance testing, the impact of using high allergy risk participants also needs to be considered because the prevalence of disease will influence the PPV and NPV. Thus if all other factors were to remain constant the PPV will increase with increasing prevalence and the NPV will decrease with increasing prevalence. Another area of consideration also relates to the sensitivity and severity of disease. Although sensitivity and specificity shouldn't really change with different groups of people, the more severe the disease, the more likely it is to make a diagnosis and thus sensitivity would be expected to go up.

The ISAAC written questionnaire is known to be a reliable and cost effective tool focused on children between 6–7 and 13–14 years of age, and although many epidemiological surveys rely on this questionnaire, some problems have been identified [4-6, 100-103].

The performance on the questionnaires in this present study was found to be different in different age groups, and the following limitations need to be considered for the infants studied: symptoms were obtained from parents rather than the child; the transient nature of signs and symptoms in the population studied, mis-diagnosis with other diseases possible, difficulties with some allergy diagnosis (food allergy) and differences in different doctor's assumptions to constitute a typical diagnoses of allergies [5, 6, 347, 355].

## 4.5 Conclusion

Parental reports of doctor diagnosed eczema questions were the most reliable for the diagnosis of eczema in infants. The comparisons for asthma, allergic rhinitis and food allergy were difficult to perform due to limitations in the study design and low incidences of some medically diagnosed allergies. Changes in the format of questions, especially for the very young infants may improve the sensitivity and ability of parents to diagnose allergy symptoms more successfully, however more studies, across different risk groups that are specifically designed to investigate validity are needed. Until then, the use of parent questionnaires capturing allergy symptoms or doctor reports of allergy symptoms to diagnose allergy in early infancy in RCTs could give a wrong picture if not accompanied by medical diagnosis.

# Chapter 5: Postnatal n-3 LCPUFA supplementation to prevent allergic disease in school age children who were born preterm

## 5.1 Introduction

Whilst the preceding chapters focussed on n-3 LCPUFA supplementation during pregnancy and allergy outcomes of children, this Chapter will focus on postnatal supplementation of n-3 LCPUFA mainly to lactating mothers who have a preterm infant/s and assessment of allergy outcomes of the children at school age. This is important because preterm infants are the most vulnerable and sickest infants and it is suggested that they are more liable to have allergies and respiratory problems including asthma [356-358]. The results of the systematic review reported in Chapter 2, identified that there is a lack of studies evaluating postnatal n-3 LCPUFA supplementation on allergy outcomes of children, especially those who were not known to be at high risk of allergies and those born preterm.

Currently, the DHA for the Improvement of Neurodevelopmental Outcomes in preterm infants (DINO) trial is the only RCT of n-3 LCPUFA supplementation in lactating women with preterm infants that has included allergy outcomes [26]. In the DINO trial, the mothers were supplemented n-3 LCPUFA within 5 days of giving birth to a preterm infant/s, until the baby reached the expected date of delivery. While the primary outcome for the DINO trial was neurodevelopment at 18 months corrected age (CA), a number of secondary outcomes related to allergy and asthma were collected based on parental recall of medical attention or medical treatment at 12 and 18 months of age [26, 283]. Overall, at 12 or 18 months of age, the results showed a reduction in risk of parent reported hay fever with DHA supplementation, but no reduction in the incidence of asthma, eczema or food allergy [283]. However, these findings need to be treated with caution as they were secondary outcomes, and the allergy questions asked were not validated questions to assess parental reports of

allergies. Based on the results in Chapter 4 of this thesis, non-validated parental reports of some allergy outcomes can yield higher incidences than study doctor diagnosed allergies in children between 0–36 months of age.

A more restricted follow-up, of 143 children from the DINO trial was conducted at 3-5 years CA, using the ISAAC questionnaire to collect parental reports of allergy diagnosis and parental reports of doctor diagnosed eczema, allergic rhinitis and asthma [284]. The incidence of asthma and rhinitis symptoms did not differ between the groups, however the incidence of eczema symptoms was significantly higher in the high DHA supplemented group [284]. These data require further follow-up to try to understand the discrepancies, any longer term effects and whether there is consistency with previously found observations. To date, there have been no studies in which allergies have been assessed at school age in children born preterm who were supplemented with n-3 LCPUFA after birth.

The ISAAC questionnaire is a well validated questionnaire for children age 6-7 years [99] and at the 7-year follow-up of the DINO children there was a great opportunity to use this validated questionnaire to assess parental reports of allergy outcomes in these children, born preterm and supplemented with n-3 LCPUFA either through breast milk or formula (if required).

## 5.2 Subjects and Methods

### 5.2.1 Study Design

This is a follow-up of the DINO trial [26]. The DINO trial was a multicentre, RCT conducted in five perinatal centres in Australia from April 2001 to October 2005. In it, 657 preterm infants (delivered by 545 women) were enrolled and randomised at the Women's & Children's Hospital and Flinders Medical Centre, Adelaide; Royal Women's Hospital, Melbourne; Royal Women and Brisbane Hospital, Brisbane; and King Edward Memorial Hospital, Perth.

The DINO trial (registered as ACTRN12606000327583 in the Australian New Zealand Clinical Trials Registry) was designed to assess the effect on neurodevelopment at 18 months CA of providing the estimated *in utero* requirement of DHA (1% total fatty acids) compared with standard dietary DHA (0.3% of total fatty acids) on preterm infants from birth until estimated due date (EDD) [26].

#### *5.2.1.1 Inclusion and exclusion criteria for the DINO trial*

Infants born before 33 weeks' gestation and who were within 5 days of commencing enteral feeds were eligible to participate. Lactating women with a blood clotting disorder where tuna oil was contraindicated, or if regularly taking anti-coagulant therapy were excluded. Infants who had major congenital or chromosomal abnormalities, infants from a multiple birth in which not all live-born infants were eligible, or mothers and infants in other trials of fatty acid supplementation were also excluded.

#### *5.2.1.2 Dietary intervention for the DINO trial*

Women and their infant(s) were randomised to either high DHA or standard DHA groups. In the high DHA group, lactating women took six 0.5 gram capsules of DHA-rich oil per day (3 gram daily dose of tuna oil, 900 mg of DHA and 195 mg of EPA) to raise the breast milk

DHA content to ~1% of total fatty acids. If formula was required, infants had a preterm formula containing DHA as 1% of total fatty acid.

In the standard DHA group, mothers took six 0.5 gram control capsules per day (3 gram daily dose of soy-oil with no DHA or EPA). The infants from the standard group received DHA at the standard dietary level (0.3% total fatty acids) from breast milk. If formula was required, infants had a standard preterm formula containing 0.3% DHA.

The dietary intervention was started within 5 days of commencing enteral feeds and continued until the infant reached 40 weeks postmenstrual age.

*5.2.1.3 Allocation of the participants to the original study*

Randomisation occurred after written informed consent was obtained. Mother-infant pairs were randomly assigned a unique study number through a computer-driven telephone randomisation service, according to an independently generated randomisation schedule. Stratification was by centre, birth weight (<1250 gram versus ≥1250 gram) and infant sex. Multiple births were considered as a single randomisation unit and randomisation of twins or triplets was according to the sex and birth weight of the first born infant.

*5.2.1.4 Blinding to the original study*

Parents, outcome assessors, investigators and clinicians were blinded to the dietary allocation. To facilitate blinding, each treatment group was separately colour coded into 2 groups. All capsules were similar in size, shape, and colour and were donated by Clover Corporation, Sydney, Australia. If formula was required, Mead Johnson Nutritionals, Evansville, Indiana, specifically manufactured ready-to-feed preterm formula to trial specifications and packaged the formula according to the colour codes.

### 5.2.2 Hypothesis

For my study the hypothesis was that postnatal n-3 LCPUFA supplementation to preterm infants (largely through maternal supplementation) will reduce the risk of parental reports of allergy symptoms (eczema, asthma and allergic rhinitis), severity of allergy symptoms in the previous 12 months and cumulative incidence of allergy symptoms/diagnosis in children at school age (7 years CA).

### 5.2.3 Objectives

The earlier allergy follow-ups in the DINO trial, identified allergy reductions related to DHA supplementation at 12 and 18 months of age. Contrary to this however, a significant increase in eczema risk with high DHA supplementation, was observed between 3-5 years of age in a small subgroup form one centre. In this Chapter, the objectives of the follow-up of the DINO infants at 7 years CA (DINO7), were to use the ISAAC questionnaire to determine whether dietary n-3 LCPUFA supplementation to preterm babies (largely through maternal supplementation) decreases the parental reported allergy incidence and severity of allergy symptoms in the previous 12 months and the cumulative incidence of allergy symptoms/diagnosis in children at school age (7 years CA).

## 5.3 Methods of DINO7 follow-up

A follow-up study of the DINO randomised controlled trial at 7 years CA.

### 5.3.1 Eligibility Criteria for DINO7 follow-up

All families who had participated in, and all children enrolled in the DINO trial that had not withdrawn or died prior to 7 years CA were eligible for this follow-up study.

### 5.3.2 Blinding - DINO7 follow-up

Participants, families, clinicians and research staff in the original DINO trial were blinded to treatment group allocation. The blinding was broken by the statistician during the analysis of the DINO results at 18 months and 3-5 years of age, however trial staff and investigators were

not aware of individual group allocations and did not directly interact with participants or staff responsible for assessments in DINO7. Participating mothers were able to request details of group allocation after the end of the 18 month follow-up, if they wished, however research staff were unaware which families had requested to be un-blinded and families were instructed not to reveal this information during study follow-up. The number of mothers requesting to be un-blinded is reported as a post-randomisation characteristic (Table 5-13).

### 5.3.3 Ethical approval – DINO7 follow-up

Ethical approval for the DINO7 follow-up study was obtained prior to the study commencement from each of the five participating perinatal Centres.

### 5.3.4 Outcomes

#### *5.3.4.1 Primary outcomes*

The primary outcomes for the allergy component of the DINO7 study were to evaluate:

- parental reports of eczema, asthma and allergic rhinitis symptoms in the previous 12 months using the ISAAC questionnaire [98].
- parental reports of severe symptoms of eczema, asthma, and allergic rhinitis in the previous 12 months using the ISAAC questionnaire [98].
- parental reports of eczema, asthma, and allergic rhinitis symptoms (ever) from 0 to 7 years CA, using the ISAAC questionnaire [98] and
- parental reports of eczema, asthma, and allergic rhinitis (doctor diagnosis, ever) from 0 to 7 years CA by using the ISAAC questionnaire [98].

#### *5.3.4.2 Secondary outcomes*

The secondary outcomes for the allergy component of the DINO7 study were to evaluate:

- parental reports of medically diagnosed eczema and asthma between 2–7 years CA (see section 5.3.5.7 for more details on the age group).

#### *5.3.4.3 Subgroup analysis*

Primary allergy outcomes were assessed only for children who were breastfed at trial entry in the hope that these data can be included in future updates of the systematic review (Chapter 2) which considers n-3 LCPUFA supplementation to mothers during lactation according to the pre-specified inclusion criteria.

### 5.3.5 Data collection and data management – DINO7 follow-up

The primary focus of DINO7 was to assess general intellectual ability of the children at 7 years CA and parental reports of asthma and allergic diseases were secondary outcomes of the DINO7 study follow-up. However, for the purpose of this thesis, the main focus was on the allergy follow-up and to achieve this, coordination with other aspects of the study was required. My role involved the recruitment and participation in the appointments of children recruited from the Women's & Children's Hospital (n=108) and the Flinders Medical Centre (n=62) in Adelaide from 2011 to 2013 and I also provided support for recruitment and follow-up in the other three centres, as needed.

#### *5.3.5.1 Contacting Families*

All eligible families were mailed an invitation pack to their most recent address containing an information letter, consent form, updated contact form and a reply-paid envelope. Families who had not returned the consent forms or contacted the study centre two weeks after the mail out, were telephoned to ensure that they had received the information pack and asked whether they would like the information pack to be resent. Families were called again four weeks later to describe the study, determine interest in participating in the study, and answer any questions. Interested participants were encouraged to discuss the study with their family and return the signed consent form, after which a convenient appointment was made to conduct the assessments, primarily at the relevant recruiting centre, or at an alternative environment when this was not possible.

#### *5.3.5.2 Informed consent*

At the start of each assessment, parent or caregivers were provided with another verbal description of the study procedure to verify consent. Consent forms were checked and completed by all parties according to study protocol before the appointment proceeded.

#### *5.3.5.3 Assessments at the appointment*

I was trained to conduct clinic appointments according to SOPs for the DINO7 study at the Women's & Children's Hospital and the Flinders Medical Centre in Adelaide. At the clinic appointment, baseline data and parental reports of allergy outcomes were collected. Baseline data were collected using a web based electronic CRF which was developed by the Chief Investigator of the DINO7 trial. In some situations (i.e. home visits) data were collected on a hard copy questionnaire and later transcribed to the electronic CRF. Parents' reports of allergy outcomes of the children were obtained using a paper-based version of the ISAAC questionnaire (Appendix 5.1) [98]. Data collection was completed by myself at WCH and FMC and by trained research assistants from the three other perinatal centres outside of Adelaide: Royal Women's Hospital, Melbourne; Royal Women and Brisbane Hospital, Brisbane and King Edward Memorial Hospital, Perth.

#### *5.3.5.4 Baseline and background data*

Baseline characteristics, including family structure, primary carer's education and occupation, other carer's education and occupation, home environment, family health information, child's education, child's general health and diet were collected using the web based electronic CRF (Appendix 5.3). Possible confounding variables such as family history of allergies for the biological parents and/or siblings, home environment including family members in the home, pets, smoker in the home and gas heating were collected. In addition, the child's recent dietary intake of DHA from fish, DHA enriched foods and fish oil supplements, consumed within one month preceding the appointment, were also collected.

Previously collected baseline characteristics, including maternal age at trial entry, maternal smoking during pregnancy, parental education, birth order, parity, gestational age at birth, birth measurements, and infants receiving breast milk at trial entry, were available from the original DINO trial [26].

#### *5.3.5.5 Assessment of primary and secondary allergy outcomes*

At the appointment, parents or care givers were asked to complete the ISAAC questionnaire (Appendix 5.1) to collect primary allergy outcomes. After parents completed the questionnaire, it was checked for any discrepancies so they could be corrected immediately. Secondary allergy outcomes were collected using CRF questions (Appendix 5.3) at the same appointment.

#### *5.3.5.6 Primary allergy outcome data – Study instrument*

The primary outcomes were assessed using the ISAAC questionnaire about parental reports of allergy outcomes (Appendix 5.1). Further details about the ISAAC questionnaire can be found in section 1.2.5.2 in Chapter 1 and in sections 4.4.3 and 4.4.4 in Chapter 4.

Current eczema was defined as having an itchy rash affecting the characteristic skin areas at any time in the past 12 months, with the itchy rash first occurring before 5 years of age (Question (Q) 16, 17, 18, Appendix 5.1). Severity of eczema was evaluated by using the negative response to complete clearance of rash (Q 19, Appendix 5.1) and positive response to sleep disturbances at night (Q 20, Appendix 5.1). Cumulative incidence of eczema symptoms and eczema diagnosis were evaluated by using positive response to questions Q 15 and Q 21 respectively from 0–7 years CA (Q 15, Q 21, Appendix 5.1).

Asthma was defined based on a positive response to question Q 2 (Appendix 5.1). The severity of asthma was evaluated by using episodes of wheezing attacks, sleep disturbances at night and speech limitation at a time between breaths (Q 3, 4 and 5, Appendix 5.1).

Cumulative incidence of asthma symptoms and asthma diagnosis was evaluated by using positive answers to questions Q 1 and Q 6 respectively at 7 years CA (Q 1, Q 6, Appendix 5.1).

Allergic rhinitis was defined as a positive response to Q 10 and rhino-conjunctivitis was defined as a positive answer to Q10 and Q11 (Appendix 5.1). The severity of the allergic rhinitis and rhino-conjunctivitis were evaluated using daily activity interference (Q13, Appendix 5.1) with severe allergic rhinitis defined by 'yes' to Q10 and 'a lot' to Q13. The presence of seasonal allergic rhinitis was defined by a positive answer to Q 10 with occurrence in September and/or October and/or November (Q 12, Appendix 5.1). Cumulative incidence of allergic rhinitis symptoms and allergic rhinitis diagnosis was evaluated by using positive answers to Q 9 and Q 14 respectively at 7 years CA (Appendix 5.1). Further details of ISAAC questions can be found in Appendix 5.1 (a).

#### *5.3.5.7 Secondary allergy outcomes – Study instrument*

Information on children with parental reports of doctor diagnosed medical conditions, including asthma and eczema, were collected using the CRF questions (Appendix 5.3) which were designed before my involvement and the time period (2-7 years CA) was chosen as the same children were assessed previously for similar outcomes until 18 months of age. The question was originally, "w*ithin the last 5 years has your child been diagnosed by a doctor with any medical conditions such as eczema, asthma, autism spectrum disorders, ADHD, seizures/epilepsy, other behavioural disorders or any other medical condition"* but as the interest of this study is allergy, only two medical conditions (Q 4) eczema (Q 4.1) and asthma (Q 4.2) were evaluated (Appendix 5.2) in this thesis.

There was no assessment of food allergy at 7 years CA. The frequency of food allergy was expected to be very low as many children outgrow their food allergies by school age.

#### *5.3.5.8 Data management*

All data from the five perinatal centres were managed by the Data Management and Analysis Centre (DMAC) at the University of Adelaide. The paper-based ISAAC questionnaires were sent to DMAC for entry. Electronic CRF data were entered directly into the database at the time of collection, except for home visits, as mentioned above. Logic checks and reasonable value limitations were applied to data fields on the electronic CRF, so that if unreasonable values were entered they were immediately flagged for checking. Data were maintained on a secure server and all information was securely backed up. Data cleaning was undertaken by a statistician and queries were sent to individual study centres where necessary. Parents were contacted to clarify answers if required.

### 5.3.6 Data analysis

I developed a data analysis plan specifically relating to the allergy part of DINO7 in consultation with Thomas Sullivan (Statistician) (Appendix 5.4). After completion of all 7 year (CA) data collection, analyses were performed on an intention to treat basis. Baseline characteristics were summarised using descriptive statistics. The proportion of children with allergic diseases was compared between treatment groups. Assuming random data, normal distribution of deviations, and independence between the observations, Pearson chi-squared and Fisher exact tests were used to compare between groups of interests (for categorical variables). For continuous variables with a normal distribution, the mean differences between the various groups were evaluated using student's t-test. Statistical significance was set at a P-value of ≤ 0.05 (two-sided). Adjustments were made for centre, birth weight strata (under 1250 g versus 1250 g or more) and sex. The relationship between treatment (and adjustment variables) and outcome was modelled using a log binomial model. A generalised estimating equation with independence correlation matrix was used to account for clustering at the maternal level (i.e., multiple births). The estimate is of the relative risk of the outcome in the

fish oil group versus the control group. In some cases, the number of events for an outcome was too small to allow for adjustment. In this case, unadjusted estimates only are presented.

There were no observed cases of severe allergic rhinitis, therefore no analyses are presented for this outcome. The primary outcome analyses were repeated for the subgroup of children whose mothers were breastfeeding at trial entry (n=531 out of total 569 in original study population) as the main focus of the thesis is on n-3 LCPUFA supplementations to pregnant and/or lactating mothers.

## 5.4 Results of DINO7 follow-up

### 5.4.1 Flow of the participants

Of the 657 preterm infants who were recruited into the DINO trial (high DHA diet n=322, standard DHA diet n=335), 18 infants died and 13 were withdrawn leaving 626 available for recruitment to this 7 year follow-up study. Six hundred and four children consented and were recruited (high-DHA diet n=291, standard-DHA diet n=313) at the 7 year CA study (Figure 5-1). Of the 604 children, 5 children were withdrawn and 11 were lost to follow-up during DINO7. Follow-up rates were between 86% - 90% (Figure 5-1) There were n=531 children breastfed (high DHA diet n=260, standard DHA diet n=271) (93.3%) at trial entry who were included in the subgroup analysis (Figure 5-1).

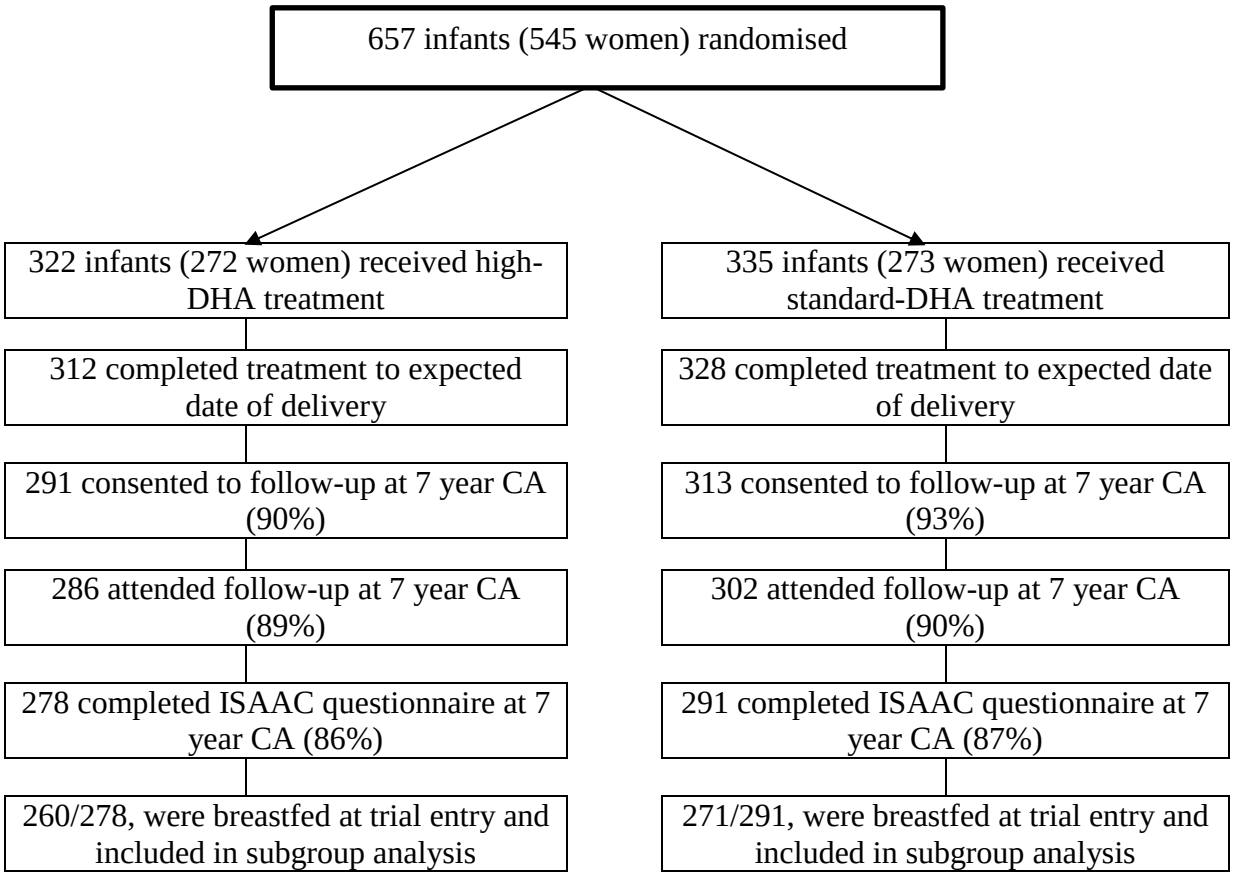


**Figure 5-1: Flow of the participants throughout DINO trial follow-up periods**
(CA= corrected age)

### 5.4.2 Baseline characteristics

Most of the demographic and baseline characteristics of the participants involved in the 7 year follow-up were comparable between the high and standard DHA groups including centre, including singleton pregnancy, maternal age, maternal education, paternal education and maternal smoking (Table 5-1). The cohort at 7 years CA was representative of the original DINO cohort [26].

Slightly higher percentages of mothers had a singleton pregnancy in the high DHA group while more mothers had multiple pregnancies in the standard DHA group (Table 5-1). This was also seen in the original DINO trial [26].

**Table 5-1: Baseline characteristics at entry into DINO trial**

| Characteristics | High DHA group (n=291) | Standard DHA group (n=313) |
|---|---|---|
| Hospital: n (%) | | |
| Flinders Medical Centre | 29 (10.0) | 31 (9.9) |
| King Edward Memorial Hospital | 58 (19.9) | 56 (7.9) |
| Royal Brisbane & Women's Hospital | 44 (15.1) | 44 (14.1) |
| Royal Women's Hospital | 50 (17.2) | 58 (18.5) |
| Women's & Children's Hospital | 110 (37.8) | 124 (39.6) |
| Singleton pregnancy: n (%) | 208 (71.5) | 195 (62.3) |
| Multiple pregnancies: n (%) | 83 (28.5) | 118 (37.7) |
| Birth order of pregnancy: n (%) | | |
| First of a multiple birth | 39 (13.4) | 56 (17.9) |
| Second of a multiple birth | 41 (14.1) | 56 (17.9) |
| Third of a multiple birth | 3 (1.0) | 6 (1.9) |
| Mothers age at trial entry (years): mean (sd) | 29.9 (5.7) | 30.5 (5.2) |
| Mother completed secondary education: n (%) | 175 (62.5) | 172 (58.7) |
| Father completed secondary education: n (%) | 146 (54.1) | 155 (55.4) |
| Mother smoked during pregnancy: n (%) | 77 (26.5) | 76 (24.3) |
| Infant receiving breast milk at trial entry: n (%) | 271 (93.1) | 291 (93.0) |

### 5.4.3 Neonatal characteristics

The neonatal characteristics of the participants involved in the 7 year CA follow-up were comparable between the treatment groups and representative of the original DINO trial [26] (Table 5-2). No differences were found in infant sex, birth weight, birth length, birth head circumferences, small for gestational age and median gestational age at birth between groups. In the standard DHA group, a slightly higher percentage of infants had a first degree relative with diagnosis of allergy (Table 5-2).

**Table 5-2: Neonatal characteristics at trial entry**

| Neonatal characteristics | High DHA group (n=291) | Standard DHA group (n=313) |
|---|---|---|
| Female infants: n (%) | 139 (47.8) | 141 (45.0) |
| Male infants: n (%) | 152 (52.2) | 172 (55.0) |
| Birth weight range: n (%) 1250g and Greater | 159 (54.6) | 174 (55.6) |
| Birth weight range: n (%) Less than 1250g | 132 (45.4) | 139 (44.4) |
| Birth weight (g): mean (sd) | 1307 (420) | 1320 (410) |
| Birth length (cm): mean (sd) | 38.2 (3.9) | 38.2 (4.1) |
| Birth head circumference (cm): mean (sd) | 27.2 (2.8) | 27.4 (2.7) |
| Gestational age at birth (weeks): median | 30.0 | 30.0 |
| Small for gestational age: n (%) | 54 (18.6) | 57 (18.2) |
| First degree relative with diagnosis of allergy: n (%) | 194 (70.5) | 222 (75.5) |

**Primary outcomes – Parental reports of allergy:**

### 5.4.4 Eczema

*5.4.4.1 Incidence of parental reports of eczema and severe eczema symptoms (between 6–7 years CA)*

No differences in the incidence of parental reports of eczema symptoms (Table 5-3) and severe eczema symptoms (Table 5-4), between 6-7 years CA were found between the high DHA supplemented group compared with the standard DHA group in unadjusted and adjusted models. Parental reports of eczema symptoms in the last 12 months including itchy rash, itchy rash in typical locations and current eczema were approximately 17%, 11% and 9% respectively (Table 5-3). There was no interaction effect between treatment and birth weight or sex for eczema symptoms (Table 5-3).

#### *5.4.4.2 Cumulative incidence of parental reports of eczema and eczema symptoms (between 0–7 years CA)*

Parental recall of eczema symptoms between birth and 7 years CA did not differ between groups with 16% in the high DHA group and 15% in the standard DHA group (Table 5-5). No interaction effect between treatment and sex or birth weight was found in interaction models (Table 5-5). Approximately 25% of children had parental reports of eczema (doctor diagnosis) between birth to age of 7 years CA (Table 5-5) and there was no difference between treatment groups detected. The percentage of cumulative incidence of eczema symptoms (itchy rash) (15%) was lower than parental reports of doctor diagnosis of eczema (25%) between birth to 7 years CA (Table 5-5). Similar observations were made when parents were asked about itchy rash symptoms and parental reports of doctor diagnosed eczema in the prenatal n-3 LCPUFA supplementation study that assessed parental allergy outcomes of children between birth to 36 months of age earlier in this thesis (see Chapter 3, Table 3-2 and Table 3-3).

**Table 5-3: Effects of high DHA supplementation on parental reports of eczema symptoms in children between 6–7 years CA**

| Parental reports of eczema symptoms in last 12 months from ISAAC questionnaire | | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|---|
| Itchy rash | [b] All children | 42/278 (15.11%) | 36/291(12.37%) | 1.22 (0.79, 1.88) | 0.36 | 1.26 (0.83, 1.91) | 0.28 |
| | [c] Male | 16/145 (11.03%) | 12/160 (7.50%) | 1.47 (0.71, 3.06) | 0.30 *0.48* [a] | 1.48 (0.72, 3.04) | 0.28 *0.59* [a] |
| | [c] Female | 26/133 (19.55%) | 24/131 (18.32%) | 1.07 (0.64, 1.78) | 0.80 | 1.16 (0.69, 1.94) | 0.57 |
| | [d] <1250 BW | 22/125 (17.60%) | 19/129 (14.73%) | 1.19 (0.66, 2.16) | 0.56 *0.92* [a] | 1.27 (0.72, 2.26) | 0.41 *0.96* [a] |
| | [d] ≥1250 BW | 20/153 (13.07%) | 17/162 (10.49%) | 1.25 (0.68, 2.29) | 0.48 | 1.24 (0.69, 2.25) | 0.47 |
| Itchy rash in typical locations (the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes) | [b] All children | 36/278 (12.95%) | 28/291 9.62%) | 1.35 (0.83, 2.19) | 0.23 | 1.38 (0.86, 2.21) | 0.19 |
| | [c] Male | 15/145 (10.34%) | 8/160 (5.00%) | 2.07 (0.89, 4.83) | 0.09 *0.18* [a] | 2.08 (0.91, 4.80) | 0.08 *0.23* [a] |
| | [c] Female | 21/133 (15.79%) | 20/131 (15.27%) | 1.03 (0.58, 1.84) | 0.91 | 1.12 (0.63, 2.00) | 0.69 |
| | [d] <1250 BW | 17/125 (13.60%) | 15/129 (11.63%) | 1.17 (0.59, 2.30) | 0.65 *0.56* [a] | 1.22 (0.63, 2.36) | 0.56 *0.59* [a] |
| | [d] ≥1250 BW | 19/153 (12.42%) | 13/162 (8.02%) | 1.55 (0.79, 3.05) | 0.21 | 1.57 (0.81, 3.03) | 0.18 |
| Current eczema (itchy rash in typical locations and start below 5 years of age) | [b] All children | 27/278 (9.71%) | 25/291 (8.59%) | 1.13 (0.66, 1.94) | 0.66 | 1.18 (0.70, 1.98) | 0.54 |
| | [c] Male | 9/145 (6.21%) | 7/160 (4.38%) | 1.42 (0.54, 3.74) | 0.48 *0.53* [a] | 1.46 (0.56, 3.80) | 0.44 *0.61* [a] |
| | [c] Female | 18/133 (13.53%) | 18/131 (13.74%) | 0.98 (0.53, 1.84) | 0.96 | 1.08 (0.58, 2.01) | 0.81 |
| | [d] <1250 BW | 10/125 (8.00%) | 13/129 (10.08%) | 0.79 (0.36, 1.77) | 0.57 *0.24* [a] | 0.85 (0.39, 1.82) | 0.67 *0.25* [a] |
| | [d] ≥1250 BW | 17/153 (11.11%) | 12/162 (7.41%) | 1.50 (0.73, 3.06) | 0.27 | 1.52 (0.76, 3.05) | 0.23 |

* Statistically significant ($P \leq 0.05$) [a] *Interaction effect* [b] Treatment effect adjusted for centre, birth weight and infant sex [c] Adjusted sex strata
[d] Adjusted birth weight strata

**Table 5-4: Effects of high DHA supplementation on parental reports of severe eczema symptoms in children between 6–7 years CA**

| Parental reports of severe eczema symptoms in last 12 months from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|
| Rash not cleared | 13/278 (4.68%) | 5/291 (1.72%) | 2.72 (0.86, 8.59) | 0.09 | 2.79 (0.89, 8.79) | 0.08 |
| Eczema kept awake | 4/278 (1.44%) | 2/291 (0.69%) | 2.09 (0.39, 11.28) | 0.39 | ND | ND |
| Severe eczema (rash has not cleared and child kept awake >one or more nights per week) | 3/278 (1.08%) | 1/291 (0.34%) | 3.14 (0.33, 29.90) | 0.32 | ND | ND |

* Statistically significant ($P \leq 0.05$) ** Treatment effect adjusted for centre, birth weight and infant sex, ND= not determined

**Table 5-5: Effects of high DHA supplementation on parental reports of eczema and eczema symptoms in children from 0–7 years CA**

| Parental reports of eczema symptoms and eczema diagnosis from 0–7 years CA from ISAAC questionnaire | | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|---|
| Ever had itchy rash | [b] All children | 43/278 (15.47%) | 43/291 (14.83%) | 1.04 (0.69, 1.58) | 0.84 | 1.08 (0.72, 1.62) | 0.71 |
| | [c] Male | 16/145 (11.03%) | 15/160 (9.38%) | 1.18 (0.59, 2.33) | 0.64 *0.61* [a] | 1.19 (0.60, 2.34) | 0.62 *0.74* [a] |
| | [c] Female | 27/133 (20.30%) | 28/131 (21.54%) | 0.94 (0.57, 1.57) | 0.82 | 1.03 (0.62, 1.71) | 0.91 |
| | [d] <1250 BW | 22/125 (17.60%) | 20/129 (15.50%) | 1.14 (0.63, 2.04) | 0.67 *0.69* [a] | 1.20 (0.68, 2.11) | 0.53 *0.60* [a] |
| | [d] ≥1250 BW | 21/153 (13.73%) | 23/162 (14.29%) | 0.96 (0.53, 1.73) | 0.89 | 0.97 (0.55, 1.72) | 0.92 |
| Ever had eczema (doctor diagnosis) | [b] All children | 70/278 (25.18%) | 74/291 (25.43%) | 0.99 (0.74, 1.33) | 0.95 | 1.01 (0.76, 1.35) | 0.94 |
| | [c] Male | 31/145 (21.38%) | 35/160 (21.88%) | 0.98 (0.64, 1.50) | 0.92 *0.98* [a] | 1.00 (0.66, 1.51) | 0.99 *0.93* [a] |
| | [c] Female | 39/133 (29.32%) | 39/131 (29.77%) | 0.98 (0.67, 1.45) | 0.94 | 1.02 (0.70, 1.51) | 0.91 |
| | [d] <1250 BW | 28/125 (22.40%) | 32/129 (24.81%) | 0.90 (0.58, 1.41) | 0.65 *0.60* [a] | 0.95 (0.61, 1.47) | 0.82 *0.73* [a] |
| | [d] ≥1250 BW | 42/153 (27.45%) | 42/162 (25.93%) | 1.06 (0.72, 1.57) | 0.77 | 1.06 (0.72, 1.54) | 0.78 |

* Statistically significant (P ≤ 0.05) [a] *Interaction effect* [b] Treatment effect adjusted for centre, birth weight and infant sex [c] Adjusted sex strata [d] Adjusted birth weight strata

### 5.4.5 Asthma

#### *5.4.5.1 Incidence of parental reports of asthma and severe asthma symptoms (between 6–7 years CA)*

No differences in parental reports of asthma or severe asthma symptoms between 6-7 years of CA were found between the high DHA group compared with the standard DHA group in unadjusted and adjusted models (Tables 5-6 and 5-7). No significant interaction effect was found in secondary analysis based on effect modification of sex and the birth weight.

#### *5.4.5.2 Cumulative incidence of parental reports of asthma symptoms (between 0–7 years CA)*

No differences in parental recall of asthma symptoms were found between the high DHA supplemented group compared with the standard DHA group in unadjusted and adjusted models (Table 5-8). Between birth and 7 years CA, 45% of children had parental reports of asthma symptoms. No significant interactions were found between sex and birth weight (Table 5-8).

#### *5.4.5.3 Cumulative incidence of parental reports of diagnosed asthma (between 0–7 years CA)*

Approximately 29% of children had cumulative incidence of asthma diagnosis between birth and 7 years CA (Table 5-8).

When all children were considered, there were no differences for parental reports of asthma diagnosis between the high DHA and standard DHA groups (Table 5-8). Although interactions suggested that boys and girls were responding differently to DHA treatment, none of the post-hoc analyses reached significance, and these interactions were not consistent with wheeze symptoms (Table 5-8).

**Table 5-6: Effects of high DHA supplementation on parental reports of asthma symptoms in children between 6–7 years CA**

| Parental reports of asthma symptoms in last 12 months from ISAAC questionnaire | | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|---|
| Wheezing or whistling in the chest | [b] All children | 75/278 (26.98%) | 72/291 (26.12%) | 1.03 (0.78, 1.37) | 0.89 | 1.04 (0.79, 1.37) | 0.79 |
| | [c] Male | 43/145 (29.66%) | 47/160 (29.38%) | 1.01 (0.71, 1.43) | 0.96 *0.80* [a] | 1.00 (0.71, 1.42) | 0.99 *0.74* [a] |
| | [c] Female | 32/133 (24.06%) | 29/131 (22.14%) | 1.09 (0.69, 1.70) | 0.72 | 1.10 (0.70, 1.72) | 0.67 |
| | [d] <1250 BW | 38/125 (30.40%) | 38/129 (29.46%) | 1.03 (0.71, 1.50) | 0.87 *1.00* [a] | 1.03 (0.71, 1.51) | 0.87 *0.97* [a] |
| | [d] ≥1250 BW | 37/153 (24.18%) | 38/162 (23.46%) | 1.03 (0.69, 1.54) | 0.88 | 1.05 (0.70, 1.56) | 0.83 |
| Wheezing accompanying by exercise | [b] All children | 43/278 (15.52%) | 39/291 (13.40%) | 1.16 (0.77, 1.73) | 0.48 | 1.14 (0.76, 1.70) | 0.53 |
| | [c] Male | 25/145 (17.24%) | 29/160 (18.13%) | 0.95 (0.58, 1.55) | 0.84 *0.16* [a] | 0.93 (0.58, 1.52) | 0.78 *0.17* [a] |
| | [c] Female | 18/133 (13.64%) | 10/131 (7.63%) | 1.79 (0.85, 3.74) | 0.12 | 1.74 (0.83, 3.67) | 0.14 |
| | [d] <1250 BW | 20/125 (16.13%) | 19/129 (14.73%) | 1.10 (0.61, 1.95) | 0.76 *0.80* [a] | 1.06 (0.59, 1.90) | 0.85 *0.73* [a] |
| | [d] ≥1250 BW | 23/153 (15.03%) | 20/162 (12.35%) | 1.22 (0.69, 2.14) | 0.49 | 1.22 (0.71, 2.10) | 0.48 |
| Nocturnal dry cough without having a cold or chest infection | [b] All children | 81/278 (29.24%) | 70/291 (24.05%) | 1.22 (0.91, 1.63) | 0.19 | 1.21 (0.91, 1.61) | 0.19 |
| | [c] Male | 43/145 (29.66%) | 45/160 (28.13%) | 1.05 (0.74, 1.51) | 0.77 *0.23* [a] | 1.03 (0.73, 1.47) | 0.85 *0.18* [a] |
| | [c] Female | 38/133 (28.79%) | 25/131 (19.08%) | 1.51 (0.93, 2.44) | 0.09 | 1.54 (0.95, 2.49) | 0.09 |
| | [d] <1250 BW | 41/125 (33.06%) | 25/129 (19.38%) | 1.71 (1.09, 2.67) | 0.02 *0.05* [a] | 1.66 (1.05, 2.61) | 0.03 *0.07* [a] |
| | [d] ≥1250 BW | 40/153 (26.14%) | 45/162 (27.78%) | 0.94 (0.64, 1.39) | 0.76 | 0.96 (0.66, 1.40) | 0.82 |

* Statistically significant (P ≤ 0.05) [a] *Interaction effect* [b] Treatment effect adjusted for centre, birth weight and infant sex [c] Adjusted sex strata
[d] Adjusted birth weight strata

**Table 5-7: Effects of high DHA supplementation on parental reports of severe asthma symptoms in children between 6–7 years CA**

| Parental reports of severe allergy symptoms in last 12 months from ISAAC Questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|
| Four or more wheezing attacks in the last 12 months | 22/278 (7.91%) | 23/291 (7.90%) | 1.00 (0.57, 1.75) | 1.00 | 0.99 (0.57, 1.74) | 0.98 |
| Sleep disturbed | 11/278 (3.96%) | 7/291 (2.41%) | 1.64 (0.63, 4.29) | 0.31 | 1.65 (0.63, 4.35) | 0.31 |
| Wheezing limited speech | 16/278 (5.76%) | 12/291 (4.12%) | 1.40 (0.65, 2.99) | 0.39 | 1.41 (0.66, 3.01) | 0.38 |
| Severe asthma (4 or more wheezing attacks, sleep disturbed and limited speech due to wheeze) | 1/278 (0.36%) | 3/291 (1.03%) | 0.35 (0.04, 3.31) | 0.36 | ND | ND |

*Statistically significant ($P \leq 0.05$) ** Treatment effect adjusted for centre, birth weight and infant sex, ND= not determined

**Table 5-8: Effects of high DHA supplementation on parental reports of asthma and asthma symptoms in children between 0–7 years CA**

| Parental reports of asthma and asthma symptoms from 0–7 years CA from ISAAC questionnaire | | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|---|
| Ever had wheeze | [b] All children | 124/278 (44.60%) | 135/291 (46.39%) | 0.96 (0.80, 1.16) | 0.68 | 0.96 (0.80, 1.16) | 0.70 |
| | [c] Male | 71/145 (48.97%) | 82/160 (51.25%) | 0.96 (0.76, 1.21) | 0.70 *0.88* [a] | 0.94 (0.74, 1.19) | 0.60 *0.69* [a] |
| | [c] Female | 53/133 (39.85%) | 53/131 (40.46%) | 0.98 (0.73, 1.33) | 0.92 | 1.01 (0.76, 1.35) | 0.93 |
| | [d] <1250 BW | 66/125 (52.80%) | 60/129 (46.51%) | 1.14 (0.88, 1.47) | 0.34 *0.09* [a] | 1.13 (0.87, 1.47) | 0.37 *0.08* [a] |
| | [d] ≥1250 BW | 58/153 (37.91%) | 75/162 (46.30%) | 0.82 (0.63, 1.07) | 0.15 | 0.81 (0.62, 1.06) | 0.12 |
| Ever had asthma (doctor diagnosis) | [b] All children | 83/278 (29.86%) | 82/291 (28.18%) | 1.06 (0.81, 1.38) | 0.67 | 1.05 (0.81, 1.36) | 0.73 |
| | [c] Male | 44/145 (30.34%) | 57/160 (35.63%) | 0.85 (0.62, 1.18) | 0.33 *0.04* [a] | 0.84 (0.61, 1.17) | 0.30 *0.03* [a] |
| | [c] Female | 39/133 (29.32%) | 25/131 (19.08%) | 1.54 (0.97, 2.43) | 0.07 | 1.56 (0.99, 2.45) | 0.06 |
| | [d] <1250 BW | 42/125 (33.60%) | 36/129 (27.91%) | 1.20 (0.82, 1.76) | 0.34 *0.37* [a] | 1.20 (0.82, 1.75) | 0.34 *0.32* [a] |
| | [d] ≥1250 BW | 41/153 (26.80%) | 46/162 (28.40%) | 0.94 (0.65, 1.36) | 0.76 | 0.92 (0.64, 1.32) | 0.65 |

* Statistically significant ($P \leq 0.05$) [a] *Interaction effect* [b] Treatment effect adjusted for centre, birth weight and infant sex [c] Adjusted sex strata [d] Adjusted birth weight strata

### 5.4.6 Allergic rhinitis

#### *5.4.6.1 Incidence of parental reports of allergic and severe allergic rhinitis symptoms (between 6–7 years CA)*

When all children were considered, no differences in parental reports of allergic or severe allergic rhinitis symptoms, between 6-7 years CA were found between the groups in unadjusted and adjusted models (Tables 5-9 and 5-10). However, there was evidence of an effect modification by birth weight strata where a significant increase of parental reports of allergic rhinitis symptoms was observed in the children who were born <1250 g birth weight that received high DHA supplementation, compared to standard DHA (control). The opposite direction was found in the children born ≥1250 g birth weight, indicating a different response.

#### *5.4.6.2 Cumulative incidence of parental reports of allergic rhinitis symptoms (between 0–7 years CA)*

No differences in parental recall of allergic rhinitis symptoms were found in the high DHA supplemented group compared with the standard DHA group in unadjusted and adjusted models between birth to 7 years CA (Table 5-11).

However, as above, there was a significant increase in cumulative incidence of parental reports of allergic rhinitis symptoms between birth and 7 years CA in children who were born <1250 g and had high DHA supplementation. The direction is consistent with parental reports of allergic rhinitis symptoms in the previous 12 months. No interaction effect was found between sex groups (Table 5-11).

#### *5.4.6.3 Cumulative incidence of parental reports of diagnosed allergic rhinitis (between 0–7 years CA)*

Parental reports of diagnosed allergic rhinitis were approximately 22% in the children between birth and 7 years CA (Table 5-11). When all children were considered, there were no significant differences for parental reports of cumulative incidence of diagnosed allergic rhinitis between the high DHA group and standard DHA group (Table 5-11).

No significant differences were found in the parental reports of cumulative incidence of diagnosed allergic rhinitis in children with ≥1250 g birth weight, in the high DHA supplemented group, compared to the standard DHA (control group) when adjusted. This finding is consistent with the parental reports of cumulative incidence of allergic rhinitis symptoms (Table 5-11).

In contrast to the parent reported symptoms of allergic rhinitis however, the cumulative incidence of parental reports of diagnosed allergic rhinitis was not significantly increased in the children who had high DHA supplementation and were in the <1250 g birth weight group (Table 5-11).

**Table 5-9: Effects of high DHA supplementation on parental reports of allergic rhinitis symptoms in children between 6–7 years CA**

| Parental reports of severe allergy symptoms in last 12 months from ISAAC questionnaire | | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|---|
| Sneezing, runny or blocked nose without cold – Allergic rhinitis symptoms | [b] All children | 69/278 (24.82%) | 67/291 (23.02%) | 1.08 (0.79, 1.47) | 0.64 | 1.09 (0.81, 1.46) | 0.59 |
| | [c] Male | 45/145 (31.03%) | 48/160 (30.00%) | 1.03 (0.73, 1.47) | 0.85 *0.59*[a] | 1.02 (0.73, 1.44) | 0.91 *0.52*[a] |
| | [c] Female | 24/133 (18.05%) | 19/131 (14.50%) | 1.24 (0.70, 2.22) | 0.46 | 1.27 (0.71, 2.28) | 0.42 |
| | [d] < 1250 BW | 36/125 (28.80%) | 23/129 (17.83%) | 1.62 (1.00, 2.62) | 0.05 *0.03*[a] | 1.62 (1.00, 2.60) | 0.05 *0.03*[a] |
| | [d] ≥1250 BW | 33/153 (21.57%) | 44/162 (27.16%) | 0.79 (0.52, 1.20) | 0.28 | 0.82 (0.55, 1.20) | 0.31 |
| Nasal symptoms accompanied with itchy watery eyes – Rhino-Conjunctivitis symptoms | [b] All children | 35/278 (12.68%) | 27/291 (9.28%) | 1.37 (0.83, 2.25) | 0.22 | 1.39 (0.84, 2.29) | 0.20 |
| | [c] Male | 22/145 (15.28% | 15/160 (9.38%) | 1.63 (0.84, 3.16) | 0.15 *0.41*[a] | 1.65 (0.85, 3.20) | 0.14 *0.40*[a] |
| | [c] Female | 13/133 (9.85% | 12/131 (9.16%) | 1.08 (0.51, 2.27) | 0.85 | 1.08 (0.50, 2.29) | 0.85 |
| | [d] < 1250 BW | 16/125 (12.90%) | 10/129 (7.75%) | 1.66 (0.78, 3.54) | 0.19 *0.51*[a] | 1.68 (0.78, 3.60) | 0.18 *0.53*[a] |
| | [d] ≥1250 BW | 19/153 (12.50%) | 17/162 (10.49%) | 1.19 (0.62, 2.28) | 0.60 | 1.22 (0.64, 2.33) | 0.54 |

*Statistically significant (P ≤ 0.05) [a] *Interaction effect* [b] Treatment effect adjusted for centre, birth weight and infant sex [c] Adjusted sex strata
[d] Adjusted birth weight strata

**Table 5-10: Effects of high DHA supplementation on parental reports of severe allergic rhinitis symptoms in children between 6–7 years CA**

| Parental reports of severe allergic rhinitis symptoms in last 12 months from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|
| Seasonal allergic rhinitis (occurred September and/or October and/or November) | 18/278 (6.47%) | 20/291 (6.87%) | 0.94 (0.49, 1.80) | 0.86 | 0.92 (0.48, 1.74) | 0.79 |
| Severe allergic rhinitis (nasal symptoms interfered 'a lot' with daily activities) | 0/278 (0.00%) | 0/291 (0.00%) | ND | ND | ND | ND |

*Statistically significant ($P \leq 0.05$)** Treatment effect adjusted for centre, birth weight and infant sex, ND= not determined

**Table 5-11: Effects of high DHA supplementation on parental reports of allergic rhinitis and allergic rhinitis symptoms in children from 0–7 years CA**

| Parental reports of allergic rhinitis and allergic rhinitis symptoms from 0–7 years of age from ISAAC questionnaire | | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|---|
| Ever had problem with sneezing or a runny , or blocked nose | [b] All children | 74/278 (26.62%) | 77/291 (26.46%) | 1.01 (0.75, 1.35) | 0.97 | 1.00 (0.75, 1.32) | 0.97 |
| | [c] Male | 48/145 (33.10%) | 54/160 (33.75%) | 0.98 (0.71, 1.36) | 0.91 *0.69* [a] | 0.94 (0.68, 1.30) | 0.72 *0.55* [a] |
| | [c] Female | 26/133 (19.55%) | 23/131 (17.56%) | 1.11 (0.65, 1.91) | 0.70 | 1.14 (0.66, 1.96) | 0.63 |
| | [d] <1250 BW | 40/125 (32.00%) | 25/129 (19.38%) | 1.65 (1.05, 2.60) | 0.03 *0.005* [a] | 1.62 (1.03, 2.53) | 0.04 *0.005* [a] |
| | [d] ≥1250 BW | 34/153 (22.22%) | 52/162 (32.10%) | 0.69 (0.47, 1.03) | 0.07 | 0.70 (0.48, 1.02) | 0.07 |
| Ever had hay fever (allergic rhinitis) (doctor diagnosis) | [b] All children | 54/278 (19.42%) | 70/291 (24.05%) | 0.81 (0.58, 1.13) | 0.22 | 0.81 (0.58, 1.13) | 0.22 |
| | [c] Male | 30/145 (20.69%) | 39/160 (24.38%) | 0.85 (0.54, 1.32) | 0.47 *0.76* [a] | 0.85 (0.55, 1.33) | 0.48 *0.72* [a] |
| | [c] Female | 24/133 (18.05%) | 31/131 (23.66%) | 0.76 (0.46, 1.27) | 0.30 | 0.76 (0.45, 1.26) | 0.28 |
| | [d] <1250 BW | 26/125 (20.80%) | 26/129 (20.16%) | 1.03 (0.61, 1.74) | 0.91 *0.21* [a] | 1.05 (0.63, 1.76) | 0.85 *0.18* [a] |
| | [d] ≥1250 BW | 28/153 (18.30%) | 44/162 (27.16%) | 0.67 (0.44, 1.04) | 0.08 | 0.67 (0.43, 1.03) | 0.07 |

* Statistically significant (P ≤ 0.05) [a] *Interaction effect* [b] Treatment effect adjusted for centre, birth weight and infant sex [c] Adjusted sex strata
[d] Adjusted birth weight strata

### 5.4.7 *Secondary outcomes – Parental reports of medically diagnosed allergy*

#### *5.4.7.1 Eczema and asthma*

Analysis of the data from CRF questions specifically asking about medical diagnosis of eczema and asthma (Appendix 5.2) in the last 5 years did not reveal any differences between treatment groups for parental reports of doctor diagnosed eczema and asthma overall in the children between 2–7 years CA in adjusted or unadjusted analyses. Children with parental reports of doctor diagnosed eczema and asthma were approximately 8% and 22% respectively (Table 5-12).

Similarly, no significant interaction effect modifications by birth weight or sex strata on parental reports of doctor diagnosed eczema and asthma in the children between 2–7 years CA were seen (Table 5-12).

**Table 5-12: Effects of high DHA supplementation on parental reports of medically diagnosed asthma and eczema in children between 2–7 years CA**

| Parent report medically diagnosed allergy outcomes from CRF Questions | | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|---|
| Eczema | [b] All children | 25/285 (8.77%) | 23/298 (7.72%) | 1.14 (0.65, 1.99) | 0.65 | 1.09 (0.63, 1.89) | 0.75 |
| | [c] Male | 8/148 (5.41%) | 7/163 (4.29%) | 1.26 (0.47,3.36) | 0.65 *0.76* [a] | 1.24 (0.47, 3.29) | 0.66 *0.77* [a] |
| | [c] Female | 17/137 (12.41%) | 16/135 (11.85%) | 1.05 (0.54, 2.04) | 0.89 | 1.04 (0.53, 2.01) | 0.91 |
| | [d] <1250 BW | 11/129 (8.53%) | 11/135 (8.15%) | 1.05 (0.46, 2.41) | 0.91 *0.79* [a] | 1.03 (0.45, 2.36) | 0.95 *0.84* [a] |
| | [d] ≥1250 BW | 14/156 (8.97%) | 12/163 (7.36%) | 1.22 (0.57. 2.62) | 0.70 | 1.15 (0.55, 2.39) | 0.70 |
| Asthma | [b] All children | 63/285 (22.11%) | 68/298 (22.82%) | 0.97 (0.71, 1.32) | 0.84 | 0.96 (0.71, 1.31) | 0.80 |
| | [c] Male | 37/148 (25.00%) | 48/163 (29.45%) | 0.85 (0.58, 1.24) | 0.39 *0.22* [a] | 0.83 (0.58, 1.21) | 0.34 *0.19* [a] |
| | [c] Female | 26/137 (18.98%) | 20/135 (14.81%) | 1.28 (0.74,2.21) | 0.37 | 1.29 (0.75, 2.22) | 0.36 |
| | [d] <1250 BW | 33/129 (25.58%) | 29/135 (21.48%) | 1.19 (0.76, 1.86) | 0.44 *0.21* [a] | 1.15 (0.74, 1.81) | 0.53 *0.25* [a] |
| | [d] ≥1250 BW | 30/156 (19.23%) | 39/163 (23.93%) | 0.80 (0.52, 1.23) | 0.31 | 0.81 (0.53, 1.23) | 0.32 |

*Statistically significant (P ≤ 0.05) [a] *Interaction effect* [b] Treatment effect adjusted for centre, birth weight and infant sex [c] Adjusted sex strata [d] Adjusted birth weight strata

### 5.4.8 Post randomisation variables associated with allergy

Post-randomisation descriptive variables which may have been associated with allergies were compared between the two groups. Mostly there were no differences, with the exception of the child's position in the family and whether a dog or cat was present in the household (Table 5-13). The number of first born children was significantly higher in high DHA group compared to standard DHA (control group) and consequently the standard DHA (control group) had significantly higher numbers of second or later born children, in addition to more dogs or cats than high DHA group (Table 5-13).

**Table 5-13: Post randomisation characteristics**

| Allergy associated conditions | High DHA group (n=291) | Standard DHA group (n=313) | P value |
|---|---|---|---|
| Position by age of child in family: n (%) | | | **0.04*** |
| First | 152 (53.3) | 134 (45.0) | |
| Second or later | 133 (46.7) | 164 (55.0) | |
| Smoker in household: n (%) | 101 (35.4) | 109 (36.6) | 0.80 |
| Dog or cat as pet: n (%) | 176 (61.8) | 215 (72.1) | **0.02*** |
| Number of children in the household: mean (sd) | 1.4 (1.0) | 1.5 (1.0) | 0.21 |
| Presence of a gas heater without a chimney at home: n (%) | 33 (11.6) | 29 (9.7) | 0.51 |
| Had a fish meal in last month: n (%) | 248 (87.0) | 267 (89.6) | 0.39 |
| Number of fish meals in last month: mean (sd) | 5.0 (3.8) | 5.1 (3.6) | 0.96 |
| DHA enriched foods in last month: n (%) | 172 (60.4) | 181 (60.7) | 0.93 |
| Number of DHA enriched foods in last month: mean (sd) | 21.2 (26.4) | 16.8 (18.2) | 0.16 |
| Supplement containing DHA in last month: n (%) | 73 (25.8) | 57 (19.5) | 0.10 |
| Child or family unblinded before the 7 year assessment: n (%) | 24 ( 8.2) | 23 (7.3) | 0.73 |

* Statistically significant ($P \leq 0.05$)

### 5.4.9 Subgroup analysis – Breastfeeding group only: Eczema, asthma and allergic rhinitis

This subgroup analyses was performed to enable inclusion of the data in future updates of the systematic review (Chapter 6). There were 93% of infants receiving breast milk at trial entry and these were balanced between the two randomised groups (Figure 5-1 and Table 5-1).

#### *5.4.9.1 Incidence of parental reports of allergic symptoms or parental reports of severe allergic symptoms in the past year (between 6–7 years CA)*

When the outcomes of eczema, asthma and allergic rhinitis were considered only in children breastfed at trial entry, there were no differences between the high DHA supplemented group compared with the standard DHA group in unadjusted and adjusted models (Table 5-14 to 5-16).

#### *5.4.9.2 Cumulative incidence of parental reports of allergic symptoms or parental reports of diagnosed allergies (between 0–7 years CA)*

No differences in parental recall of allergic symptoms and parental reports of allergy diagnosis were found between birth and 7 years CA in the high DHA supplemented group compared with the standard DHA group in unadjusted and adjusted models in the breastfed children (Table 5-14 to 5-16).

### 5.4.10 Comparison of parental reports of medically diagnosed allergy – Breastfeeding group only: Eczema and Asthma

#### *5.4.10.1 Comparison of parental reports of doctor diagnosed allergy (between 2–7 years CA)*

No differences were found for parental reports of doctor diagnosed eczema and asthma between 2–7 years CA in the breastfed children in the high DHA supplemented group compared with the standard DHA group in unadjusted and adjusted models (Table 5-17). Overall, the subgroup analyses showed that the results from the breastfeeding group are consistent with the complete group, which is expected given that the majority were breastfed at trial entry.

**Table 5-14: Effects of high DHA supplementation on parental reports of eczema and eczema symptoms in children between 6–7 years CA and 0–7 years of age - breastfeeding group**

| Parental reports of eczema symptoms between 6–7 years CA from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|
| Itchy rash | 40/260 (15.38%) | 34/271 (12.55%) | 1.23 (0.79, 1.91) | 0.37 | 1.26 (0.82, 1.94) | 0.29 |
| Itchy rash in typical places (the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes) | 34/260 (13.08%) | 27/271 (9.96%) | 1.31 (0.80, 2.16) | 0.28 | 1.35 (0.83, 2.19) | 0.23 |
| Current eczema (itchy rash in typical locations and start below 5 years of age) | 26/260 (10.00%) | 24/271 (8.86%) | 1.13 (0.65, 1.96) | 0.67 | 1.18 (0.69, 2.02) | 0.54 |
| Rash not cleared | 11/260 (4.23%) | 5/271 (1.85%) | 2.29 (0.71, 7.39) | 0.16 | 2.36 (0.73, 7.64) | 0.15 |
| Eczema kept awake | 4/260 (1.54%) | 2/271 (0.74%) | 2.08 (0.39, 11.22) | 0.39 | ND | ND |
| Severe eczema (rash has not cleared and child kept awake >one or more nights per week) | 3/260 (1.15%) | 1/271 (0.37%) | 3.13 (0.33, 29.75) | 0.32 | ND | ND |
| Parental reports of eczema outcomes from birth to 7 years CA from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
| Ever had itchy rash | 41/260 (15.77%) | 40/271 (14.81%) | 1.06 (0.69, 1.64) | 0.78 | 1.10 (0.72, 1.68) | 0.67 |
| Ever had eczema (doctor diagnosis) | 65/260 (25.00%) | 72/271 (26.57%) | 0.94 (0.69, 1.27) | 0.69 | 0.96 (0.71, 1.29) | 0.78 |

*Statistically significant ($P \leq 0.05$) **Treatment effect adjusted for centre, birth weight and infant sex, ND= not determined

**Table 5-15: Effects of high DHA supplementation on parental reports of asthma and asthma symptoms in children between 6–7 years CA and 0–7 years of age - breastfeeding group**

| Parental reports of asthma symptoms between 6–7 years CA from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|
| Wheezing or whistling in the chest | 71/260 (27.31%) | 71/271 (26.20%) | 1.04 (0.78, 1.39) | 0.78 | 1.05 (0.79, 1.40) | 0.74 |
| Wheezing accompanying by exercise | 41/260 (15.83%) | 37/271 (13.65%) | 1.16 (0.77, 1.75) | 0.48 | 1.14 (0.76, 1.71) | 0.54 |
| Nocturnal dry cough without having a cold or chest infection | 77/260 (29.73%) | 65/271 (23.99%) | 1.24 (0.92, 1.67) | 0.16 | 1.23 (0.92, 1.66) | 0.16 |
| 4 or more wheezing attacks in the last 12 months | 21/260 (8.08%) | 22/271(8.12%) | 0.99 (0.56, 1.76) | 0.99 | 1.00 (0.57, 1.77) | 0.99 |
| Sleep disturbed due to wheezing in the last 12 months | 10/260 (3.85%) | 7/271 (2.59%) | 1.48 (0.55, 3.97) | 0.43 | 1.53 (0.57, 4.10) | 0.39 |
| Wheezing limited speech | 16/260 (6.15%) | 9/271 (3.32%) | 1.85 (0.84, 4.10) | 0.13 | 1.88 (0.85, 4.17) | 0.12 |
| Severe asthma (4 or more wheezing attacks, sleep disturbed and limited speech due to wheeze) | 1/260 (0.38%) | 3/271 (1.11%) | 0.35 (0.04, 3.29) | 0.36 | ND | ND |
| Parental reports of asthma outcomes from birth to 7 years CA from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
| Ever had wheeze | 117/260 (45.00%) | 124/271(45.76%) | 0.98 (0.81, 1.19) | 0.86 | 0.99 (0.81, 1.20) | 0.89 |
| Ever had asthma (doctor diagnosis) | 78/260 (30.00%) | 75/271 (27.68%) | 1.08 (0.83, 1.42) | 0.56 | 1.07 (0.82, 1.41) | 0.60 |

* Statistically significant ($P \leq 0.05$) **Treatment effect adjusted for centre, birth weight and infant sex ND= not determined

**Table 5-16: Effects of high DHA supplementation on parental reports of allergic rhinitis and allergic rhinitis symptoms in children between 6–7 years CA and 0–7 years CA – breastfeeding group**

| Parental reports of allergic rhinitis symptoms between 6–7 years CA from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|
| Sneezing, runny or blocked nose without cold - Allergic rhinitis symptoms | 61/260 (23.46%) | 65/271(23.99%) | 0.98 (0.71, 1.35) | 0.89 | 0.99 (0.72, 1.34) | 0.93 |
| Nasal symptoms accompanied with itchy watery eyes - Rhino-Conjunctivitis symptoms | 34/260 (13.18%) | 26/271 (9.59%) | 1.37 (0.83, 2.28) | 0.22 | 1.40 (0.84, 2.33) | 0.20 |
| Seasonal allergic rhinitis (occurred September and/or October and/or November) | 13/260 (05.00%) | 19/271 (7.01%) | 0.71 (0.35, 1.44) | 0.34 | 0.69 (0.35, 1.39) | 0.30 |
| Severe allergic rhinitis (nasal symptoms interfered ‘a lot’ with daily activities) | 0/260 (00.00%) | 0/271 (0.00%) | ND | ND | ND | ND |
| Parental reports of allergic rhinitis outcome from birth to 7 years CA from ISAAC questionnaire | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
| Ever had problem with sneezing or a runny , or blocked nose | 66/260 (25.38%) | 74/271 (27.31%) | 0.93 (0.69, 1.26) | 0.64 | 0.92 (0.69, 1.23) | 0.56 |
| Ever had hay fever (allergic rhinitis) (doctor diagnosis) | 52/260 (20.00%) | 64/271 (23.62%) | 0.85 (0.60, 1.20) | 0.35 | 0.85 (0.60, 1.21) | 0.37 |

*Statistically significant ($P \leq 0.05$) **Treatment effect adjusted for centre, birth weight and infant sex, ND= not determined

**Table 5-17: Effects of high DHA supplementation on parental reports of medically diagnosed asthma and eczema in children between 2–7 years CA – breastfeeding group**

| Parent report medically diagnosed allergy outcomes from CRF | High DHA group n/N (%) | Standard DHA group n/N (%) | Unadjusted RR (95% CI) | Unadjusted P-value* | Adjusted** RR (95% CI) | Adjusted P-value* |
|---|---|---|---|---|---|---|
| Eczema | 23/265 (8.68%) | 23/278 (8.27%) | 1.05 (0.60, 1.85) | 0.87 | 1.02 (0.59, 1.79) | 0.93 |
| Asthma | 60/265 (22.64%) | 62/278 (22.30%) | 1.02 (0.74, 1.40) | 0.93 | 1.01 (0.73, 1.39) | 0.96 |

*Statistically significant ($P \leq 0.05$) **Treatment effect adjusted for centre, birth weight and infant sex

## 5.5 Discussion

These results, from the 7 year CA follow-up of the DINO trial, show that supplementation with 1% DHA during the preterm period (through breast milk or formula) does not reduce the overall parental reports of incidence of allergies at 7 CA, compared to the standard feeding practice of ~ 0.3% DHA. Specifically, high DHA supplementation to preterm infants did not significantly reduce the incidence, severity or cumulative incidence of eczema, asthma and allergic rhinitis symptoms in children.

This study has minimal bias (see Chapter 2) and had good follow-up rates at all-time points, including at the 7 year CA in this Chapter. The DINO trial was a large trial (n=657 preterm infants) with infants recruited from five major perinatal centres covering four geographically distinct major cities in Australia [26] and the DINO7 participants followed up in this Chapter were representative of the DINO trial overall. While there are no other comparable data testing DHA supplementation in preterm children, it is important to note the strength of the data and the similarity to the Australian section of ISAAC which was conducted to collect allergy outcomes from 6–7 year old school children (assumed to be mainly term born) in metropolitan cities including Adelaide, Melbourne, Perth and Sydney [6, 359]. However, as these studies were published in 1998-1999 and the prevalence of allergies would be different since then (see Chapter 1), caution is needed with these comparisons. Nevertheless, the Australian arm of ISAAC reported a prevalence of itchy rash located in flexural folds of 11% of children in the Adelaide cohort and 10.9% in other Australian cities [359], which agrees well with the DINO7 allergy follow-up where the incidence of itchy rash and itchy rash located in typical locations (flexural folds) at 7 years of age were 14% and 11% respectively. Similarly, Williams et al reported that the cumulative incidence of eczema from birth to 7 years old was 24.9% in the Adelaide cohort and 22.8% in other Australian cities and in the

present DINO7 allergy follow-up the cumulative incidence of eczema was 25% from birth to 7 years (CA) old children [359]. The incidence of wheeze in the last 12 months was 26.6% in all children in the DINO7 allergy follow-up and there was a 6% higher incidence in boys than girls (boys 29.5% and girls 23.1%). Interestingly, this finding appears to be supported by the Australian arm of the ISAAC study, which showed a prevalence of current wheeze of 24.6% in Australian children and also reported that current wheeze was significantly more common (5.7% higher incidence) in boys than girls (27.4% and 21.7% respectively) at 7 years of age [6].

In the DINO7 allergy follow-up, the cumulative incidence of asthma diagnosis was 29% from birth to 7 years (CA). Interestingly, no differences were seen in the asthma diagnosis (ever) in the preterm children in this study compared with all children in the Oceania region including Australia (29%) despite recent systematic reviews showing that childhood asthma was higher in preterm born children [282, 360]. The incidence of rhinitis and rhino-conjunctivitis symptoms in the preterm infants studied in this thesis was 24% and 11% respectively, which was comparable to the Australian study which showed rhinitis and rhino-conjunctivitis was 26% and 12% respectively for 6-7 year olds [6]. The cumulative incidence of allergic rhinitis diagnosis was 22% in this study compared to the Australian study which showed 23.5% in Adelaide cohort and 18% when all of the other Australian cities were combined [6].

The data provided from the ISAAC for the preterm infants in this DINO7 follow-up are consistent with children who were not selected based on prematurity in other ISAAC studies. No differences were seen in values typical of what would be expected from mostly term children assessed with the ISAAC study. Most of the allergy outcomes in the preterm infants were comparable to children in the general population. The reasons for this are unknown and were not investigated further.

Despite the similarity of the overall data in this study with the ISAAC report, there were some significant interactions revealed in subgroup analyses suggesting that boys and girls, and infants of different birth weight strata may respond differently to high doses of DHA treatment in the ex-utero neonatal period. Although there may be a trend towards potential benefit on some types of allergic airway problems in supplemented children with birthweights ≥1250 g, this appeared to be at the expense of increased risk in those with a very low birth weight.

However the effect on allergic rhinitis in the high DHA treatment group in this study is consistent with parent reported secondary outcome data at 18 months in the same cohort which showed a reduction in the cumulative incidence of allergic rhinitis that approached significance in the high DHA supplemented group in the ≥1250 g birth weight strata [283]. Manley et al also observed a significant reduction in parental reported hay fever in children at either 12 or 18 months CA but no effect on asthma, eczema, or food allergy [283]. In a subgroup analyses based on sex, Manley et al also reported a significant reduction in hay fever in boys in the high-DHA supplemented group at 12 or 18 months CA [283]. No significant differences were found in the risk of having asthma, eczema and food allergy on girls and children with a birth weight <1250 g. Although there was a high retention rate (93.5%) in the Manley follow-up, allergy data were incomplete at 12 months CA, largely because the families who participated in the pilot phase of the trial (n=143 of the 657 infants) were not asked the allergy questions. In addition the allergy questions that were asked were not validated questions to assess parental reports of allergies and based on the results from Chapter 4 of this thesis, non-validated parental reports of allergy outcomes overestimate the prevalence of allergies compared with study doctor diagnosed allergies in children between 0–36 months of age.

Aside from their observation on allergies, Manley et al reported an effect of DHA treatment on bronchopulmonary dysplasia (BPD), which may have clinical relevance. High DHA supplementation during the ex-utero preterm period reduced the incidence of BPD in boys and low birth weight infants [283]. It is unknown whether the lower incidence of BPD in the high DHA group may have an effect on later respiratory outcomes, but BPD is an important predictor of morbidity in the longer term as well as chronic lung disease [361-364].

Sex and birth weight interactions have also been reported for the primary outcomes of both the DINO and DOMInO trials [26, 27]. For example, in the DOMInO trial girls whose mothers received the high DHA treatment during pregnancy were more likely to have delayed language development and lower language scores at 18 months of age than girls whose mothers received standard DHA [27]. In contrast, in the DINO trial the Mental Development Index (MDI) at 18 months CA of girls born preterm who received the high DHA ex-utero was higher than girls that received the standard DHA and the MDI in the high DHA treated group of infants born weighing <1250 g was higher than the standard DHA group in unadjusted (but not adjusted) comparisons [26].

The mechanisms underlying how sex and birth weight influence the response to DHA treatment have not been elucidated and further research is needed in this area. However mechanisms including rates of endogenous synthesis, which is higher in adult females than males [136, 365] and metabolic rates as well as use of dietary DHA for energy have been suggested [283, 366].

In the present follow-up of the DINO children at 7 years CA no significant effect of high DHA in the ex-utero period was seen on the parent reports of the symptoms of eczema. This finding is in contrast to that observed in a small sample (n=143) of the DINO children assessed at 3-5 years of age which showed an increase in eczema in children in the high DHA

group (10/61, 16%) compared to the standard DHA group (3/64, 5%) [284]. No differences between sexes or other allergy outcomes were reported in this earlier age follow-up [284]. The differences between the findings at the 3-5 year follow-up and the present 7 year CA follow-up may be associated with the small sample (n=143) at age 3-5 years, the change in allergy status with age of assessment and the study instrument used in the earlier assessment (non-validated ISAAC questionnaire) [284].

It is important to consider that the allergy data presented in this Chapter were not primary outcome data for the DINO trial and thus the trial may not have been adequately powered to detect differences between the two treatment methods for the allergy outcomes. In addition the many analyses and comparisons conducted on sub-groups may increase the chance of both type I and type II statistical errors, as discussed in more depth in Chapter 2. Also, there were some significant differences in post-randomisation variables (Table 5-13- first born children vs. subsequent children, dog or cat present vs. not present in households) between the two groups. Although these variables were not explored, it has been reported that households with pets are less likely to have allergies and first born children may be more likely to have some forms of allergies [367-371]. Food allergies and allergic rhinitis, but not asthma or eczema at school age, seem to be higher in the first born child [368, 371].

It is interesting to speculate whether prenatal (in utero) supplementation of infants with DHA may produce similar results to postnatal supplementation of preterm infants ex-utero until they reach 40 weeks of postmenstrual age. Makrides has investigated this comparison for the neurological outcomes of both the DINO and DOMInO trials [26, 27, 366]. This review revealed that DHA supplementation ex-utero, targeted at achieving the level of DHA accumulation expected to occur in the womb, appeared more effective in improving the neurodevelopmental outcome in preterm infants rather than in utero DHA supplementation of

term infants [366]. The time and dosage of n-3 LCPUFA supplementation in the DINO trial (ex-utero) and DOMInO trial (in utero) were different with the ex-utero supplementation being~9 weeks and 3 grams of fish oil containing DHA 900 mg and EPA 195 mg/day compared to in utero~20 weeks and 0.9 grams of fish oil containing DHA 800 mg and EPA 100 mg/day. However preterm infants may be likely to be more responsive to the actions of DHA, as their supply in utero is terminated at an early stage, along with the supply of other nutrients that it normally receives across the placenta [366].

In the DOMInO trial, the allergy follow-up was only in younger children (0-3 years of age) but it does seem that the parent reports of doctor diagnosis of eczema and asthma between 0-3 years of age was about ~31% and ~13% respectively, compared to parent reports of doctor diagnosis of eczema and asthma between 0-7 years CA was ~25% and ~29% respectively in the DINO7 trial. Also, parent reports of cumulative allergic rhinitis symptoms between 0-3 years of age was ~23% in term born infants in the DOMInO study compared to ~27% in 0-7 years CA children in the DINO7. However, it is important to consider that the 'allergic march' discussed in Chapter 1 may play a part here and whether parent reporting of allergies is as accurate in the long term as through the shorter period versus longer period. So, the results need treating with caution due to different ages assessed and different methodologies, as discussed earlier.

However, maternal n-3 LCPUFA supplementation in DINO ex-utero and DOMInO in utero showed parent report doctor diagnosed eczema between 0-12 months of age were ~13% in DINO ex-utero (data from Manley et al) [283] compared to ~23% in DOMInO in utero as well as ~24% medically diagnosed eczema in DOMInO in utero [303]. These results either support that preterm children were more sensitive to the preventive action of n-3 LCPUFA for eczema or they may be due to the selection of participants in a high allergy risk group in the

DOMInO cohort. However, parent report doctor diagnosed asthma between 0-12 months of age was ~8% in DINO (ex-utero supplementation) [283] and ~2% in DOMInO (in utero supplementation) [303], which is the opposite direction to the eczema comparison. This may be due to the fact that preterm infants are more vulnerable to respiratory disease including asthma than term infants [372-374] or it may reflect differences in capture of the information due to different questions, or different supplementing time/period (in utero vs. ex-utero).

Two studies [262, 263] which directly supplemented n-3 LCPUFA to infants (discussed in Chapter 1) can be compared with this study. However, comparisons are difficult due to the following differences between studies: supplemented mode, dosage and duration, allergy assessment methods and assessed age, high allergy risk vs. not selected allergy risk, the prematurity of infants in this study. Although a reduction of allergy outcomes seen in two direct supplementation studies, eczema at 6 months of age [262] and wheeze at 18 months of age [263], in high risk of allergy children who were assessed clinically, the reduction of allergy outcomes was not seen in later follow-ups (12 months of age [262] and 5 years of age [214]). This may also raise questions whether n-3 LCPUFA supplementation has transient effects only when children are supplemented postnatally or continuous supplementation is needed after child birth or supplementation is required before birth which continues postnatally to reduce allergies.

### 5.5.1 Strengths and limitation of the study

One of the strengths of this DINO7 follow-up allergy study was the study instrument used - the uniform internationally validated ISAAC questionnaire which is used routinely in large cross-sectional surveys to collect parental reports of allergy outcomes in children [5, 98]. ISAAC methodology is simple, the protocol was rigorously applied, and a number of validation studies have indicated that the ISAAC core questions have acceptable sensitivity

and specificity when compared with other indicators of physician diagnosis of allergy, other questionnaires and physiological measures [5, 98]. To date, there have been no other studies in which allergies have been assessed at school age in children born preterm who were supplemented with n-3 LCPUFA after birth. Adjustments were made in the analyses for centre, birth weight and infant sex and n-3 LCPUFA intake or supplementation during the past month at the appointment was balanced between groups. However, the more modest sample size of the DINO trial, with n= 657 children, may be a limiting factor when compared to large cross-sectional studies which use the ISAAC questionnaire [5]. Other potential weaknesses relate to the parental reporting of allergy outcomes in general, which may be either over or under reported (discussed in detail in Chapter 3) and unforeseen selection bias' in the children enrolled in the RCT, however as discussed earlier, this did not seem evident.

The sub groups of breast fed preterm infants were evaluated separately and no overall changes were observed. Although, 93% of mothers were providing breast milk at trial entry, no data were available on how long preterm infants had been breast fed. In interpreting data from all of the subgroup analyses, it is important to be mindful that many comparisons were conducted and as discussed in Chapter 2, this will increase the possibility of chance findings.

## 5.6 Conclusion

Of the n=604 preterm born children, the n=291 children in the high DHA group had no overall significant change in parental reports of current allergies within the past 12 months at 7 years CA, or the cumulative incidence of allergies from birth to 7 years CA, compared with the n= 313 children in the standard DHA group. Although the sub-group analyses suggested that there may be some potential benefits on some types of allergic airway problems in supplemented children born ≥1250 g, this may be at the expense of increased risk in those with a very low birth weight. However, further appropriately powered trials with medically diagnosed allergy primary outcomes are needed to determine that these observations are not just random effects.

# Chapter 6: Maternal n-3 LCPUFA supplementation for prevention of allergies in early childhood – an updated meta-analysis

## 6.1 Introduction

This Chapter updates the systematic review and meta-analysis described in Chapter 2 to include the new data relating to n-3 LCPUFA supplementation in pregnancy and lactation from Chapters 3 and 5 of this thesis. An updated search was also conducted to assess whether new studies were published during the period in which I undertook the studies outlined in Chapters 3 and 5. One additional study was found and included [268] as was a further follow-up of an earlier trial [285]. The compiled systematic review is now published in The Cochrane Database Systematic Reviews, Issue 7, 2015 [375] (see Appendix 6).

## 6.2 Aims and objectives

The aim of the systematic review was to assess the effect of maternal n-3 LCPUFA supplementation during pregnancy and/or lactation on the allergy outcomes in their children.

## 6.3 Methods

The methods of the systematic review are described in Chapter 2. A few methodological modifications were included in this updated review, following the recommendations of the Cochrane peer reviewers. The methodology deviations are described below and in the published review in the Cochrane Library, Issue7, 2015 [375] (see Appendix 6- under Differences between protocol and review- page 91).

### 6.3.1 Types of outcome measures

In this updated review, cumulative incidences were not reported across time points due to variation in reporting between studies. The data are reported at the last time point (oldest age) in the subgroup analysis comparisons.

### 6.3.2 Primary outcomes

In this updated review, primary outcomes were:

1. Medically diagnosed any allergy with sensitisation, i.e. IgE mediated allergies where both the signs and symptoms of the allergic disease and a positive SPT and/or RAST test are present.
2. Medical diagnosis or parental report (using validated questionnaire) of any allergy, +/- IgE sensitisation.

Any allergy included children with one or more allergy types including food allergy, atopic dermatitis (eczema), asthma/wheeze and allergic rhinitis (hay fever).

### 6.3.3 Secondary outcomes

Secondary outcome measures included children with specific forms of allergy, including food allergy, atopic dermatitis (eczema), asthma/wheeze, allergic rhinitis (hay fever) with IgE sensitisation and +/- IgE sensitisation, SPT results, and parent-reported allergies using non-validated questionnaires. Secondary safety outcomes included infant safety (e.g. infections) and maternal safety (e.g. postpartum haemorrhage or infection) due to the theoretical risk associated with higher doses of n-3 LCPUFA.

### 6.3.4 Search methods for identification of the studies

The literature search detailed in Chapter 2 was updated to determine if any additional trials had been published. All the searches described in Chapter 2 were repeated as follows: the Cochrane Pregnancy and Childbirth Group's Trials Register (to 6 August 2014), PubMed (1966 to 1 August 2014), CINAHL via EBSCOhost (1984 to 1 August 2014), Scopus (1995 to 1 August 2014), Web of Knowledge (1864 to 1 August 2014) and ClinicalTrials.gov (1 August 2014).

### 6.3.5 Data synthesis

Statistical analysis was performed using Review Manager software 5.2 [297] and the same methods described in Chapter 2.

## 6.4 Results of the updated systematic review

### 6.4.1 Results of the updated search

Electronic searches yielded 2290 records. The Cochrane Pregnancy and Childbirth Trials Register search (to 06 August 2014) retrieved 144 reports. Other electronic databases, PubMed (1966 to 01 August 2014), CINAHL via EBSCOhost (1984 to 01 August 2014), Scopus (1995 to 01 August 2014), Web of Knowledge (1864 to 01 August 2014) and ClinicalTrials.gov (01 August 2014) retrieved 159, 18, 1817, 140 and 12 reports respectively. Parental reports of allergy outcome data from the current data in this thesis – based on Makrides et al 2009 [26] and Makrides et al 2010 [27] are also included.

Of the 2292 records, duplicates and irrelevant reports were removed and 141 titles and abstracts were identified by considering the inclusion criteria. Sixteen trials (with 43 reports) were excluded, leaving eight trials (with 94 reports) for inclusion in this review. Four trials (with four reports) are ongoing (Figure 6-1). All reports from the first (Chapter 2) and second search (this Chapter) are combined and included in this updated review (see Appendix 6-References to studies included, excluded and on-going).

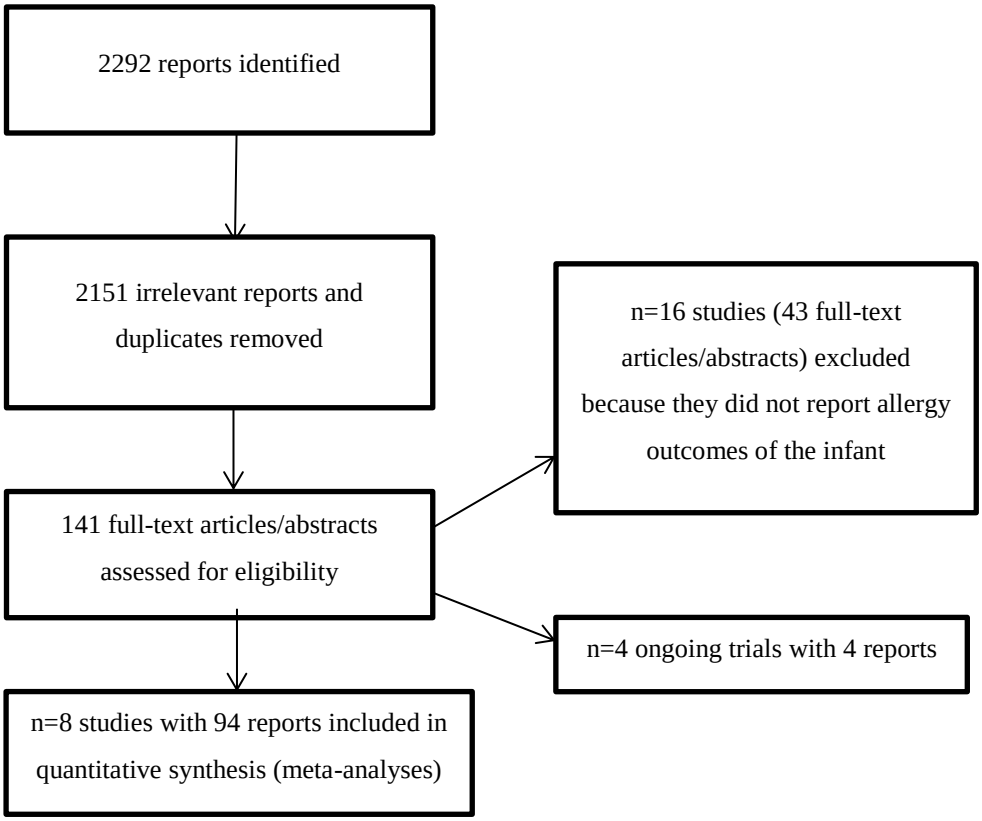


**Figure 6-1: Flow chart presenting process for the selection of included studies**

### 6.4.2 Description of the studies

The characteristics of the RCTs that provided new data for this updated review are described in Table 6-1 and the existing RCTs are as described in Chapter 2. Data from the two follow-up studies (7 year allergy follow-up from the DINO trial (Chapter 5) and parental reports of doctor diagnosed allergy outcomes of children below three years of age from the DOMInO trial (Chapter 3)) as well as IgE mediated allergies from the same trial (Palmer et al 2013) were included [285]. One of the ongoing studies identified in Chapter 2 also became eligible for inclusion in this update [268].

### 6.4.3 Summary of the study characteristics

The new study included in this update, but not in Chapter 2 was a parallel randomised controlled trial, conducted in Mexico and published in English on 1094 pregnant women, not specifically selected on the basis of allergy risk [268]. Supplementation commenced between 18 to 22 weeks' gestation with either 400 mg of DHA daily as the intervention, or 400 mg of a blend of corn and soy oil daily, as the control.

**Table 6-1: Trial characteristics with new study components (which are underlined)**

| **Description of Makrides et al 2009 [26]** | |
|---|---|
| **Methods** | Randomised controlled trial<br>Unpublished data from Makrides 2009 is included in this systematic review. There are 11 publications and one dissertation from this trial. All publications and the dissertation details are included in the references to included studies (Appendix 6-Reference to studies included). |
| **Participants** | Setting: 5 Australian perinatal centres.<br>Infants born before 33 weeks gestation and within five days of any enteral feeds were eligible to participate (included n = 657 infants and 545 women).<br>Infants with major congenital or chromosomal abnormalities, from a multiple birth in which not all live-born infants were eligible, or were in other trials of fatty acid supplementation were excluded. Lactating women in whom tuna oil was contraindicated (for example, because of bleeding disorders or therapy with anticoagulants) were also excluded.<br>To meet the inclusion criteria for this systematic review only infants whose mothers were supplying breast milk at trial entry (n = 603 infants) were included. A follow-up of the first 143 infants who participated in the pilot phase was conducted at three to five years corrected age (Simmonds 2007), again only infants whose mothers were providing breast milk at trial entry are included in this systematic review (n = 125).<br><u>A follow-up of was conducted at 7 years corrected age and n = 569 children completed ISAAC questionnaires, again only infants whose mothers were providing breast milk at trial entry are included in this systematic review (n = 531).</u> |
| **Interventions** | Intervention: Lactating women whose infants were randomly assigned to the high-DHA group consumed six 500 mg DHA-rich tuna oil capsules per day which provided 900 mg DHA and 195 mg EPA. The intent was to achieve breast milk DHA |

| | |
|---|---|
| | concentration that was~1% of total fatty acids without altering the naturally occurring concentration of AA in breast milk. If supplementary formula was required, infants were given a high-DHA preterm formula (1% DHA and 0.6% AA).<br>Control: Lactating women with infants allocated to the standard-DHA group consumed six 500 mg placebo soy oil capsules with no n-3 LCPUFA. If supplementary formula was required in this group, a standard preterm infant formula was used (0.35% DHA and 0.6% AA).<br>Duration of the intervention: within five days from the infant receiving any enteral feeds until infants reached their expected date of delivery. |
| **Outcomes** | 1. Makrides 2009<br>Primary outcome: Neurodevelopment at 18 months corrected age.<br>2. Manley 2012<br>Secondary outcomes: Parental reported food allergy, eczema, asthma and allergic rhinitis at 18 months corrected age<br>3. Simmonds 2010<br>ISAAC questionnaire was used to collect parent report of allergy diagnosis and parent report of doctor diagnosis eczema, allergic rhinitis and asthma at three to five years corrected age. (The ISAAC questionnaire is not validated for this age group).<br>4. Current study 2013<br>The ISAAC questionnaire was used to collect parent report of allergy diagnosis and parent report of doctor diagnosed eczema, allergic rhinitis and asthma at 7 year of corrected age. (The ISAAC questionnaire is validated for this age group). |
| **Notes** | This study was supported by a grant from the Australian National Health and Medical Research Council (grant 250322) and by the Channel seven Children's Research Foundation of South Australia Inc. Treatment and placebo capsules were donated by Clover Corp and infant formula was donated by Mead Johnson Nutritionals and Nutricia Australasia. |
| **Description of Makrides 2010 [27]** | |
| **Methods** | Randomised control trial |

| | |
|---|---|
| | Makrides 2010 [27] was the main trial with four publications. All seven publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: Australian maternity hospitals.<br>Recruitment to primary 'DOMInO' trial [27]: Women with singleton pregnancies at less than 21 weeks' gestation. Women already taking a prenatal supplement with DHA, with a bleeding disorder in which tuna oil was contraindicated, were taking anticoagulant therapy, had a documented history of drug or alcohol abuse, were participating in another fatty acid trial, fetus had a known major abnormality, or were unable to give written informed consent or if English was not the main language spoken at home were excluded (n = 2399).<br>Pregnant women were approached to enter the allergy follow-up (Palmer 2012) after randomisation into the DOMInO trial. Only Adelaide based women were eligible for the allergy follow-up. Women were eligible if the unborn baby had a mother, father, or sibling with a history of any medically diagnosed allergic disease (asthma, allergic rhinitis, eczema) (n = 706). |
| **Interventions** | Intervention: Three 500 mg capsules daily of DHA rich n-3 LCPUFA concentrate, providing 800 mg of DHA and 100 mg of EPA per day.<br>Control: Three 500 mg vegetable oil blend capsules daily without DHA. |
| **Outcomes** | 1. Makrides 2010 [27]<br>Primary outcome: Maternal postnatal depression at six weeks and six months; child neurodevelopment at 18 months of age.<br>Secondary outcomes included a range of clinical outcomes including post-partum haemorrhage.<br>2. Palmer 2012 [303]<br>Primary outcome: IgE associated allergic diseases – food allergy, eczema, asthma, allergic rhinitis and any allergy with sensitisation at one year of age. The data were imputed for all IgE mediated allergies.<br>Reported medically diagnosed allergic diseases with or without IgE mediated allergic diseases – food allergy, eczema, asthma, allergic rhinitis and any allergy with or without sensitisation based on medically diagnosed allergy at the age of one year. The |

| | |
|---|---|
| | data were imputed for food allergy, eczema and any allergies but not for asthma (wheeze) and allergic rhinitis.<br>Secondary outcome: IgE sensitisation – SPT at one year of age. Imputed data were used for egg, cow's milk, peanut, any SPT sensitisation but not for wheat, fish, pollen, house dust mite and cat allergens.<br>3. Palmer 2013 [285]<br>Three year follow-up of the same children in Palmer 2012, to evaluate medically diagnosed allergy.<br>Reported Immunoglobulin E associated (IgE) allergic diseases – food allergy, eczema, asthma, allergic rhinitis and any allergy with sensitisation at three years of age. Missing data were imputed for all IgE mediated allergies.<br>Obtained medically diagnosed allergic diseases with or without IgE mediated allergic diseases;<br>Food allergy, eczema, asthma, allergic rhinitis and any allergies with or without sensitisation based on medically diagnosed allergy at the age of three years. Missing data were imputed for medically diagnosed food allergy, eczema and any allergy but not imputed for medically diagnosed asthma (wheeze) and allergic rhinitis. In secondary outcomes missing data were imputed for egg, cow's milk. peanut and any SPT sensitisation but not for wheat, fish, pollen, house dust mite and cat allergens.<br>4. Current study 2013<br>Parental reports of doctor diagnosed allergy outcomes including food allergy, eczema, asthma, allergic rhinitis and any allergy below 36 months of age. Missing data were not imputed for any parent reported allergy outcomes.<br>Results using imputed data were reported to differ little from raw data [285, 303]. Modified ISAAC questions were used to collect parent report of doctor diagnosed eczema, allergic rhinitis and asthma from birth and three years of age. (The ISAAC questions are not validated for this age group). |
| **Notes** | Supported by grants from the Australian National Health and Medical Research council and Australian Egg corporation Limited. Treatment and placebo capsules were donated by Efamol, UK. |
| **Ramakrishnan 2010** [268] | |

| | |
|---|---|
| **Methods** | Randomised controlled trial.<br>Ramakrishnan 2010 was the main trial with 14 publications. All 14 publications are included in the references to included studies (Appendix 6- Reference to studies included). |
| **Participants** | Setting: Mexico.<br>Women were recruited at the Mexican Institute of Social Security (Instituto Mexicano del Seguro Social [IMSS]) General Hospital in Cuernavaca, Mexico, and 3 small health clinics within the IMSS system in Cuernavaca during routine prenatal care visits. Women were recruited for inclusion in the study if they were in gestation week 18 to 22, were aged 18 to 35 years, planned to deliver at the IMSS General Hospital in Cuernavaca, planned to predominantly breastfeed for at least 3 months, and planned to live in the area for 2 years after delivery. Women were excluded, if they had a (1) high-risk pregnancy, (2) lipid metabolism/ absorption disorders, (3) regular intake of fish oil or DHA supplements, or (4) chronic use of certain medications. (n = 1094) |
| **Interventions** | Intervention: Two 200 mg capsules daily of DHA rich algal oil concentrate, providing 400 mg of DHA per day.<br>Control: Two 200 mg corn oil and soy oil capsules daily without DHA. |
| **Outcomes** | Ramakrishnan 2010<br>Primary outcome measures: birth size and gestational age.<br>Imhoff-Kunsch 2011<br>Outcome measures: immune function and morbidity.<br>Parental reports of wheeze were reported at 1 month, 3 months and 6 months of age. A non-validated questionnaire was used to collect data.<br>Upper respiratory tract infections and fever were reported at 1 month, 3 months and 6 months of age.<br>Escamilla-Nunez 2014 |

| | |
|---|---|
| | Outcome measures: respiratory symptoms in children at 18 months of age (reported as incidence rate according to atopy). |
| **Notes** | Contacted authors to obtain incidence of 18 month allergy outcomes.<br>The research was supported by NIH (HD-043099) and the March of Dimes foundation. |

### 6.4.4 Updated risk of bias in included studies

The new included study and follow-up studies were assessed using the criteria in section 2.3.10.

#### *6.4.4.1 Summary of the risk of bias in included studies*

The risk of bias for the seven included studies remains similar to that in Chapter 2 (Figure 6-2 and 6-3). In the two follow-ups conducted in this thesis, there were withdrawals and dropouts at different time points. The Makrides et al 2009 trial [26] had 89.5% follow-up rates at the assessment at seven years CA and Makrides et al 2010 [27] had above 90% follow-up rates at 3 years of age. The new trial included in the update in this Chapter [268] had a low risk of bias for sequence generation, blinding, incomplete outcome data and other potential sources of bias, however allocation concealment was rated as an unclear risk of bias as the method of allocation concealment was not described and selective reporting was rated as high risk of bias as expected outcomes of interest to this review were not reported or were not reported completely (Figure 6-2 and 6-3).

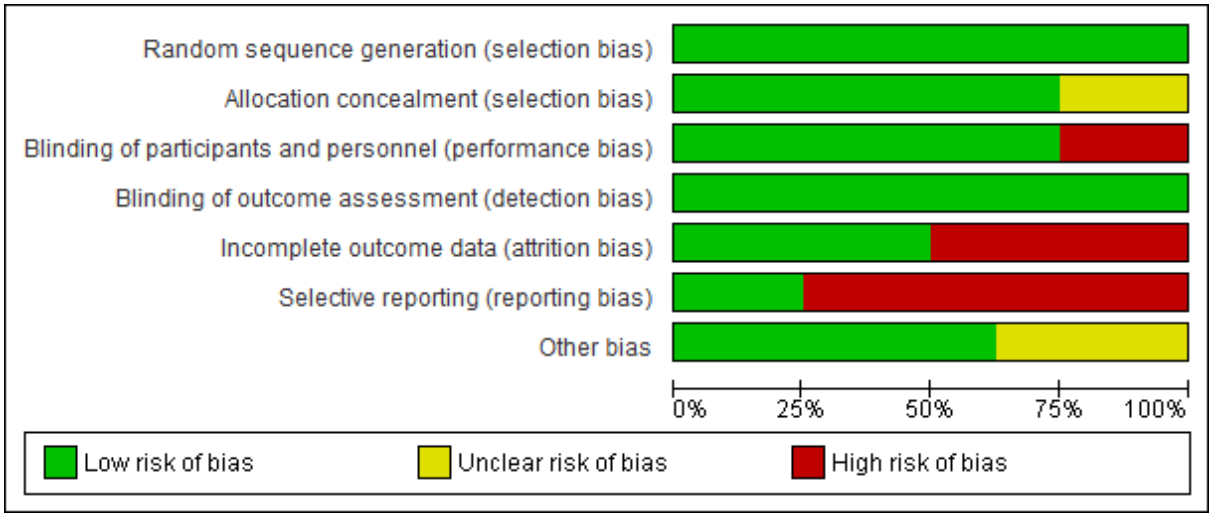


**Figure 6-2: Risk of bias graph: judgements about each risk of bias item presented as percentages across all included studies.**

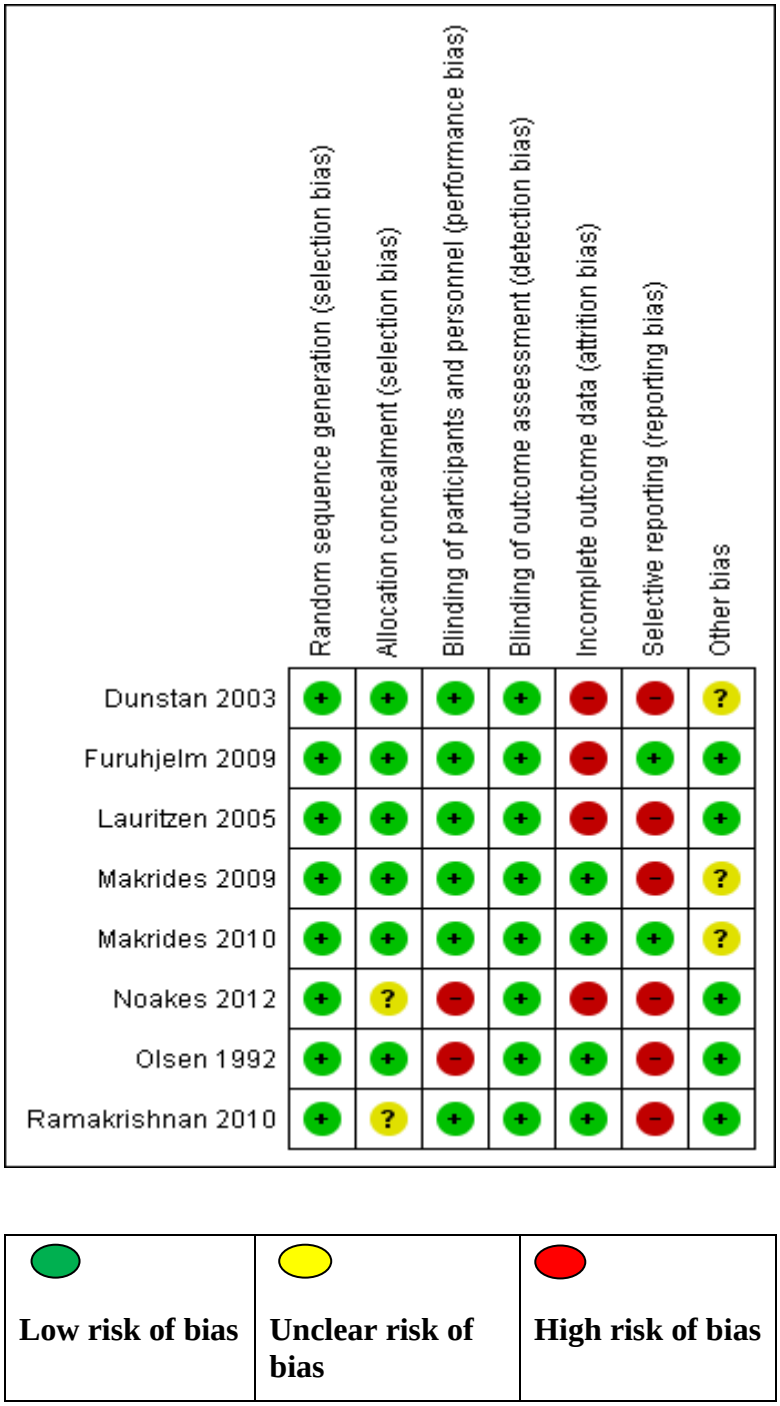


**Figure 6-3: Risk of bias summary: judgments about each risk of bias item for each included study.**

### 6.4.5 Excluded studies updated review

Studies were excluded if they were maternal n-3 LCPUFA supplemented RCTs, but allergy outcomes of the infants and/or children were not reported. Previously excluded studies (detailed in Chapter 2, section 2.4.5) that were not RCTs, not maternal studies or not n-3 LCPUFA interventions were not re-categorised under this updated section. Sixteen trials were excluded [306, 308, 309, 311, 315-319, 321, 325, 376-380] (see Appendix 6- characteristics to the excluded studies).

### 6.4.6 On-going studies updated review

There are four ongoing trials [328, 329, 381, 382] (see Appendix 6- characteristics to the on-going studies).

### 6.4.7 Main results updated review

In this updated review, published in the Cochrane Database of Systematic Reviews, Issue 7, 2015, [375] the peer review recommendations were to not report the cumulative incidences of allergic diseases outcomes due to concern over variation in reporting between studies. Therefore, primary and secondary allergy outcomes and sensitisation data were updated at short term (up to 12 months of age), medium term (12 to 36 months) and long term (36 months and beyond) time points only in the updated review. Primary outcome measures were medically diagnosed any allergy with sensitisation and medical diagnosis or parental report (using validated questionnaire) of any allergy, +/- IgE sensitisation. Secondary outcome measures were children with specific forms of allergy, including food allergy, atopic dermatitis (eczema), asthma/wheeze, allergic rhinitis (hay fever) with IgE sensitisation and +/- IgE sensitisation, SPT results, and parent-reported allergies using non-validated questionnaires. Secondary safety outcomes included infant safety (e.g. infections) and maternal safety (e.g. postpartum haemorrhage or infection).

**Primary outcome measures:**

The new updates to the primary outcome measures (medically diagnosed IgE mediated any allergies and medically diagnosed +/- IgE mediated any allergies) were reported by Palmer et al 2013 [285] for children assessed at 3 years of age (main trial- Makrides 2010) and parental reports of any allergy outcomes at 7 years of age, obtained using the validated questionnaire (Chapter 5) (main trial- Makrides 2009).

*6.4.7.1 Any allergies*

6.4.7.1.1 IgE mediated any allergy (Table 6-2 and Figure 6-4)

N-3 LCPUFA supplementation showed a clear reduction in IgE mediated allergies in children aged 12 to 36 months of age, compared with the control group (see Appendix 6- Analysis 1.1; Furuhjelm 2009; Makrides 2010, 823 children, risk ratio (RR) 0.66, 95% confidence interval (CI) 0.44 to 0.98). Makrides et al was the only trial to provide information about IgE mediated allergies to 3 years of age [27]. No clear differences were found in IgE mediated allergies between treatments in 36 months of age or older children (Makrides 2010, 706 children, RR 0.86, 95% CI 0.0.61 to 1.20). No included trials reported on combined IgE mediated allergies in infants under 12 months of age.

6.4.7.1.2 Any allergy +/- IgE mediation (Table 6-3 and Figure 6-5)

When all allergies (+/- IgE sensitivity) were considered, n-3 LCPUFA supplementation did not show clear differences in allergies in children at 12 to 36 months (Furuhjelm 2009, Makrides 2010, RR 0.89, 95% CI 0.71 to 1.11, 823 children) or 36 months and beyond (Makrides 2009, Makrides 2010, Olsen 1992, RR 0.96, 95% CI 0.84 to 1.09, 1765 children) (see Appendix 6- Analysis 1.2). No included trials reported on combined +/- IgE mediated allergies in infants under 12 months of age.

**Table 6-2: The effects of n-3 LCPUFA supplementation on IgE mediated allergies using pooled analysis RR (M-H, Fixed/Random, 95% CI)**

| ***Allergy*** *(IgE mediated)* | ***Assessed age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Control*** | | ***Effect estimate RR (95% CI)*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Food allergy* | < 12 months | 1 | 117 | 1 | 52 | 10 | 65 | **0.13 [0.02 to 0.95]** * |
| | 12-36 months | 2 | 825 | 13 | 422 | 20 | 403 | 0.58 [0.18 to 1.88] R |
| | ≥ 36 months | 1 | 706 | 14 | 368 | 9 | 338 | 1.43 [0.63 to 3.26] |
| *Eczema* | < 12 months | 1 | 117 | 4 | 52 | 13 | 65 | 0.38 [0.13 to 1.11] |
| | 12-36 months | 2 | 823 | 29 | 422 | 45 | 401 | **0.61 [0.39 to 0.95]** * |
| | ≥ 36 months | 1 | 706 | 44 | 368 | 48 | 338 | 0.84 [0.57 to 1.23] |
| *Allergic rhinitis* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 825 | 1 | 422 | 3 | 403 | 0.47 [0.07 to 3.06] |
| | ≥ 36 months | 1 | 706 | 18 | 368 | 20 | 338 | 0.83 [0.44 to 1.54] |
| *Asthma* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 824 | 3 | 422 | 4 | 402 | 0.86 [0.21 to 3.49] |
| | ≥ 36 months | 1 | 706 | 6 | 368 | 5 | 338 | 1.10 [0.34 to 3.58] |
| *Any allergies* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 823 | 36 | 422 | 52 | 401 | **0.66 [0.44 to 0.98]** * |
| | ≥ 36 months | 1 | 706 | 55 | 368 | 59 | 338 | 0.86 [0.61 to 1.20] |

* Statistically significant P ≤ 0.05, NE= Not estimable, R= Random effect estimate

**Table 6-3: The effects of n-3 LCPUFA supplementation on all allergies (+/- IgE sensitisation) using pooled analysis RR (M-H, Fixed, 95% CI)**

| ***Allergy*** *(IgE mediated or not)* | ***Assessed age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Control*** | | ***Effect estimate RR (95% CI)*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Food allergy* | < 12 months | 1 | 117 | 1 | 52 | 10 | 65 | **0.13 [0.02 to 0.95]** * |
| | 12-36 months | 4 | 973 | 19 | 499 | 26 | 474 | 0.72 [0.40 to 1.30] |
| | ≥ 36 months | 1 | 706 | 14 | 368 | 9 | 338 | 1.43 [0.63 to 3.26] |
| *Eczema* | < 12 months | 2 | 203 | 16 | 100 | 20 | 103 | 0.76 [0.22 to 2.62]$^{R}$ |
| | 12-36 months | 4 | 973 | 118 | 499 | 122 | 474 | 0.96 [0.69 to 1.33]$^{R}$ |
| | ≥ 36 months | 2 | 1237 | 122 | 628 | 131 | 609 | 0.88 [0.68 to 1.13]$^{R}$ |
| *Allergic rhinitis* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 805 | 10 | 414 | 18 | 391 | 0.53 [0.25 to 1.12] |
| | ≥ 36 months | 2 | 1169 | 114 | 593 | 109 | 576 | 1.03 [0.81 to 1.30] |
| *Asthma* | < 12 months | 1 | 83 | 11 | 46 | 7 | 37 | 1.26 [0.54 to 2.94] |
| | 12-36 months | 4 | 955 | 106 | 491 | 105 | 464 | 0.93 [0.73 to 1.18] |
| | ≥ 36 months | 3 | 1697 | 165 | 856 | 171 | 841 | 0.94 [0.78 to 1.13] |
| *Any allergies* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 823 | 109 | 422 | 117 | 401 | 0.89 [0.71 to 1.11] |
| | ≥ 36 months | 3 | 1765 | 270 | 891 | 274 | 874 | 0.96 [0.84 to 1.09] |

* Statistically significant P ≤ 0.05, NE= Not estimable, $^{R=}$ Random effect estimate

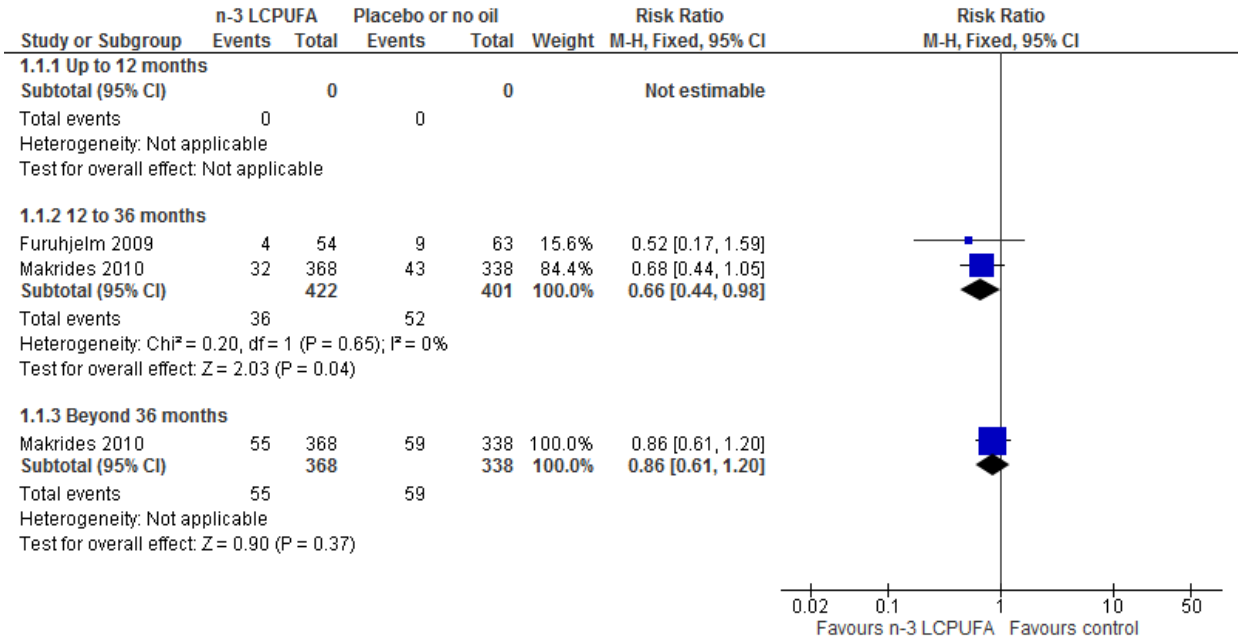


**Figure 6-4: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-One or more allergies with IgE sensitisation**

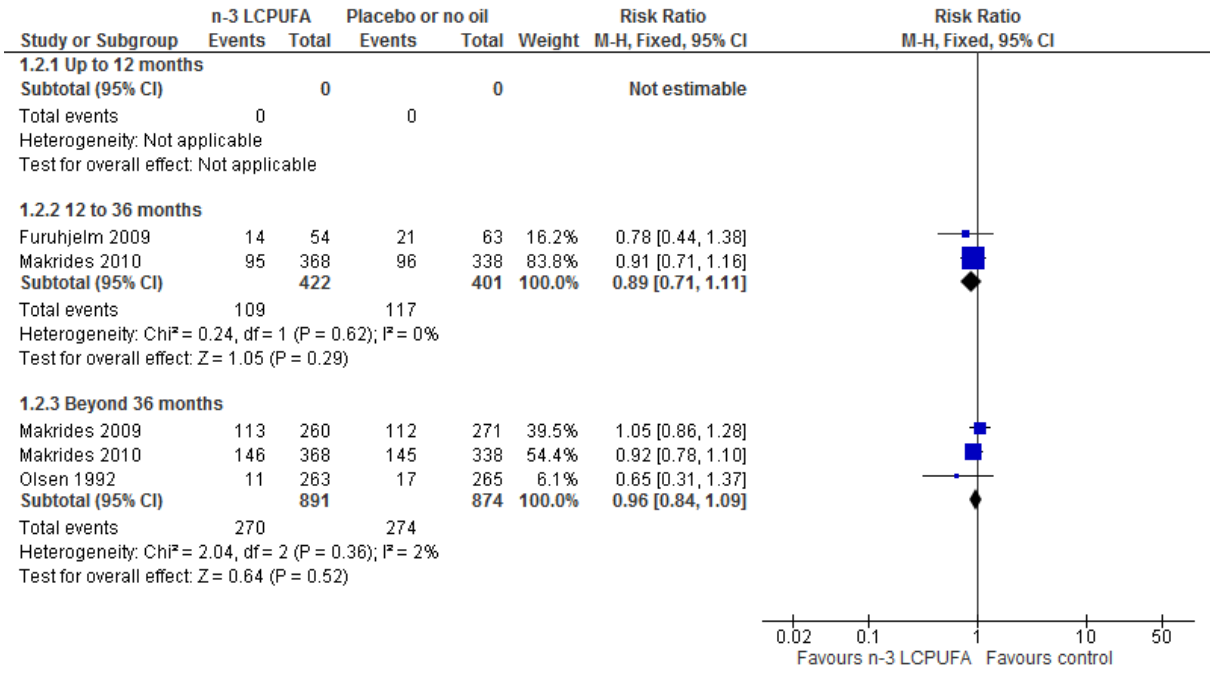


**Figure 6-5: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-One or more allergies with/without IgE sensitisation**

**Secondary outcomes:**

Medically diagnosed IgE mediated allergies (food allergy, eczema, asthma and allergic rhinitis), +/- IgE mediated allergy outcomes (food allergy, eczema, asthma/wheeze and allergic rhinitis) and SPT results from Palmer et al 2013 [285] for children assessed at 3 years of age (main trial-Makrides 2010) and parental reports of allergy outcomes (eczema, asthma and allergic rhinitis) at 7 years of age, obtained using the validated questionnaire (Chapter 5) (main trial-Makrides 2009) were included under secondary outcome measures in this updated review. In addition, new data on the parental reports of doctor diagnosed allergy outcomes (food allergy, eczema, asthma and allergic rhinitis) which were obtained using the non-validated questionnaire between birth and three years of age (Chapter 3) (main trial- Makrides 2010) and parental reports of allergy outcomes (wheeze) which were obtained using the non-validated questionnaire at 18 months of age from Ramakrishnan 2010 [268] were also included for the secondary outcome measures in the updated meta-analyses.

*6.4.7.2 Specific forms of allergy outcomes*

6.4.7.2.1 IgE mediated food allergy (Table 6-2 and Figure 6-6)

N-3 LCPUFA supplementation reduced the incidence of IgE mediated food allergies in children up to 12 months of age (Furuhjelm 2009, 117 infants, RR 0.13, 95% 0.02 to 0.95; see Appendix 6- Analysis 2.1), but there were no clear differences found between the intervention and control groups at any other age (12 to 36 months Furuhjelm 2009; Makrides 2010, 825 children, average RR 0.58, 95% CI 0.18 to 1.88; > 36 months of age Makrides 2010, 706 children, RR 1.43, 95% CI 0.63 to 3.26). A random-effects analysis was used as substantial heterogeneity was noted at the 12- to 36-month time point ($Tau^2 = 0.39$; P = 0.15; $I^2 = 51\%$) (see Appendix 6- Analysis 2.1). The heterogeneity may be due to the duration of the intervention, the dose used and the difference in assessment ages.

6.4.7.2.2 Food allergy +/- IgE mediation (Table 6-3 and Figure 6-7)

When food allergies +/- IgE sensitivity were considered (see Appendix 6- Analysis 2.2), results showed few differences from those for IgE mediated allergies (see Appendix 6- Analysis 2.1) with no differences in the direction of findings from those for IgE mediated allergies (up to 12 months of age, Furuhjelm 2009, 117 infants, RR 0.13, 95% CI 0.02 to 0.95; between 12 to 36 months, Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2010, 973 children, RR 0.72, 95% CI 0.40 to 1.30; > 36 months of age Makrides 2010, 706 children, RR 1.43, 95% CI 0.63 to 3.26).

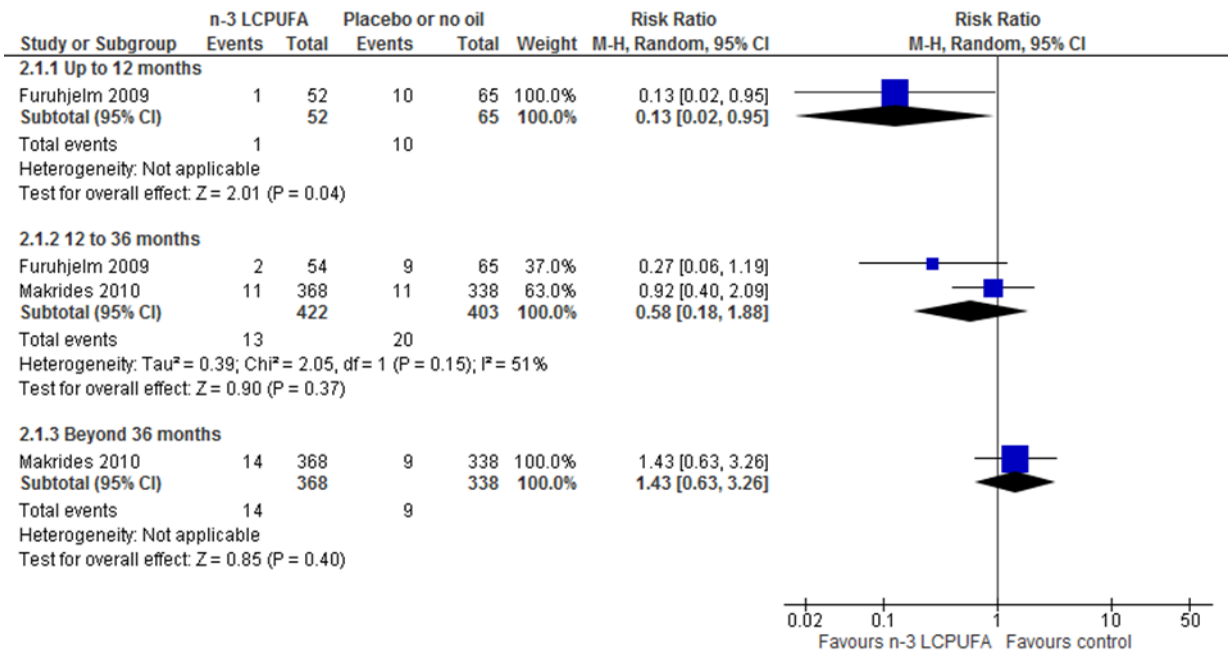


**Figure 6-6: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-Food allergies with IgE sensitisation**

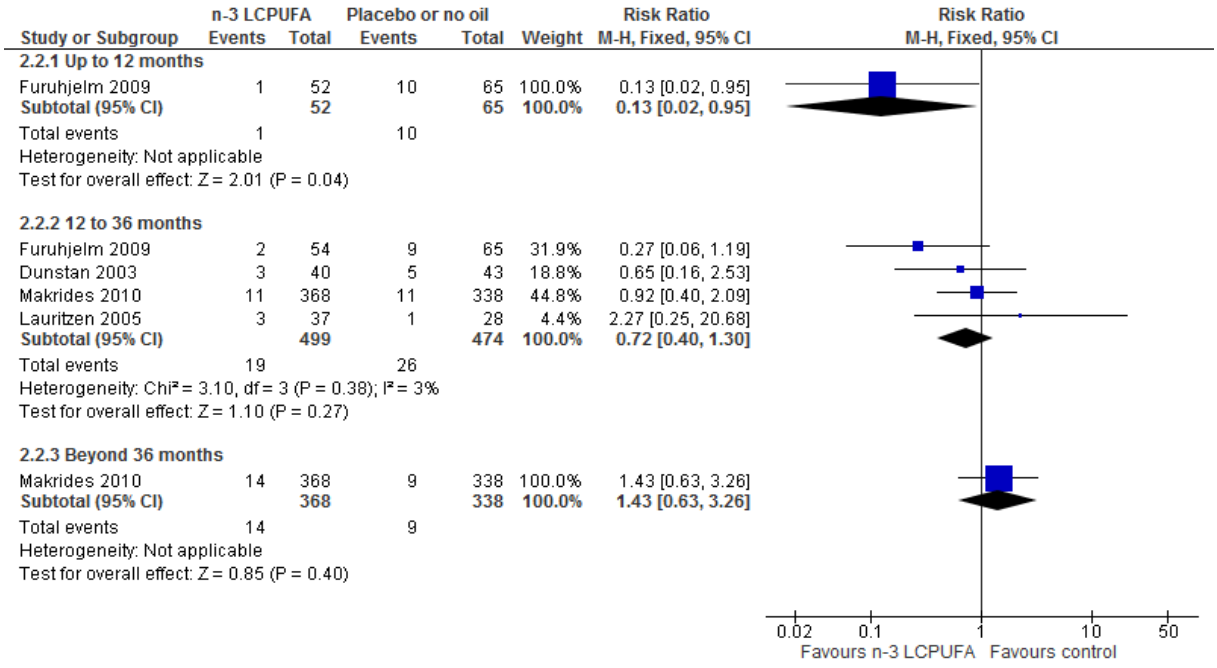


**Figure 6-7: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-Food allergies with/without IgE sensitisation**

6.4.7.2.3 IgE mediated eczema (Table 6-2 and Figure 6-8)

N-3 LCPUFA supplementation reduced the incidence of IgE mediated eczema in children at 12 to 36 months of age (Furuhjelm 2009; Makrides 2010; 823 children, RR 0.61, 95% CI 0.39 to 0.95, see Appendix 6- Analysis 2.3). There were no clear differences between groups at the other time points (< 12 months Furuhjelm 2009; 117 children, RR 0.38, 95% CI 0.13 to 1.11 or > 36 months of age Makrides 2010, 706 children, RR 0.84, 95% CI 0.57 to 1.23).

6.4.7.2.4 Eczema +/- IgE mediation (Table 6-3 and Figure 6-9)

When eczema outcomes +/- IgE sensitivity were considered (see Appendix 6- Analysis 2.4), results showed few differences from those for IgE mediated eczema (see Appendix 6- Analysis 2.3), however the direction of effect was reversed for the 12- to 36-month age group (up to 12 months of age (Furuhjelm 2009; Noakes 2012, 203 infants, average RR 0.76, 95% CI 0.22 to 2.62; between 12 to 36 months, Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2010, 973 children, average RR 0.96, 95% CI 0.69 to 1.33; > 36 months of age,

Makrides 2009; Makrides 2010, 1237 children, average RR 0.88, 95% CI 0.68 to 1.13). A random-effects analysis was used all time points as substantial heterogeneity was noted at the 12-month time point (Tau² = 0.57; P = 0.06; I² = 71%) (see Appendix 6- Analysis 2.4).

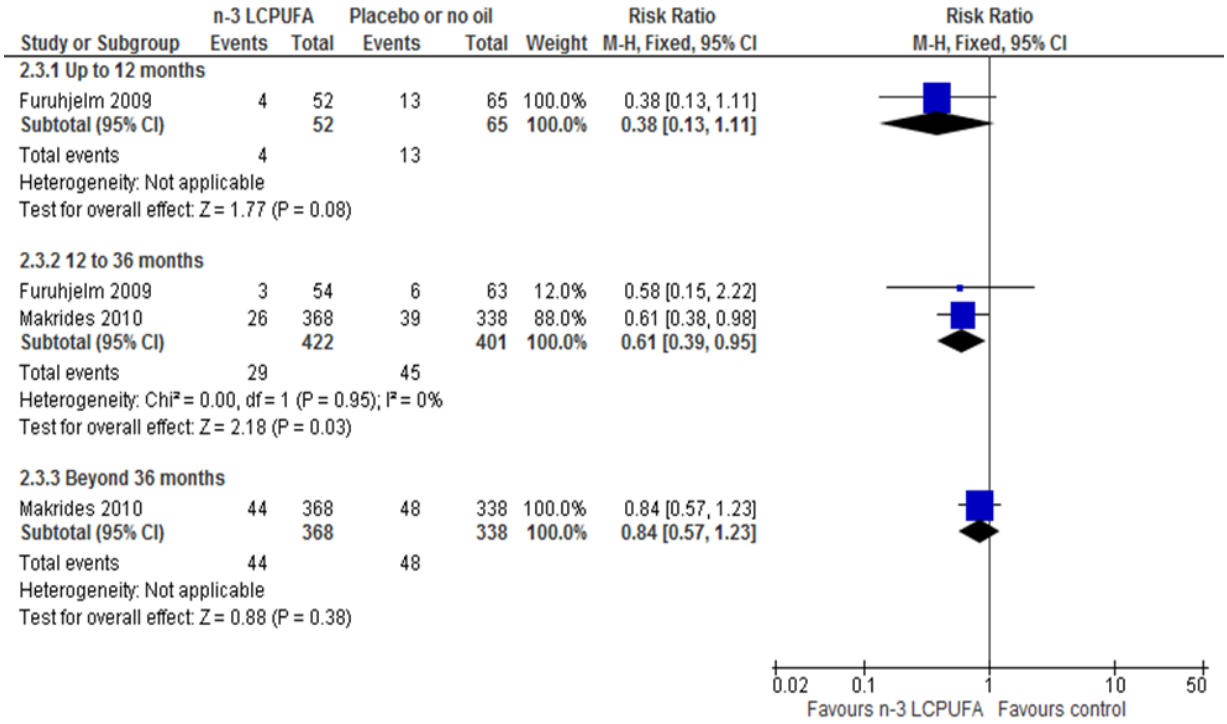


**Figure 6-8: Comparison: n-3 LCPUFA supplementation versus control (placebo or no oil)-Eczema with IgE sensitisation**

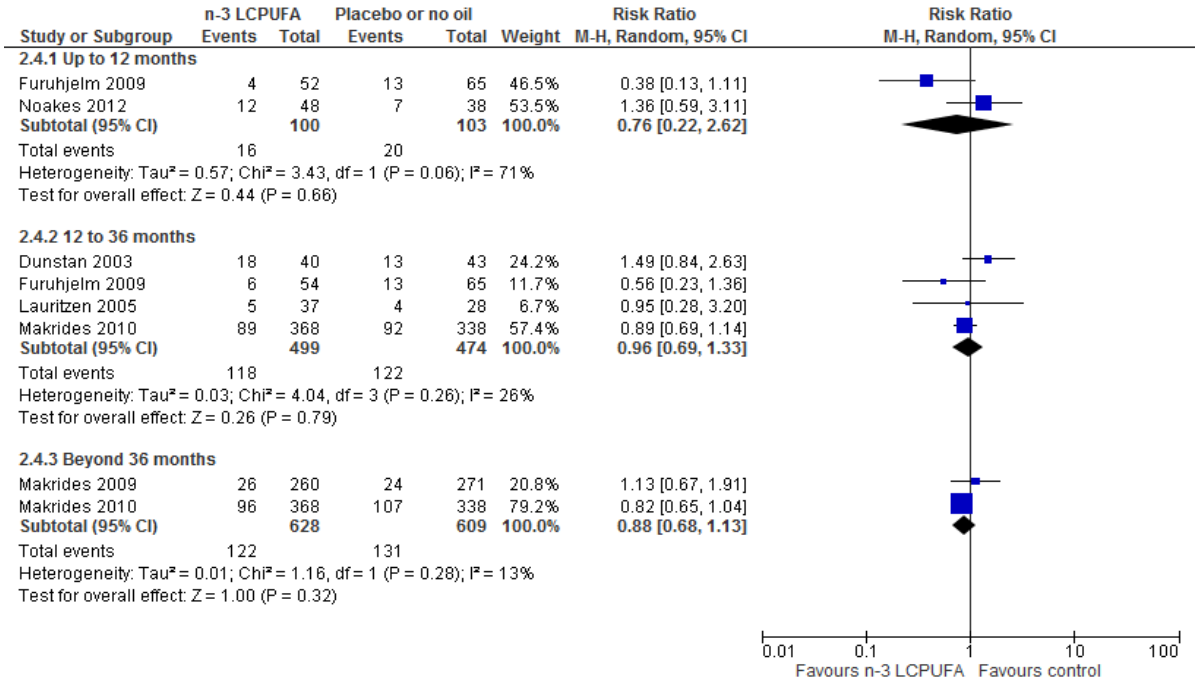


**Figure 6-9: Comparison: n-3 LCPUFA (fish or fish oil) supplementation versus control (placebo or no oil)-Eczema with/without IgE sensitisation**

6.4.7.2.5 IgE mediated allergic rhinitis or allergic rhinitis +/- IgE sensitivity (Tables 6-2 and 6-3)

No clear difference was seen between n-3 LCPUFA and control groups in either IgE mediated allergic rhinitis (see Appendix 6- Analysis 2.5: 12 to 36 months, Furuhjelm 2009; Makrides 2010, 825 children, RR 0.47, 95% CI 0.07 to 3.06; > 36 months of age, Makrides 2010, 706 children, RR 0.83, 95% CI 0.44 to 1.54 or allergic rhinitis +/- IgE sensitivity (see Appendix 6- Analysis 2.6: 12 to 36 months, Furuhjelm 2009, Makrides 2010, 805 children, RR 0.53, 95% CI 0.25 to 1.12; > 36 months of age, Makrides 2009, Makrides 2010, 1169 children, RR 1.03, 95% CI 0.81 to 1.30) across any age group. No included trials reported allergic rhinitis outcomes in infants under 12 months of age.

6.4.7.2.6 IgE mediated asthma or asthma +/- IgE sensitivity (Tables 6-2 and 6-3)

No clear differences were found between n-3 LCPUFA and control groups in children with either IgE mediated asthma (see Appendix 6- Analysis 2.7; 12 to 36 months, Furuhjelm 2009; Makrides 2010, 824 children, RR 0.86, 95% CI 0.21 to 3.49; > 36 months of age, Makrides 2010, 706 children, RR 1.10, 95% CI 0.34 to 3.58), or asthma +/- IgE sensitivity (see Appendix 6- Analysis 2.8; < 12 months, Noakes 2012, 83 infants, RR 1.26, 95% CI 0.54 to 2.94; 12 to 36 months, Dunstan 2003, Furuhjelm 2009, Lauritzen 2005, Makrides 2010, 955 children, RR 0.93, 95% CI 0.73 to 1.18; > 36 months of age, Makrides 2009, Makrides 2010, Olsen 1992, 1697 children, RR 0.94, 95% CI 0.78 to 1.13) across any age group. No included trials reported IgE mediated asthma outcomes in infants under 12 months of age.

*6.4.7.3 Sensitisation to allergens*

SPT results from Palmer et al 2013 [285] for children assessed at 3 years of age (main trial- Makrides 2010) were used to update sensitisation data which are summarised in Table 6-4. Sensitisation is the strongest predictor of IgE mediated allergy and was defined by a positive skin prick test to an allergen [129].

6.4.7.3.1 Skin prick test results for food allergens (Table 6-4)

Sensitisation to egg (Figure 6-10 and see Appendix 6- Analysis 4.1) was reduced in the n-3 LCPUFA group compared with the control in 12- to 36 month-old children (Dunstan 2003; Furuhjelm 2009; Makrides 2010, 893 children, RR 0.55; 95% CI 0.39 to 0.77). No clear differences between groups were seen in egg sensitisation in children up to 12 months of age [267, 269] or in children 36 months or older [27].

Sensitisation to cows' milk was not different between the treatment groups at any age of assessment (see Appendix 6- Analysis 4.2), although no trials contained data for children aged

36 months or older. Given the substantial heterogeneity at 12 to 36 months, a random-effects model was used ($Tau^2 = 0.48$; $P = 0.19$; $I^2 = 40\%$).

There were no clear differences between groups in peanut sensitisation at any time point (see Appendix 6- Analysis 4.3; no trials in infants under 12 months of age),

The effect of n-3 LCPUFA supplementation on wheat sensitisation was not different from the control group at any age (see Appendix 6- Analysis 4.4) and similar results were seen with sensitisation to fish (see Appendix 6- Analysis 4.5; no trials up to 12 months of age).

#### 6.4.7.3.2 Skin prick test results for inhalant allergens (Table 6-4)

The effect of n-3 LCPUFA supplementation on inhalant allergens, pollens (see Appendix 6- Analysis 4.6; no trials up to 12 months of age), dust mites (see Appendix 6- Analysis 4.7; no trials up to 12 months of age) and cats (see Appendix 6- Analysis 4.8) were not different from the control group at any age.

#### 6.4.7.3.3 Skin prick test results for food and inhalant allergens (Table 6-4)

When all allergens were considered (Figure 6-11 and see Appendix 6- Analysis 4.9), no clear differences were found between treatments for infants up to 12 months and beyond 36 months. However, n-3 LCPUFA showed a clear reduction in sensitisation in 12 to 36 months of age children (Dunstan 2003; Furuhjelm 2009; Makrides 2010, 892 children, RR 0.70; 95% CI 0.53 to 0.94).

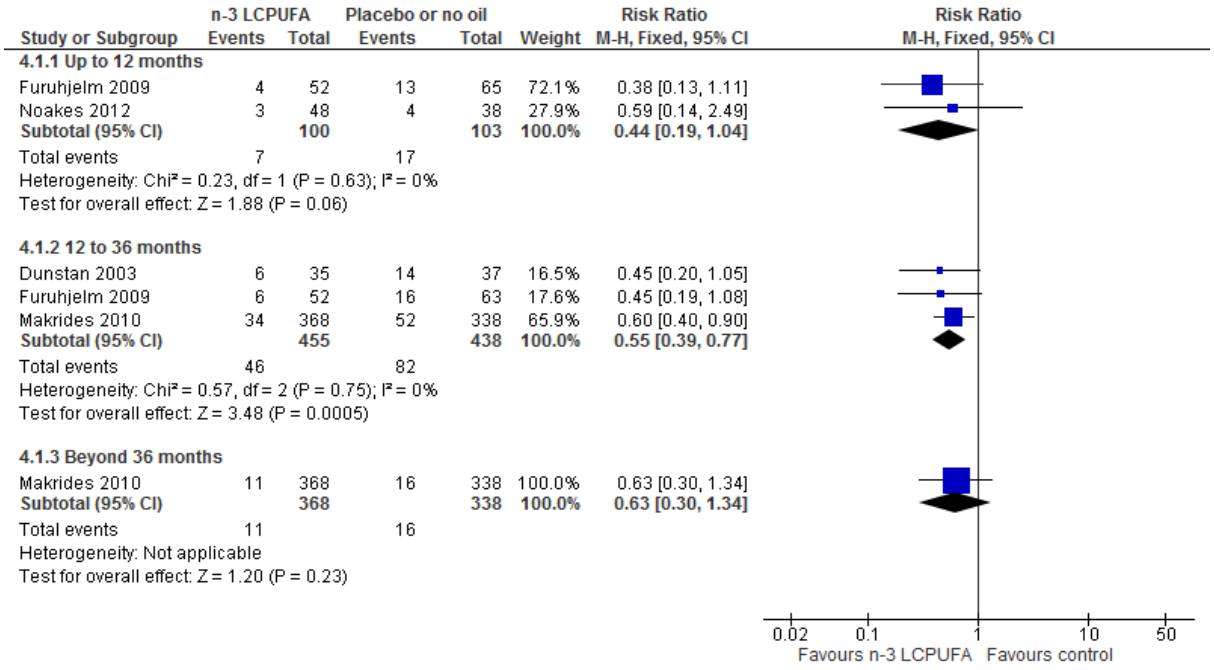


**Figure 6-10: Comparison: n-3 LCPUFA (fish or fish oil) supplementation versus control (placebo or no oil)-Skin prick sensitisation to egg**

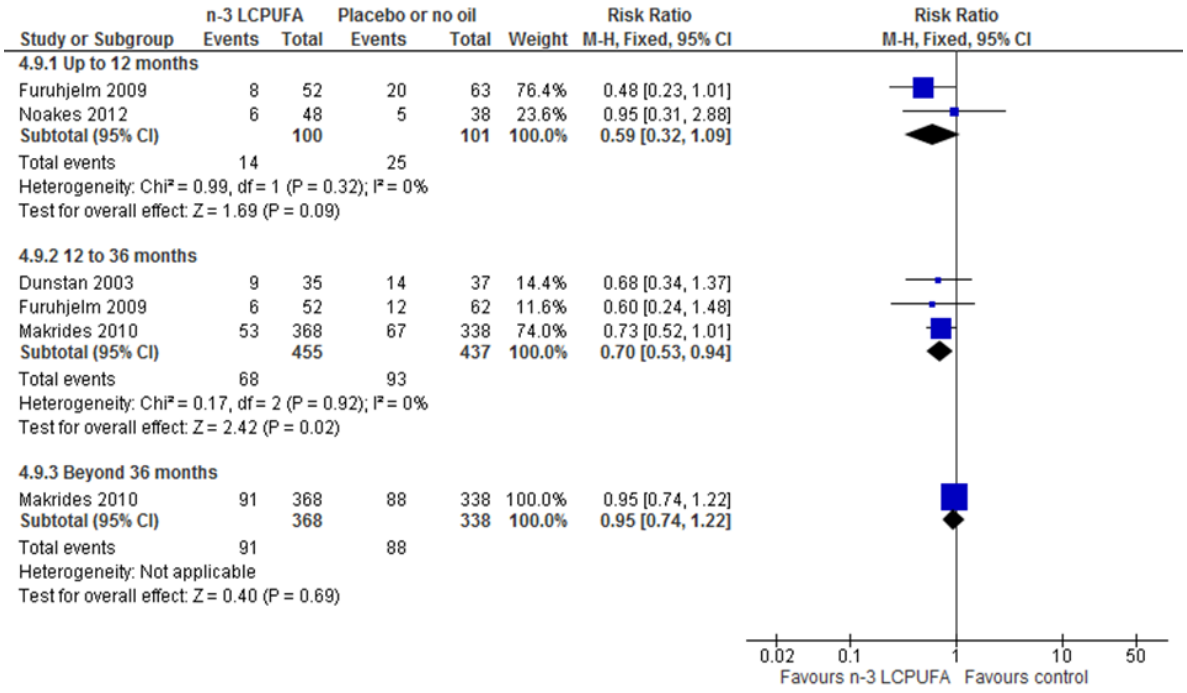


**Figure 6-11: Comparison: n-3 LCPUFA (fish or fish oil) supplementation versus control (placebo or no oil)-Skin prick sensitisation one or more allergen**

**Table 6-4: The effects of n-3 LCPUFA supplementation on skin prick results for allergens using pooled analysis RR (M-H, Fixed, 95%)**

| ***Skin prick results*** | ***Assessed age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Control*** | | ***Effect estimate RR (95% CI)*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Egg* | < 12 months | 2 | 203 | 7 | 100 | 17 | 103 | 0.44 [0.19 to 1.04] |
| | 12-36 months | **3** | **893** | **46** | **455** | **82** | **438** | **0.55 [0.39 to 0.77]** ** |
| | ≥ 36 months | 1 | 706 | 11 | 368 | 16 | 338 | 0.63 [0.30 to 1.34] |
| *Cow's milk* | < 12 months | 2 | 205 | 5 | 102 | 7 | 103 | 0.75 [0.24 to 2.29][R] |
| | 12-36 months | 3 | 897 | 9 | 457 | 13 | 440 | 0.68 [0.20 to 2.34][R] |
| | ≥ 36 months | 0 | 0 | | | | | NE |
| *Peanut* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 778 | 18 | 403 | 28 | 375 | 0.61 [0.34 to 1.08] |
| | ≥ 36 months | 1 | 706 | 13 | 368 | 20 | 338 | 0.60 [0.30 to 1.18] |
| *Wheat* | < 12 months | 1 | 117 | 1 | 52 | 0 | 65 | 3.74 [0.16 to 89.85] |
| | 12-36 months | 2 | 783 | 3 | 401 | 2 | 382 | 1.40 [0.29 to 6.84] |
| | ≥ 36 months | 1 | 706 | 6 | 368 | 2 | 338 | 2.76 [0.56 to 13.56] |
| *Fish* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 666 | 3 | 349 | 0 | 317 | 6.36 [0.33 to 122.65] |
| | ≥ 36 months | 1 | 706 | 2 | 368 | 1 | 338 | 1.84 [0.17 to 20.17] |
| *Inhalant allergens (pollens)* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 779 | 2 | 400 | 5 | 379 | 0.44 [0.08 to 2.30] |
| | ≥ 36 months | 1 | 580 | 21 | 303 | 25 | 277 | 0.77 [0.44 to 1.34] |
| *House dust mite* | < 12 months | 0 | 0 | | | | | NE |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12-36 months | 2 | 738 | 3 | 384 | 4 | 354 | 0.76 [0.18 to 3.28] |
| | ≥ 36 months | 1 | 580 | 22 | 303 | 24 | 277 | 0.84 [0.48 to 1.46] |
| *Cat* | < 12 months | 1 | 86 | 1 | 48 | 1 | 38 | 0.79 [0.05 to 12.25] |
| | 12-36 months | 2 | 738 | 8 | 384 | 7 | 354 | 1.07 [0.39 to 2.94] |
| | ≥ 36 months | 1 | 706 | 25 | 368 | 12 | 338 | 1.91 [0.98 to 3.75] |
| *Any allergen (one or more allergen)* | < 12 months | 2 | 201 | 14 | 100 | 25 | 101 | 0.59 [0.32 to 1.09] |
| | 12-36 months | **3** | **892** | **68** | **455** | **93** | **437** | **0.70 [0.53 to 0.94]** * |
| | ≥ 36 months | 1 | 706 | 91 | 368 | 88 | 338 | 0.95 [0.74 to 1.22] |

* Statistically significant P ≤ 0.05, 500.0 > P tnacifingis **, NE= Not estimable, $^{R=}$ Random effect estimate

#### *6.4.7.4 Parent reports of allergy outcomes*

For this updated meta-analysis, results from the follow-up study which collected parental reports of allergy outcomes using non-validated questionnaires were included. Parental reports of allergy outcomes included parental reports of allergy symptoms as well as parental reports of doctor diagnosed allergies following prenatal supplementation (Chapter 3). As parental reports of doctor diagnosed allergies were found to be more reliable than parental reports of allergy symptoms at early ages (Chapter 4), only these were included in the updated review. Parental reports of doctor diagnosed eczema and asthma outcomes were included for all subgroup analyses including below the age of 12 months, between 12–36 months and above 36 months of age. For the other outcomes – including parental reports of doctor diagnosed food allergy, allergic rhinitis and any allergies – data were included only below 12 months of age as no data were available for the other time points (as described in Chapter 3). Parental reports of wheeze symptoms at 18 months of age were also included from one trial [268]. Although the large data set was included in the updated meta-analyses no significant changes to the original meta-analysis were observed (Table 2-10, Table 6-5).

No clear differences were found between n-3 LCPUFA supplementation and control at any age in the incidence of parental reports of food allergies (see Appendix 6- Analysis 5.1), eczema (see Appendix 6- Analysis 5.2), allergic rhinitis (see Appendix 6- Analysis 5.3), asthma/wheeze (see Appendix 6- Analysis 5.4), or any allergies (see Appendix 6- Analysis 5.5).

**Table 6-5: Updated meta-analyses of n-3 LCPUFA supplementation on parental reports of allergy (non-validated questionnaires) using pooled analysis RR (M-H, Fixed, 95% CI)**

| *Outcomes* | *Assessed Age* | *No of studies* | *No of participants* | *n-3 LCPUFA* | | *Control* | | *Fixed Effect estimate RR (95% CI)* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Events* | *Total* | *Events* | *Total* | |
| *Food Allergy* | <12 months | 1 | 695 | 3 | 362 | 0 | 333 | 6.44 [0.33, 124.23] |
| | 12-36 months | 1 | 565 | 10 | 274 | 13 | 291 | 0.82 [0.36, 1.83] |
| | ≥36 months | 0 | 0 | | | | | NE |
| *Eczema* | <12 months | 1 | 695 | 57 | 362 | 58 | 333 | 0.90 [0.65, 1.26] |
| | 12-36 months | 2 | 1263 | 127 | 639 | 134 | 624 | 0.91 [0.74, 1.13] |
| | ≥36 months | 2 | 746 | 118 | 382 | 118 | 364 | 0.95 [0.77, 1.18] |
| *Allergic rhinitis* | <12 months | 1 | 695 | 2 | 362 | 0 | 333 | 4.60 [0.22, 95.48] |
| | 12-36 months | 1 | 565 | 6 | 274 | 9 | 291 | 0.71 [0.26, 1.96] |
| | ≥36 months | 1 | 109 | 14 | 49 | 11 | 60 | 1.56 [0.78, 3.12] |
| *Asthma/wheeze* | <12 months | 1 | 695 | 0 | 362 | 1 | 333 | 0.31 [0.01, 7.50] |
| | 12-36 months | 3 | 2134 | 187 | 1069 | 205 | 1065 | 0.94 [0.79, 1.11] |
| | ≥36 months | 2 | 745 | 56 | 382 | 53 | 363 | 1.02 [0.72, 1.45] |
| *Any allergies* | <12 months | 1 | 695 | 58 | 362 | 59 | 333 | 0.90 [0.65, 1.26] |
| | 12-36 months | 1 | 565 | 82 | 274 | 92 | 291 | 0.95 [0.74, 1.21] |
| | ≥36 months | 1 | 110 | 25 | 49 | 34 | 61 | 0.92 [0.64, 1.30] |

NE=Not estimable

*6.4.7.5 Safety outcomes*

6.4.7.5.1 Maternal safety

There was no clear difference in postpartum haemorrhage (defined as > 500 mL of blood loss post-delivery) in women supplemented with n-3 LCPUFA compared with those in the control group (see Appendix 6- Analysis 3.1; n = 2932, average RR 0.73, 95% CI 0.49 to 1.10). Given the substantial heterogeneity between trials, a random-effects model was used ($Tau^2$ = 0.05, P = 0.11; $I^2$ = 60%; see Appendix 6- Analysis 3.1). Postpartum infection was not reported in any of the included trials.

6.4.7.5.2 Infant safety

Infant safety was assessed using early childhood infections. Four trials reported this outcome [26, 27, 267, 268] (2280 infants) with no clear difference between the n-3 LCPUFA and control group (RR 0.99; 95% CI 0.87 to 1.12). Ramakrishnan 2010 [268] reported fever in 834 infants and found no differences between groups (RR 0.99; 95% CI 0.74 to 1.31; see Appendix 6- Analysis 3.2).

*6.4.7.6 Subgroup analysis*

The Cochrane peer reviewer recommendations were to include only the data at the oldest age time point, for the subgroup analyses. Thus, data are reported for the last time point only for primary allergy outcomes (IgE mediated any allergy with sensitisation or any allergy +/- sensitisation) in the subgroup analyses comparisons in this updated review.

6.4.7.6.1 Timing of supplementation

Five trials included in the review confined supplementation with n-3 LCPUFA to the prenatal period only [27, 259, 266-268]. Ramakrishnan 2010 [268] collected allergy outcome data using a non-validated questionnaire, therefore these data did not meet the inclusion criteria for

the primary outcome. Two of the included trials supplemented women with n-3 LCPUFA in the postnatal period only [26, 260], and only one trial [269] supplemented women with n-3 LCPUFA through both prenatal and postnatal periods.

There were no significant subgroup differences for primary allergy outcomes (see Appendix 6- Analysis 6.1; Analysis 6.2).

6.4.7.6.2 Allergy risk of the offspring

Four trials [27, 259, 267, 269] provided n-3 LCPUFA supplements to women whose fetuses were at high risk of allergy development with two reporting only IgE mediated allergies [27, 269] and three trials studied the effect of n-3 LCPUFA supplementation on childhood allergy in women with fetuses or infants who were not selected on the basis of increased allergy risk [26, 260, 266]. There were no significant subgroup differences for primary allergy outcomes (see Appendix 6- Analysis 7.1-7.5).

6.4.7.6.3 Infant maturity

Subgroup analyses based on infant maturity were not able to be conducted. Although data from preterm children were available for Makrides 2009, it was not possible to separate out children according to their gestational age in the remaining included trials [27, 260, 266, 267, 269]. Dunstan 2003 excluded preterm infants after randomisation [259].

#### *6.4.7.7 Sensitivity analysis*

Sensitivity analyses were conducted for the primary outcome, removing trials with high or unclear risk of selection, performance or attrition bias; Makrides 2009 and Makrides 2010 [26, 27] were the only trials with low risk of bias across these parameters. Removing trials with high or unclear risk of bias changed the direction for IgE mediated any allergy at the 12 to 36 months time point, but not beyond the 36 months time point, or for medically diagnosed IgE mediated and/or parental report any allergy outcome at any time points (see Appendix 6-Analysis 8.1; Analysis 8.2).

## 6.5 Discussion

### 6.5.1 Summary of main results

Eight trials involving 3366 women with 3175 children were included in this review. Supplementation occurred during pregnancy, lactation or both pregnancy and lactation. Overall, this review has synthesised the most up-to-date published data, as well as data collected in the chapters of this thesis and shows that maternal n-3 LCPUFA (long chain polyunsaturated fatty acid) supplementation provided little benefit in the reduction of childhood allergic disease. There was no clear overall effect of maternal n-3 LCPUFA supplementation on the incidence of medically diagnosed or parental reports of allergy (+/- IgE sensitisation) including food allergy, eczema, allergic rhinitis, asthma/wheeze or any allergy. No reduction was observed with maternal n-3 LCPUFA supplementation on IgE mediated allergic rhinitis or IgE mediated asthma.

However, there were reductions in some outcomes such as IgE mediated food allergy up to 12 months of age, IgE mediated eczema between one and three years of age and the risk of developing any IgE mediated allergic disease between one and three years of age. These findings need to be interpreted with caution however, as IgE mediated allergies were reported in only two trials which focused on high-risk populations of allergy.

There was also a reduction in the incidence of sensitisation to egg and any allergen in children between one and three years of age in the n-3 LCPUFA group.

### 6.5.2 Overall completeness and applicability of evidence

The review rigorously assessed trial quality, used Cochrane systematic review methods and focused on separating IgE mediated and all allergies (with/without IgE mediation).

The majority of the evidence came from children of women supplemented with n-3 LCPUFA during pregnancy and/or lactation and from women with a fetus at high risk of allergy (who were supplemented during pregnancy). Most of the trials included in this review were conducted in high-income industrialised countries and the findings are therefore applicable to the most affluent societies where the burden of allergy is known to be high.

IgE mediated allergies, where both the signs and symptoms of the allergic disease and a positive skin prick test (SPT) and/or radioallergosorbent test (RAST) were present, were reported in two trials [27, 269] and all trials except three [26, 260, 268], used medical diagnosis of allergy for the analyses (with IgE status not tested or unknown).

Most allergic responses are mediated by IgE antibodies, specific to the trigger allergen [81, 82]. However, although the presence of IgE antibodies indicates a sensitised state, the most reliable diagnosis of allergic disease should take into account clinical history as well [28, 81]. Thus, diagnoses involving laboratory tests (RAST) or SPT and assessment of clinical symptoms are more reliable than clinical presentation or parental reports (using validated questionnaires), or laboratory reports alone.

The medical diagnosis of wheeze outcomes (regardless of IgE mediation) to three years of age was available and included in the updated review, from unpublished data from Palmer et al 2012 and 2013 [285, 303]. As asthma is difficult to diagnose in young children, authors often report 'wheeze' [26, 260, 267, 268] therefore reports of wheeze were included in asthma outcomes. There were however substantial differences in the ages of asthma outcome

measures. Noakes et al 2012 [267] reported wheeze below one year of age, while Lauritzen et al 2005 [260] reported it at two and half years of age and in this thesis study the follow-up of Makrides et al 2009 [26] reported wheeze at 7 year of CA. Olsen et al [305] reported asthma outcomes in children at 16 years of age.

The selection of women differed with respect to fish intake, with three trials targeting women with a low fish intake [259, 260, 267], while fish intake was not related to inclusion criteria in other trials [26, 27, 266, 268, 269]. One of the reasons for some of the mixed results in this review may be due to the mothers baseline intake of n-3 LCPUFA not being considered in some studies. In the two trials [266, 267] where there was not an assigned control and women continued their 'usual diet', the women in these groups may have had a high intake of dietary n-3 LCPUFA as the benefits of dietary n-3 LCPUFA in pregnancy was promoted in the countries in which these two trials were conducted [266, 267].

In relation to diet and supplementation, some studies excluded women who were known to be allergic to fish [259, 266], as a safety precaution in case they may have reacted to fish oil capsules. Consequently, not many studies looked at fish allergy and hence data in this area may be limited [259, 266].

The evidence is incomplete for subgroup comparisons on the timing of supplementation, allergy risk of infants and maturity of infants. The trial in which n-3 LCPUFA supplementation started during pregnancy and continued through to lactation was limited by small sample size and high risk of attrition bias [269].

### 6.5.3 Overall strengths and weaknesses of evidence

The updated search strategy was comprehensive and was not limited by language and publication status. The major international and local bibliographic databases were covered, as well as hand searches of major journals, the proceedings of major conferences in the field, weekly current awareness alerts for a further 44 journals and monthly BioMed Central email alerts. Clear inclusion criteria and thorough quality assessment methodology was used to appraise the studies. As discussed in Chapter 2, two independent reviewers (AWG, CTC) screened and appraised the trials, and extracted data, using pre-designed data extraction forms. Therefore biases in the review process are unlikely. MM and CTC were investigators on two trials – Makrides et al 2009 [26] and Makrides et al 2010 [27] – that were included in the review. These trials were independently assessed for risk of bias and data extracted by AWG and an independent researcher Karen Best (KB).

The majority of the comparisons in this review were based on data from two trials [27, 269] and thus the quality of the evidence for each of the comparisons greatly depends on the quality of these two trials. However, other primary outcome data regardless of IgE mediation were based on all included trials except Ramakrishnan 2010 [268]. The risk of bias varied across the eight included trials with only two trials [26, 27] with a low risk of selection, performance and attrition bias.

Women's adherence to the supplementation may also impact on the outcomes. Blood analysis was used to check adherence to the supplementation in most trials [27, 259, 260, 266, 267, 269], and all reported a significant n-3 LCPUFA increase in the intervention group. The use of fish, rather than fish oil as a supplement also needs consideration as it may contribute to the energy and protein content in the maternal diet and influence outcomes in a different way. In this review one trial [267] supplemented with fish, however this trial had been designed to

overcome any additional effect of the diet by using it as a replacement for white fish, chicken and some red meat, thus minimising any additional energy and protein contributions (personal communication Noakes 2012).

The inclusion of data collected in this thesis allowed some of the gaps identified in Chapter 2 to be addressed, i.e. data were available for IgE mediated allergies and sensitisation to 3 years of age, parent reports of allergy data were available at 7 years of age and data from preterm infants was obtained.

However, all of the gaps identified in Chapter 2 were not fully answered and evidence is still incomplete for subgroup comparisons on the timing of supplementation, allergy risk of infants and maturity of infants. There is insufficient evidence to determine the effect of n-3 LCPUFA supplementation during lactation alone. The two postnatal trials [26, 260] included in the systematic review were based on parental reports of allergies and as discussed in Chapter 4, parents may over report some allergies.

## 6.6 Conclusion

### 6.6.1 Implications for practice

In this thesis, data has been created and synthesised with published data of others in the most up-to-date review of the effects of maternal prenatal and/or postnatal n-3 LCPUFA supplementation for preventing allergies in early childhood. Eight trials involving 3366 women and their 3175 children were included in the review. Women were supplemented with n-3 LCPUFA during pregnancy (five trials), lactation (two trials) or both pregnancy and lactation (one trial). Overall, there is limited evidence to support maternal n-3 LCPUFA supplementation during pregnancy and/or lactation for the reduction of allergic disease in the children with few differences seen in allergic disease in children between women who were supplemented with n-3 LCPUFA and those who were not. However, at some time points there

were reductions in some outcomes such as IgE mediated food allergy, IgE mediated eczema and IgE mediated any allergy, with n-3 LCPUFA supplementation in women with a fetus at high risk of allergy; therefore, further research is warranted.

In terms of safety for mothers and their infants, the use of n-3 LCPUFA supplementation during pregnancy and lactation was not associated with increased risk of postpartum haemorrhage and early childhood infections.

### 6.6.2 Implications for further research

More trials are needed to investigate the effects of n-3 LCPUFA supplementation during lactation, with studies in different allergy risk groups that assess allergy outcomes at different ages, to avoid the potential to skew data by repeated inclusion of one trial with multiple follow-up points.

As the studies included in this review used differing DHA to EPA ratios and duration of n-3 LCPUFA supplementation, further research is needed to investigate the influence of these factors, as it has been suggested that the DHA to EPA ratio may be important in determining allergy outcomes [301]. This may be especially important in studies involving supplementation during lactation as these studies tended to use lower doses and for a shorter period of time. The larger postnatal supplementation study included in my thesis used high DHA but less EPA (Table 2-1), had a different study population (preterm infants) and allergy outcomes were secondary, thus for the allergy point of view, further research is suggested for postnatal n-3 LCPUFA supplementation studies, including varying DHA and EPA supplements and longer supplementation periods in children with both normal and high risk of allergies.

One of the reasons for some of the mixed results in this review may be due to mothers' baseline intake of n-3 LCPUFA not being considered in some studies [26, 27, 266, 268, 269].

Further studies should consider taking into account baseline n-3 LCPUFA status as mothers with low baseline status may respond differently to the supplementation compared to women with high baseline status.

Trials should clearly differentiate between children at high risk of allergy and children at low risk of allergy given the suggestion of benefit to children who were at high risk of allergy. Trials should report both IgE mediated allergy and allergy +/- IgE mediation and should include follow-up into the school years.

It is not possible to generalise the applicability of findings worldwide because the mothers participating in this review and the two follow-ups were from high/upper-middle income industrialised countries and most probably with a low dietary intake of fish. Further research should be conducted in different populations of the world.

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# APPENDICES

## Appendices to chapter 2

### Appendix 2.1 – Published Systematic Review Protocol (pages 271-285)

# Statement of Authorship

| | |
|---|---|
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## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidature's thesis.

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| Signature | Date 19/08/2015 |

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# Maternal prenatal and/or postnatal n-3 fish oil supplementation for preventing allergies in early childhood (Protocol)

**Gunaratne AW, Makrides M, Collins CT**

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[Intervention Protocol]

# Maternal prenatal and/or postnatal n-3 fish oil supplementation for preventing allergies in early childhood

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## ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To assess the effect of n-3 LCPUFA supplementation in mothers during pregnancy or lactation on allergy outcomes in their children.

## BACKGROUND

### Description of the condition

Allergy prevention is a challenge for the Western world (Strong 2005). Over the past 20 years the prevalence of allergies has increased worldwide in industrialised countries, from approximately 4% to the current level of 20% (Asher 1998; Asher 2006). Allergic diseases include food allergies, allergic dermatitis (eczema), asthma and allergic rhinitis (hay fever) Arkwright 2008. The pattern of allergy expression differs with the age of the child. Food allergies and atopic eczema are common in children under three years of age while asthma and hay fever are common between the ages of three and 15 years (Saarinen 1995). Regardless of these changing patterns of allergic disease in childhood, many childhood allergies persist with 50% of childhood asthma sufferers and 80% of hay fever sufferers continuing to experience allergic symptoms into adulthood (Asher 2006; Barbee 1998; Greisner 1998).

Atopy is defined as a genetic predisposition to the production of the immunoglobulin E (IgE) antibody (Sears 1996). People with atopy who are exposed to allergens can develop an immune reaction that leads to allergies and therefore are defined as atopic (Sears 1996). The risk of allergy is 30% greater if one first degree relative (parent or sibling) is atopic. If both parents are atopic, the allergy risk increases to 70% (Sears 1996). Allergic responses are reactions to an extrinsic substance (allergen) that is mediated by an immunological response (Arkwright 2008). This immunological response may cause mild to severe reactions in different individuals and can be life threatening (Arkwright 2008). Thus allergy is an important public health problem which is burdensome to individuals, society and the healthcare system (Gupta 2004).
Environmental factors seem to be an important influence on the increasing incidence of allergies. Possible contributing environmental factors include lack of breastfeeding, higher socio-economic conditions with better hygiene standards, fewer respiratory infections, greater use of antibiotics early in life, fewer older sib-

lings in the household, less contact with farm animals, general lack of microbial exposure and changes in dietary patterns (Gupta 2004; Strong 2005).

Among the changes in dietary patterns, it has been hypothesised that the balance of long chain polyunsaturated fatty acids (LCPUFA), specifically the n-3 to n-6 ratio, may be a major factor in the increased incidence of childhood allergies (Calder 2000; Prescott 2004). Fish and fish oil are the major sources of n-3 LCPUFA, and vegetable oils are the major source of n-6 LCPUFA. Recent data suggest that dietary consumption of n-3 LCPUFA has declined in Western diets to favour the intake of n-6 fatty acids (Meyer 2003). The n-3 to n-6 ratio is now estimated as 1:20-30 compared with the hunter and gatherer traditional diet ratio of 1:1-2 (Simopoulos 1999). Furthermore, maternal fish consumption during pregnancy has also reduced due to precautionary public health advice given regarding the consumption of specific fish which may contain methyl mercury (Oken 2003). Epidemiological data have shown that a higher fish intake during pregnancy is associated with fewer symptoms of allergic diseases in early childhood (Calvani 2006; Romieu 2007; Sausenthaler 2007; Willers 2007). Thus, the reduction in maternal consumption of n-3 LCPUFA may be an important factor in the increased incidence of allergies.

## Description of the intervention

Dietary n-3 LCPUFA supplementation during pregnancy and lactation is thought to modulate the immune system of the fetus, neonate or infant before allergic responses are established Denburg 2005. Supporting evidence from mechanistic studies (Denburg 2005; Prescott 2007) observational studies (Hodge 1996; Oddy 2004) and small scale intervention trials show that allergy markers and allergy mechanisms are influenced by n-3 LCPUFA (Dunstan 2003). Evidence from epidemiological studies (Calvani 2006; Romieu 2007; Salam 2005; Sausenthaler 2007; Willers 2007) showing an association between maternal fish consumption in pregnancy and reduction of allergies in the offspring are promising. But there is conflicting evidence with reports of no association between maternal fish intake during lactation and childhood allergy (Huang 2001). Observational studies with maternal n-3 fish oil supplementation showed that there was modulation of a range of inflammatory events in women (Calder 2010; Caughey 2008; James 2000). Supporting evidence also comes from studies where infants consumption of n-3 fish oil lowers the risk of allergic diseases (Hodge 1998; Mihrshahi 2004; Oddy 2004)

## How the intervention might work

When diets are high in n-3 LCPUFA (for example in fish), n-3 LCPUFA is incorporated into cellular phospholipids, altering T-cell function. Several mechanisms by which n-3 LCPUFA influence immune cell function mechanisms have been identified. For example, modification of lipid rafts inhibits T cell responses, decreased prostaglandin 2 (PGE2) influences lymphocyte proliferation, Natural killer T cell activity and Th1 cytokine production (Gottrand 2008). Studies also support a role for n-3 LCPUFA in allergy prevention (Gottrand 2008).

n-3 LCPUFA supplementation has been shown to lead to a reduction in prostaglandin 2 (PGE2) and induction of Type 1 T cells (Th1) cytokines production (James 2000). In utero variations in the LCPUFA composition of fetal cell membranes has the potential to influence immune programming (Prescott 2007). The early programming of fetal immune responses to allergens possibly begins in the epithelial tissue where antigen (allergen) proteins first encounter antigen-presenting cells (APC) (Prescott 2007). The pattern of APC cytokine production determines the pattern of T helper cell differentiation (Prescott 2007). Th1 cells develop under the influence of IL-12 and IL-2, whereas T cells producing Type 2 (Th2) T cell develop in the relative absence of pro-Th2 factors such as IL-4 (Snijdewint 1993). The differences in T cell phenotypes also determines the pattern of B-cell antibody production with Th2 cytokines (IL-4, IL-5, and IL-13) prompting IgE production and allergic inflammation whereas, Th1 cytokines (IFN-$\gamma$) largely inhibit this in favour of low level IgG production (Simopoulos 1999). Th2 cytokines are also important in determining whether these immune responses result in clinically relevant diseases such as asthma, allergic rhinitis, atopic dermatitis or allergic inflammation (Georas 2005; Prescott 2007). A well regulated placental balance between the Th1 and Th2 responses is important for developing a robust immune system during pregnancy (Wilczynski 2005). There are two fish oil intervention studies that support this immune programming hypothesis. One study investigated Th1/Th2 related molecules in cord blood (Krauss-Etschmann 2008) and the other investigated cytokine production at two and a half years of age (Lauritzen 2005). These studies showed that allergy-related immune parameters were decreased in the offspring of women who had fish oil supplementation during pregnancy (Krauss-Etschmann 2008; Lauritzen 2005).

## Why it is important to do this review

There is a rising interest in prenatal and postnatal fish oil supplementation for preventing allergies. There is uncertainty as to whether n-3 LCPUFA supplementation during pregnancy or lactation does reduce allergy in children. It is for these reasons that we need clear evidence from intervention studies. Therefore, in this systematic review we aim to evaluate the effects of maternal fish oil supplementation during pregnancy or lactation on allergy outcomes in children.

## OBJECTIVES

To assess the effect of n-3 LCPUFA supplementation in mothers during pregnancy or lactation on allergy outcomes in their children.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

We will include studies in this review if they are randomised controlled trials (RCTs) or quasi-randomised trials focusing on n-3 LCPUFA fish oil supplementation of pregnant or lactating women compared with placebo or no treatment and assessing allergy outcomes of the infants or children. Cluster-randomised trials will be included. Trials with a cross-over design will be excluded. Trials presented only as abstracts will not be included unless unpublished data from the authors can be obtained.
Trials with only biochemical outcomes will be excluded.

#### Types of participants

Women, and their children, at either a normal or high risk of allergies will be included. A fetus or a child with a first degree relative with medically diagnosed allergies or who has positive a skin prick test or positive radio allergy sorbent test (RAST) will be defined as at high risk of allergies. Infants will also be considered at high risk of allergies, if their cord blood IgE level is above 0.70 IU/mL.

#### Types of interventions

We will consider all randomised comparisons of marine oil supplementation given to pregnant or lactating women (either with or without Arachidonic acid) with placebo or no supplementation, regardless of dose regimens, and durations of intervention. Studies in which fish was the intervention will be included if appropriately controlled, for example, with appropriate adjustments/matching of the protein contribution of fish.

#### Types of outcome measures

Primary outcome measures will be children with food allergy, allergic dermatitis (eczema), asthma, allergic rhinitis (hay fever), or any allergies. Outcomes will be assessed as short term (less than 12 months), medium term (12 to less than 36 months) and long term (36 months and older). Outcomes will be also assessed combining short term, medium term and long term (any follow-ups) to assess the cumulative incidence of allergies.

##### Primary outcomes

1. Medically diagnosed allergy with sensitisation, i.e. IgE mediated allergies that have both signs and symptoms of the allergic disease and a positive skin prick test,RAST, or both) and,
2. medical diagnosis or parental report (using validated questionnaire).

##### Secondary outcomes

1. Skin prick test results; infant safety; e.g. infections, parent-reported allergies using non-validated questionnaires.

### Search methods for identification of studies

#### Electronic searches

We will contact the Trials Search Co-ordinator to search the Cochrane Pregnancy and Childbirth Group's Trials Register.
The Cochrane Pregnancy and Childbirth Group's Trials Register is maintained by the Trials Search Co-ordinator and contains trials identified from:

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE;
3. weekly searches of EMBASE;
4. handsearches of 30 journals and the proceedings of major conferences;
5. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Details of the search strategies for CENTRAL, MEDLINE and EMBASE, the list of handsearched journals and conference proceedings, and the list of journals reviewed via the current awareness service can be found in the 'Specialized Register' section within the editorial information about the Cochrane Pregnancy and Childbirth Group.
Trials identified through the searching activities described above are each assigned to a review topic (or topics). The Trials Search Co-ordinator searches the register for each review using the topic list rather than keywords.
In addition, we plan to search PubMed (1966 to current) (Appendix 1), CINAHL via EBSCOhost (1984 to current) ( Appendix 2), Scopus (Appendix 3), Web of Knowledge (Appendix 4) and ClinicalTrials.gov (Appendix 5).
We will not apply any language restrictions.

### Data collection and analysis

#### Selection of studies

Two review authors Anoja W Gunaratne (AWG) and Carmel T Collins (CTC) will independently assess the eligibility of studies identified by the search. Disagreements will be resolved through discussion or, if required, by consultation with the third review author Maria Makrides (MM).

## Data extraction and management

We will design a form to extract data. For eligible studies, two review authors (AWG, CTC) will extract the data independently using the agreed form. Discrepancies will be resolved through discussion or, if required, through consultation with the third author (MM). When information regarding any of the above is unclear or incomplete, we will attempt to contact authors of the original reports to provide further details. We will enter data into Review Manager software (RevMan 2011) and check for accuracy.

## Assessment of risk of bias in included studies

Two review authors (AWG, CTC) will independently assess risk of bias for each study using the criteria outlined in *the Cochrane Handbook for Systematic Reviews of Interventions (Handbook) (*Higgins 2011*)*. We will resolve any disagreement by discussion or by involving a third assessor (MM). MM and CTC are Investigators on LCPUFA trials that could potentially be included in the review. These studies will be independently assessed for risk of bias and if included, AWG and Karen Best (KB) will extract the data.

### (1) Random sequence generation (checking for possible selection bias)

We will describe for each included study the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.
We will assess the method as:

- low risk of bias (any truly random process, e.g. random number table; computer random number generator);
- high risk of bias (any non-random process, e.g. odd or even date of birth; hospital or clinic record number);
- unclear risk of bias.

### (2) Allocation concealment (checking for possible selection bias)

We will describe for each included study the method used to conceal allocation to interventions prior to assignment and will assess whether intervention allocation could have been foreseen in advance of, or during recruitment, or changed after assignment.
We will assess the methods as:

- low risk of bias (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk of bias (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear risk of bias.

### (3.1) Blinding of participants and personnel (checking for possible performance bias)

We will describe for each included study the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We will consider that studies are at low risk of bias if they were blinded, or if we judge that the lack of blinding would be unlikely to affect results. We will assess blinding separately for different outcomes or classes of outcomes.
We will assess the methods as:

- low, high or unclear risk of bias for participants;
- low, high or unclear risk of bias for personnel.

### (3.2) Blinding of outcome assessment (checking for possible detection bias)

We will describe for each included study the methods used, if any, to blind outcome assessors from knowledge of which intervention a participant received. We will assess blinding separately for different outcomes or classes of outcomes.
We will assess methods used to blind outcome assessment as:

- low, high or unclear risk of bias.

### (4) Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)

We will describe for each included study, and for each outcome or class of outcomes, the completeness of data including attrition and exclusions from the analysis. We will state whether attrition and exclusions were reported and the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information is reported, or can be supplied by the trial authors, we will re-include missing data in the analyses which we undertake.
We will assess methods as:

- low risk of bias (e.g. no missing outcome data; missing outcome data balanced across groups);
- high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups; 'as treated' analysis done with substantial departure of intervention received from that assigned at randomisation);
- unclear risk of bias.

### (5) Selective reporting (checking for reporting bias)

We will describe for each included study how we investigated the possibility of selective outcome reporting bias and what we found.

We will assess the methods as:

- low risk of bias (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- high risk of bias (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported);
- unclear risk of bias.

#### (6) Other bias (checking for bias due to problems not covered by (1) to (5) above)

We will describe for each included study any important concerns we have about other possible sources of bias.

We will assess whether each study was free of other problems that could put it at risk of bias:

- low risk of other bias;
- high risk of other bias;
- unclear whether there is risk of other bias.

#### (7) Overall risk of bias

We will make explicit judgements about whether studies are at high risk of bias, according to the criteria given in the *Handbook* (Higgins 2011). With reference to (1) to (6) above, we will assess the likely magnitude and direction of the bias and whether we consider it is likely to impact on the findings. We will explore the impact of the level of bias through undertaking sensitivity analyses - *see* Sensitivity analysis.

### Measures of treatment effect

#### Dichotomous data

For dichotomous data, we will present results as summary risk ratio with 95% confidence intervals.

#### Continuous data

For continuous data, we will use the mean difference if outcomes are measured in the same way between trials. We will use the standardised mean difference to combine trials that measure the same outcome, but use different methods.

### Unit of analysis issues

#### Cluster-randomised trials

We will include cluster-randomised trials in the analyses along with individually-randomised trials. We will adjust their sample sizes using the methods described in the *Handbook* using an estimate of the intracluster correlation co-efficient (ICC) derived from the trial (if possible), from a similar trial or from a study of a similar population. If we use ICCs from other sources, we will report this and conduct sensitivity analyses to investigate the effect of variation in the ICC. If we identify both cluster-randomised trials and individually-randomised trials, we plan to synthesise the relevant information. We will consider it reasonable to combine the results from both if there is little heterogeneity between the study designs and the interaction between the effect of intervention and the choice of randomisation unit is considered to be unlikely.

We will also acknowledge heterogeneity in the randomisation unit and perform a sensitivity analysis to investigate the effects of the randomisation unit.

#### Cross-over trials

Cross-over trials are not an appropriate design for this review.

#### Other unit of analysis issues

#### Studies with more than two treatment groups

If we include studies using one or more treatment groups (multi-arm studies), where appropriate, we will combine groups to create a single pair-wise comparison. We will use the methods described in the *Handbook* (Higgins 2011) to ensure that we do not double count participants.

### Dealing with missing data

For included studies, we will note levels of attrition. We will explore the impact of including studies with high levels of missing data in the overall assessment of treatment effect by using sensitivity analysis.

For all outcomes, we will carry out analyses, as far as possible, on an intention-to-treat basis, i.e. we will attempt to include all participants randomised to each group in the analyses, and all participants will be analysed in the group to which they were allocated, regardless of whether or not they received the allocated intervention. The denominator for each outcome in each trial will be the number randomised minus any participants whose outcomes are known to be missing.

### Assessment of heterogeneity

We will assess statistical heterogeneity in each meta-analysis using the $T^2$, $I^2$ and $Chi^2$ statistics. We will regard heterogeneity as substantial if $I^2$ is greater than 30% and either $T^2$ is greater than

zero, or there is a low P value (less than 0.10) in the Chi² test for heterogeneity. We will conduct a random-effects analysis.

### Assessment of reporting biases

If there are 10 or more studies in the meta-analysis, we will investigate reporting biases (such as publication bias) using funnel plots. We will assess funnel plot asymmetry visually, and use formal tests for funnel plot asymmetry. For continuous outcomes we will use the test proposed by Egger 1997, and for dichotomous outcomes we will use the test proposed by Harbord 2006. If asymmetry is detected in any of these tests or is suggested by a visual assessment, we will perform exploratory analyses to investigate it.

### Data synthesis

We will carry out statistical analysis using the Review Manager software (RevMan 2011). We will use fixed-effect meta-analysis for combining data where it is reasonable to assume that studies are estimating the same underlying treatment effect: i.e. where trials are examining the same intervention, and the trials' populations and methods are judged sufficiently similar. If there is clinical heterogeneity sufficient to expect that the underlying treatment effects differ between trials, or if substantial statistical heterogeneity is detected, we will use random-effects meta-analysis to produce an overall summary if an average treatment effect across trials is considered clinically meaningful. The random-effects summary will be treated as the average range of possible treatment effects and we will discuss the clinical implications of treatment effects differing between trials. If the average treatment effect is not clinically meaningful we will not combine trials.
If we use random-effects analyses, the results will be presented as the average treatment effect with 95% confidence intervals, and the estimates of $T^2$ and $I^2$.

### Subgroup analysis and investigation of heterogeneity

If we identify substantial heterogeneity, we will investigate it using subgroup analyses and sensitivity analyses. We will consider whether an overall summary is meaningful, and if it is, use random-effects analysis to produce it.
We plan to carry out the following subgroup analyses.

1. Timing of supplementation:
    - n-3 LCPUFA fish oil supplementation during pregnancy versus placebo or no supplementation during pregnancy;
    - n-3 LCPUFA fish oil supplementation during lactation versus placebo or no supplementation during lactation;
    - n-3 LCPUFA fish oil supplementation during pregnancy and lactation versus placebo or no supplementation during pregnancy and lactation;
2. Allergy risk:
    - maternal n-3 LCPUFA fish oil supplementation in women at high risk of allergies versus placebo or no supplementation;
    - maternal n-3 LCPUFA fish oil supplementation in women at normal risk of allergies versus placebo or no supplementation;
3. Maturity:
    - maternal n-3 LCPUFA fish oil supplementation in term born infants versus placebo or no supplementation;
    - maternal n-3 LCPUFA fish oil supplementation in pre-term born infants versus placebo or no supplementation;

We will restrict subgroup analyses to the primary outcomes.
We will assess differences between subgroups by interaction tests available within RevMan (RevMan 2011).

### Sensitivity analysis

We will carry out sensitivity analyses to investigate the effect of trial quality by removing those trials rated as 'high risk of bias' or 'unclear risk of bias' to establish whether it is likely to impact on the findings.
We will restrict sensitivity analyses to the primary outcomes.

## ACKNOWLEDGEMENTS

As part of the pre-publication editorial process, this protocol has been commented on by two peers (an editor and referee who is external to the editorial team), a member of the Pregnancy and Childbirth Group's international panel of consumers and the Group's Statistical Adviser.

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* *Indicates the major publication for the study*

# APPENDICES

## Appendix 1. PubMed search strategy

1. pregnancy[mh]
2. pregnan*[tiab]
3. maternal exchange*[tiab]
4. transplacental exposure*[tiab]
5. gestat*[tiab]
6. fetal Development [mesh]
7. Fetal Development [tiab]
8. Fetal Programming* [tiab]
9. fetal growth[tiab]
10. Foetal Development [tiab]
11. Foetal Programming* [tiab]
12. foetal growth[tiab]
13. Gestational Age*[tiab]
14. Fetal Age*[tiab]
15. foetal age*[tiab]
16. Breast Feeding[mh]
17. breast feeding[tiab]
18. breast fed[tiab]
19. lactating mother*[tiab]
20. breastfeeding[tiab]
21. Postpartum Period[mh]
22. Postpartum[tiab])
23. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16OR 17 OR 18 OR 19 OR 20 OR 21 OR 22
24. fish oils[mh]
25. fish oil*[tiab]
26. docosahexaenoic acid*[tiab]
27. cod liver oil[tiab]
28. omega 3 fatty acid*[tiab]
29. n 3 fatty acid*[tiab]
30. eicosapentaenoic acid[tiab]
31. n 3 pufa)
32. 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31
33. hypersensitivity[mh]
34. hypersensitivit*[tiab]
35. allerg*[tiab]
36. environmental illness*[tiab]
37. atopic dermatitis[tiab]
38. anaphyla*[tiab]
39. atopic dermatitis[tiab]
40. eczema[tiab]
41. urticaria*[tiab]
42. hives[tiab])
43. 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39 OR 40 OR 41 OR 42
44. 23 AND 32 AND 43
45. randomized controlled trial[pt]
46. controlled clinical trial[pt]
47. randomized controlled trials[mh]

48. random allocation[mh]
49. double-blind method[mh]
50. single-blind method[mh]
51. clinical trial[pt]
52. clinical trials[mh]
53. clinical trial[tw]
54. ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw]))
55. (placebos[mh] OR placebo*[tw] OR random*[tw] OR research design [mh:noexp] OR comparative study[pt] OR evaluation studies as topic[mh] OR follow-up studies[mh] OR prospective studies[mh] OR control[tw] OR controlled[tw] OR prospectiv*[tw] OR volunteer*[tw])
56. 45 OR 46 OR 47 OR 48 OR 49 OR 50 OR 51 OR 52 OR 53 OR 54 OR 55
57. NOT animals[mh] NOT (human[mh] and animals[mh])
58. 44 AND 56
59. 58 NOT 57

## Appendix 2. CINAHL (via EBSCOhost) search strategy

1. (((singl* OR doubl* OR trebl* OR tripl*) AND (mask* OR blind*)) OR (placebo* OR random* OR "research design*" OR "comparative stud*" OR "evaluation stud*" OR "follow-up stud*" OR control OR controlled OR prospectiv* OR volunteer*)) NOT (animals NOT human))
2. (pregnan* OR "maternal exchange*" OR "transplacental exposure*" OR gestat* OR "fetal development" OR "fetal programming*" OR "fetal growth" OR "foetal development" OR "foetal programming*" OR "foetal growth" OR "fetal age*" OR "foetal age*" OR "breast feeding" OR "breast fed" OR "lactating mother*" OR breastfeeding OR postpartum)
3. ("fish oil*" OR "docosahexaenoic acid*" OR "cod liver oil" OR "omega 3 fatty acid*" OR "n 3 fatty acid*" OR "eicosapentaenoic acid" OR "n 3 pufa")
4. (hypersensitivit* OR allerg* OR "environmental illness*" OR "atopic dermatitis" OR anaphyla* OR eczema OR urticaria* OR hives)
5. 1 AND 2 AND 3 AND 4

## Appendix 3. Scopus search strategy

1. (pregnan* OR "maternal exchange" OR "transplacental exposure" OR gestat* OR "fetal development" OR "fetal programming" OR "fetal growth" OR "foetal development" OR "foetal programming" OR "foetal growth" OR "Gestational Age" OR "fetal age" OR "foetal age" OR "breast feeding" OR "breast fed" OR "lactating mother" OR breastfeeding OR postpartum)
2. ("fish oil" OR "docosahexaenoic acid" OR "cod liver oil" OR "omega 3 fatty acid" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "n 3 pufa")
3. (hypersensitivit* OR allerg* OR "environmental illness" OR "atopic dermatitis" OR anaphyla* OR "atopic dermatitis" OR eczema OR urticaria* OR hives)
4. ("randomized controlled trial" OR "randomised controlled trial" OR "random allocation" OR "double blind" OR "single blind" OR "clinical trial*" OR ((singl* OR doubl* OR trebl* OR tripl*) AND (mask* OR blind*)) OR placebo* OR "comparative stud" OR "follow up stud" OR "prospective stud" OR rct OR "systematic review" OR "meta analys" OR metaanalys*)
5. 1 AND 2 AND 3 AND 4

## Appendix 4. Web of Knowledge search strategy

1. pregnan*
2. "maternal exchange*"
3. "transplacental exposure*"
4. gestat*
5. "Fetal Development*"
6. "Fetal Programming*"
7. "fetal growth"
8. "Foetal Development*"
9. "Foetal Programming*"
10. "foetal growth"
11. "Gestational Age*"
12. "Fetal Age*"
13. "foetal age*"
14. "breast feeding*"
15. "breast fed"
16. "lactating mother*"
17. breastfeeding
18. postpartum
19. 1 OR 2 OR 3 OR4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18
20. "fish oil*"
21. "docosahexaenoic acid*"
22. "cod liver oil*"
23. "omega 3 fatty acid*"
24. "n 3 fatty acid*"
25. "eicosapentaenoic acid*"
26. "n 3 pufa*"
27. 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26
28. hypersensitivit*
29. allerg*
30. "environmental illness*"
31. "atopic dermatitis"
32. anaphyla*
33. "atopic dermatitis"
34. eczema
35. urticaria*
36. hives
37. 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36
38. 19 AND 27 AND 37
39. ("randomized controlled trial*" OR "controlled clinical trial*" OR "randomised controlled trial*" OR "random allocation*" OR "double blind" OR "single blind" OR "clinical trial*" OR ((singl* OR doubl* OR trebl* OR tripl*) AND (mask* OR blind*)) OR placebo* OR "comparative stud*" OR"follow up stud*" OR "prospective stud*" OR rct OR "systematic review*" OR "meta analys*" OR metaanalys*)
40. 38 AND 39

## Appendix 5. ClinicalTrials.gov search strategy

**http://clinicaltrials.gov**

(hypersensitivity OR hypersensitive OR allergy) AND ("fish oil" OR docosahexaenoic OR "omega 3 fatty acid") AND (pregnancy OR pregnant OR postpartum OR fetal OR foetal OR "breast feeding" OR breastfeeding)

# HISTORY

Protocol first published: Issue 9, 2012

# CONTRIBUTIONS OF AUTHORS

AWG and MM were responsible for conceiving the review. AWG, MM and CTC designed, developed and wrote the protocol. MM and CTC provided a methodological perspective and a clinical perspective and provided general advice on the protocol. AWG and CTC were responsible for coordinating the review.

# DECLARATIONS OF INTEREST

MM and CTC are Investigators on LCPUFA trials that could potentially be included in the review. These studies will be independently assessed for risk of bias and if included, data extraction by AWG and KB. AWG has no conflict of interest.

# SOURCES OF SUPPORT


## Internal sources

- The University of Adelaide, Adelaide, Australia Women's & Children's Health Research Institute, Adelaide, Australia, Not specified.

## External sources

- No sources of support supplied

**Appendix 2.2 – Data Extraction Form**

**Review title: Maternal fish oil supplementation for prevention of allergies in early childhood**

| | | |
|---|---|---|
| **Review ID:** | **Study ID**: | **Reference ID:** |
| **Person extracting data:** | **Date of date extraction:** | **Year of study publication:** |
| **Title:** | | |
| **Author:** | | |
| **Reference:** | | |

**Study design**

| |
|---|
| **Type of study design (cluster RCT; block randomisation; stratified randomisation; multi-arm; factorial etc):** |
| **Unit of randomisation:** |

**Participants and setting**

| |
|---|
| **Describe setting**: |
| **Exclusion criteria:** |

**Intervention**

| |
|---|
| **Experimental intervention:** |

**Comparison**

| |
|---|
| **Control/Comparison intervention:** |

**Outcomes:**

| |
|---|
| **Outcomes:** |

**Study methods-Risk of bias**

| | |
|---|---|
| **Adequate sequence generation**<br>Was the allocation sequence adequately generated? | Yes / Unclear / No<br>Describe: |
| **Allocation concealment**<br>Was allocation concealment adequate? | Yes / Unclear / No<br>Describe: |
| **Blinding**<br>Was knowledge of the allocated intervention adequately prevented during the study? | Participant: Yes / Unclear / No<br>Clinician: Yes / Unclear / No<br>Outcome assessor: Yes / Unclear / No<br>Describe: |
| **Incomplete outcome data addressed**<br>Were complete outcome data adequately addressed? | Yes / Unclear / No<br>Describe any loss of participants to follow up at each data collection point:<br>Describe any exclusion of participants after randomisation:<br>Was the analysis intention to treat? If not has the data been able to be re-included? |
| **Free of selective reporting bias**<br>Are reports of study free of suggestions of selective reporting bias? | Yes / Unclear / No<br>Describe: |
| **Free of other bias**<br>Was the study apparently free of other problems that could put it at high risk of bias? | Yes / Unclear / No<br>If the study was stopped early, explain the reasons:<br>Describe any baseline in balance:<br>Describe any differential diagnosis: |

**Additional information requested**

| |
|---|
| |

**Outcomes for main analysis**

| | **Outcome Measures (Dichotomous)**<br>**Primary:** | Total number of participants in study = | | | |
|---|---|---|---|---|---|
| | | **Intervention group**<br>Total no. in study = | | **Control group**<br>Total no. in study = | |
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated (Any allergy follow up -cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| | **Outcome Measures (Dichotomous)**<br>**Secondary:** | Total number of participants in study = | | | |
| | | **<u>Intervention group</u>**<br>Total no. in study= | | **<u>Control group</u>**<br>Total no. in study= | |
| | | events | Total | events | total |
| 1 | Post Partum Bleeding | | | | |
| 2 | Post Partum Infections/sepsis | | | | |
| 3 | Early childhood infections >12 months | | | | |
| 4 | Early childhood infections 12- <36 months | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 5 | Early childhood infections ≥ 36 months | | | | |
| 6 | Others | | | | |
| | **Skin Prick Test** | Total number of participants in study = | | | |
| | | **Intervention group**<br>Total no. in study = | | **Control group**<br>Total no. in study = | |
| | **< 12 months of age** | events | Total | events | Total |
| | Egg | | | | |
| | Cow's milk | | | | |
| | Wheat | | | | |
| | Pea nut | | | | |
| | Fish | | | | |
| | Inhalant (rye grass pollen, olive tree pollen etc.) | | | | |
| | HDM (house dust mite) | | | | |
| | Cat | | | | |
| | Any positive SPT | | | | |
| | **12- <36 months of age** | | | | |
| | Egg | | | | |
| | Cow's milk | | | | |
| | Wheat | | | | |
| | Pea nut | | | | |
| | Fish | | | | |
| | Inhalant (rye grass pollen, olive tree pollen etc.) | | | | |
| | HDM (house dust mite) | | | | |
| | Cat | | | | |
| | Any positive SPT | | | | |
| | **≥36 months of age** | | | | |
| | Egg | | | | |
| | Cow's milk | | | | |
| | Wheat | | | | |
| | Pea nut | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Fish | | | | |
| | Inhalant (rye grass pollen, Olive tree pollen etc.) | | | | |
| | HDM (house dust mite) | | | | |
| | Cat | | | | |
| | Any positive SPT | | | | |

**Outcomes for sub-group analyses**

| | **Outcome Measures (Dichotomous)**<br>**Primary: High risk of allergies** | Total number of participants in study = | | | |
|---|---|---|---|---|---|
| | | **Intervention group**<br>Total no. in study = | | **Control group**<br>Total no. in study = | |
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- < 36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated (Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| | **Outcome Measures (Dichotomous)** | Total number of participants in study = | | | |

| | **Primary: Low risk of allergies** | **Intervention group**<br>Total no. in study = | | **Control group**<br>Total no. in study = | |
|---|---|---|---|---|---|
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- < 36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated (Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| | **Outcome Measures (Dichotomous)**<br>Primary**: Pregnancy** | Total number of participants in study = | | | |
| | | **<u>Intervention group</u>**<br>Total no. in study = | | **<u>Control group</u>**<br>Total no. in study = | |
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| | **Outcome Measures (Dichotomous)** | Total number of participants in study = | | | |
| | **Primary: Pregnancy and Lactation** | **<u>Intervention group</u>**<br>Total no. in study = | | **<u>Control group</u>**<br>Total no. in study = | |
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- < 36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |

| | **Outcome Measures (Dichotomous)**<br>**Primary: Lactation** | Total number of participants in study = | | | |
|---|---|---|---|---|---|
| | | **<u>Intervention group</u>**<br>Total no. in study = | | **<u>Control group</u>**<br>Total no. in study = | |
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- < 36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| | **Outcome Measures (Dichotomous)**<br>**Primary: Term infants** | Total number of participants in study = | | | |
| | | **<u>Intervention group</u>**<br>Total no. in study = | | **<u>Control group</u>**<br>Total no. in study = | |
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Asthma | | | | |
| | Any allergies | | | | |
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- < 36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated (Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| | **Outcome Measures (Dichotomous)**<br>Primary: **Preterm Infants** | Total number of participants in study = | | | |
| | | **<u>Intervention group</u>**<br>Total no. in study = | | **<u>Control group</u>**<br>Total no. in study = | |
| | | events | Total | events | total |
| 1 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 2 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases 12- < 36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 3 | **IgE mediated (+ve SPT or/and +ve RAST) medically diagnosed allergic diseases ≥36 months** | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 4 | **IgE mediated Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 5 | **Medically diagnosed or Parental reported allergic diseases < 12 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 6 | **Medically diagnosed or Parental reported allergic diseases 12- <36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 7 | **Medically diagnosed or Parental reported allergic diseases ≥36 months** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |
| 8 | **Medically diagnosed or Parental reported Any allergy follow up (cumulative incidence)** | | | | |
| | Food allergy | | | | |
| | Eczema | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Allergic rhinitis/Hay fever | | | | |
| | Asthma | | | | |
| | Any allergies | | | | |

**General conclusions**

| **Very brief summary of main findings/conclusions:** |
|---|
| |

**Notes study authors**

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| |

**Exclusion after data extraction**

| **Reasons for exclusion: (study design? participants? interventions/ outcomes? attrition? bias?)** |
|---|

**Dates:**

**Date entered into RevMan and by whom?**

**Date checked and by whom?**

**Date copy sent to editorial base and by whom?**

## Appendix 2.3 – Risk of bias table for included studies

## Table 2.3 Risk of bias in included studies

### Dunstan 2003 [1]

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "The groups were block-randomised according to parity, pre-pregnancy BMI, age and maternal allergy". |
| Allocation concealment (selection bias) | Low risk | Quote: "Randomization and allocation of capsules occurred at a different centre separate from the recruitment of participants. Capsules were administered to the participants by someone separate from those doing the allocation". |
| Blinding of participants and personnel (performance bias) | Low risk | The capsules in the 2 groups were image matched, and the participants, research scientists, and paediatrician remained blinded to the groups for the duration of the study. |
| Blinding of outcome assessment (detection bias) | Low risk | Participants, research scientists, and paediatrician remained blinded to the groups for the duration of the study. |
| Incomplete outcome data (attrition bias) | High risk | Randomised n=98 (52 intervention, 46 control)<br>At birth 15 (15%) excluded (12 treatment, three control): eight discontinued intervention due to nausea (seven treatment, one control), one cord blood not collected (control), four gestation <36 weeks (three treatment, one control), two unrelated infant disease (treatment), (85% follow-up rate; intervention 77%, control 94%).<br>Outcomes were reported on n=83 (85%) at one year of age (fish oil group n=40, 77% and control group n=43, 94%). Of the 83, telephone interviewed n=11, clinic visit and skin prick test n=72 |
| Selective reporting (reporting bias) | High risk | Trial registered at anzctr.org.au Identifier: ACTRN12611000041954<br>Prespecified outcomes were reported in this trial according to their protocol. However, most of the prespecified review outcomes were not reported in this trial. Skin Prick Test was performed but did not report the IgE mediated allergies. |
| Other bias | Unclear risk | Preterm infants were excluded in their analysis after randomisation. |

### Furuhjelm 2009 [2]

| Bias | Authors' judgement | Support for judgement |
|---|---|---|

| | | |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "The mothers were randomly allocated to dietary supplementation either with Ω-3 fatty acids or placebo." |
| Allocation concealment (selection bias) | Low risk | Quote: "Producer performed the block randomisation". We interpreted this as central allocation. |
| Blinding of participants and personnel (performance bias) | Low risk | Quote: "Active and placebo capsules could not be distinguished from each other." |
| Blinding of outcome assessment (detection bias) | Low risk | Quote: "The research nurses, the paediatricians and the person performing the laboratory analyses were blinded during the intervention and follow up." |
| Incomplete outcome data (attrition bias) | High risk | 25 did not complete the requested 15 week period (16, 23% in treatment group and 9, 12% in placebo group), 1 withdrew post-delivery, 2 not followed as moved before 6/12 follow up (group not stated). Total 28 (19%) not included in analysis, 117 included. Analysis was not intention-to-treat<br>Skin prick test 117 at 6 months, 115 at 12 months.<br>Medically diagnosed allergy outcomes were reported on n=117 (fish oil group n=52 and control group n=65), at 6 months and at 1 year of age)<br>Medically diagnosed allergy outcomes were reported on n=119 (fish oil group n=54 and control group n=65), at 2 year of age). |
| Selective reporting (reporting bias) | Low risk | Trial registered at ClinicalTrials.gov identifier: NCT00892684<br>Prespecified outcomes were reported in this trial according to their protocol. Outcomes of interest to the review are reported. |
| Other bias | Low risk | No obvious risk of other bias. |

## Lauritzen 2005 [3]

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "After birth, the women with fish intakes below the 50th percentile were randomly assigned to a supplementation group by a randomization schedule prepared by a person uninvolved in the study." |
| Allocation concealment (selection bias) | Low risk | Quote: "Owing to the non-identical appearance of the capsules for the two groups, a person who was not otherwise involved in the project handled the capsules in order to avoid braking the blinding of the investigators." |
| Blinding of participants and | Low risk | Quote: "Investigators and families were blinded to the randomisation". |

| | | |
|---|---|---|
| personnel (performance bias) | | |
| Blinding of outcome assessment (detection bias) | Low risk | Quote: "A person not otherwise involved in the study handled the capsules in order to avoid breaking the blinding of the investigators". |
| Incomplete outcome data (attrition bias) | High risk | Randomised: Study entry n=122 (Intervention 62, control 60) At 2.5 years of age: n=72 (50, 41% withdrew, Intervention 20, 32% Control 30, 50%)<br>Ref group study entry: n=53<br>At 2.5 years of age: n=29 (24, 45% withdrew)<br>Large losses to follow up, all noted as 'withdrawals' or no reason given. |
| Selective reporting (reporting bias) | High risk | Trial registered at ClinicalTrials.gov identifier: NCT00266305<br>Most of the prespecified review outcomes were not reported in this trial. |
| Other bias | Low risk | No obvious risk of other bias. |

## Makrides 2009 [4]

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done by a computer-driven telephone randomisation service according to an independently generated randomisation schedule. A unique study number was given through stratification by centre, birth weight (1250 g vs1250 g), and infant sex. Multiple births were considered a single randomisation unit and randomisation of twins or triplets was according to the sex and birth weight of the first born infant. |
| Allocation concealment (selection bias) | Low risk | To facilitate blinding, each treatment group was separately colour coded into 2 groups. All capsules were similar in size, shape, and colour, It formula feeding needed ready-to-feed preterm formula to trial specifications and packaged the formula according to the colour codes. |
| Blinding of outcome assessment (detection bias) | Low risk | Clinicians and all research personnel were blinded to participant study group. |
| Incomplete outcome data (attrition bias) | Low risk | A total of 657 infants were enrolled (high-DHA diet: 322; standard-DHA diet: 335), and 614 infants (93.5%) completed the 18-month follow up. Although there was a high retention rate in the trial, allergy data were incomplete at 12-months and 18-month corrected age, largely because the families who anticipated in the pilot phase of the trial did not |

| | | |
|---|---|---|
| | | complete the allergy questionnaires. Data were analysed at the age of 12 months (n=471) and the age of 18 months n=503). All analyses were conducted according to the intention-to-treat principle. |
| Selective reporting (reporting bias) | High risk | Trial registered at anzctr.org.au Identifier: ACTRN12606000327583<br>Most of the prespecified review outcomes were not reported in this trial. |
| Other bias | Unclear risk | Subgroup of mothers who were providing breast milk at trial entry and their children were included in this review. |

## Noakes 2012 [5]

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | "Women were randomly assigned to 1 of 2 groups; random assignment was according to a random number table" |
| Allocation concealment (selection bias) | Unclear risk | Not described. |
| Blinding of participants and personnel (performance bias) | High risk | Participants were not blinded. |
| Blinding of outcome assessment (detection bias) | Low risk | Researchers responsible for assessing outcome measures (both laboratory and clinical) remained blinded. |
| Incomplete outcome data (attrition bias) | High risk | Enrolled 123 (62 salmon, 61 control)<br>At delivery: 107 (87%): 53 (85%) salmon, 54 (89%) control<br>Birth samples: 101 (82%): 51 (82%) salmon, 50 (82%) control<br>6 month clinic visit: 86 (70%): 48 (77%) salmon, 38 (62%) control |
| Selective reporting (reporting bias) | High risk | Trial registered at clinicaltrials.gov as NCT00801502<br>Only limited data were reported on some of the prespecified review outcome. |
| Other bias | Low risk | There is no obvious risk of other bias. |

## Olsen 1992 [6]

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|

| | | |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | 'Women were randomly assigned to the three groups in the ratio 2/1/1. Randomisation was stratified by parity and arranged in balanced blocks of between 8 and 12" |
| Allocation concealment (selection bias) | Low risk | " Sealed, opaque envelope for that study number contained a randomisation number that either identified a particular package of oil capsules or showed that the woman should receive no oil supplement" |
| Blinding of participants and personnel (performance bias) | High risk | While group 1 and 2 were blinded, group 3 received no supplement was unblinded |
| Blinding of outcome assessment (detection bias) | Low risk | Independent evaluation, registry based diagnosis |
| Incomplete outcome data (attrition bias) | Low risk | Randomised 402 (Intervention 266 and Olive oil 136)<br>16 years 399, 99% (Intervention 263 [1 still born, 2 not identified in registry missing DOB], Olive oil 133) |
| Selective reporting (reporting bias) | High risk | Trial registered at ClinicalTrials.gov identifier: NCT01353807<br>Most of the prespecified outcomes were reported in this trial according to their protocol. However, outcomes of interest to the review are not reported completely. Only limited data were reported on some of the prespecified review outcomes. |
| Other bias | Low risk | Placebo group and no oil group were combined in the review. There were no differences in results between the two control groups. |

## Makrides 2010 [7]

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Independently generated randomisation schedule, with balanced variable-sized blocks |
| Allocation concealment (selection bias) | Low risk | Computer driven telephone randomisation service. |
| Blinding of participants and personnel (performance bias) | Low risk | To maintain the blind, both active and placebo capsules are identical in appearance |
| Blinding of outcome assessment (detection bias) | Low risk | Neither the parents nor the research staff members were aware of the treatment allocated. Described as "double-blind" but not defined. |
| Incomplete outcome | Low risk | 681/706 (96.5%) infants (intervention: 357/368, placebo: 324/338) attended their one year medical review and 666/706 (94.3%) infants had |

| | | |
|---|---|---|
| data (attrition bias) | | skin prick tests results. The analysis was by intention to treat. These are subgroups and there is some imbalance between the intervention and the placebo (368 vs 338 respectively). |
| Selective reporting (reporting bias) | Low risk | Trial registered at anzctr.org.au Identifier: ACTRN12605000569606 Prespecified outcomes were reported in this trial according to their protocol. Most of the outcomes of interest to the review are reported. |
| Other bias | Unclear risk | Of the original DOMInO trial (n=2399), a subgroup of mothers whose unborn child had a family history of allergies were included. |


1. Dunstan, J.A., et al., *Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial.* Journal of Allergy and Clinical Immunology, 2003. **112**(6): p. 1178-84.
2. Furuhjelm, C., et al., *Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy.* Acta Paediatrica, 2009. **98**(9): p. 1461-1467.
3. Lauritzen, L., et al., *Fish oil supplementation of lactating mothers affects cytokine production in 2 1/2-year-old children.* Lipids, 2005. **40**(7): p. 669-676.
4. Makrides, M., et al., *Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial.* Journal of American Medical Association, 2009. **301**(2): p. 175-82.
5. Noakes, P.S., et al., *Increased intake of oily fish in pregnancy: effects on neonatal immune responses and on clinical outcomes in infants at 6 mo.* American Journal of Clinical Nutrition, 2012. **95**(2): p. 395-404.
6. Olsen, S.F., et al., *Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration.* Lancet, 1992. **339**: p. 1003-1007.
7. Makrides, M., et al., *Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial.* Journal of American Medical Association, 2010. **304**(15): p. 1675-1683.

## Appendix 2.4 – Characteristics of excluded studies

### Table 2.4 Excluded studies with the reasons for exclusion

| Excluded studies | Reason for exclusion |
|---|---|
| Bergmann 2008 [1] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Bertschi 2005 [2] | Excluded because the intervention was not a n-3 LCPUFA supplementation. |
| Borod 1999 [3] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Carlson 2006 [4] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Colombo 2004 [5] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Courville 2011 [6] | Excluded because of the intervention was not a RCT. |
| D'Vaz 2012 [7] | Excluded because the intervention was not a maternal intervention. |
| Damsgaard 2007 [8] | Excluded because the intervention was not a maternal intervention. |
| de Groot 2004 [9] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Granot 2011 [10] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Hauner 2009 [11] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Helland 2001 [12] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Hesselmar 2010 [13] | Excluded because of the intervention was not a RCT. |
| Innis 2007 [14] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Judge 2007 [15] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Karlsson 2010 [16] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Kitz 2006 [17] | Excluded because the intervention was not a maternal intervention. |
| Knudsen 2006 [18] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Krauss-Etschmann 2007 [19] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Mihrshahi 2001 [20] | Excluded because the intervention was not a maternal intervention. |
| Nwaru 2012 [21] | Excluded because of the intervention was not a RCT. |
| Oien 2010 [22] | Excluded because of the intervention was not a RCT. |
| Pena-Quintana 2011 [23] | Excluded because of the intervention was not a RCT. |
| Ribeiro 2012 [24] | Excluded because the trial does not report allergy outcome of the infants and/or children. |
| Romieu 2007 [25] | Excluded because of the intervention was not a RCT. |
| Salam 2005 [26] | Excluded because of the intervention was not a RCT. |
| Sausenthaler 2007 [27] | Excluded because of the intervention was not a RCT. |

1. Bergmann, R.L., et al., *Supplementation with 200 mg/day docosahexaenoic acid from mid-pregnancy through lactation improves the docosahexaenoic acid status of mothers with a habitually low fish intake and of their infants.* Annals of Nutrition and Metabolism, 2008. **52**(2): p. 157-66.
2. Bertschi, I., et al., *Maternal dietary Alpine butter intake affects human milk: fatty acids and conjugated linoleic acid isomers.* Lipids, 2005. **40**(6): p. 581-7.
3. Borod, E., et al., *Effects of third trimester consumption of eggs high in docosahexaenoic acid on docosahexaenoic acid status and pregnancy.* Lipids, 1999. **34 Suppl**: p. S231.
4. Carlson, S.E., *DHA supplementation and pregnancy outcome (ongoing trial)*, in http://clinicaltrials.gov/ct2/show/NCT002668252006.
5. Colombo, J., et al., *Maternal DHA and the development of attention in infancy and toddlerhood.* Child Development, 2004. **75**(4): p. 1254-67.
6. Courville, A.B., O. Harel, and C.J. Lammi-Keefe, *Consumption of a DHA-containing functional food during pregnancy is associated with lower infant ponderal index and cord plasma insulin concentration.* British Journal of Nutrition, 2011. **106**(2): p. 208-12.
7. D'Vaz, N., et al., *Fish oil supplementation in early infancy modulates developing infant immune responses.* Clinical and Experimental Allergy, 2012. **42**(8): p. 1206-16.
8. Damsgaard, C.T., et al., *Fish oil supplementation modulates immune function in healthy infants.* Journal of Nutrition, 2007. **137**(4): p. 1031-6.
9. de Groot, R.H., et al., *Effect of alpha-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome.* American Journal of Clinical Nutrition, 2004. **79**(2): p. 251-60.
10. Granot, E., et al., *DHA supplementation during pregnancy and lactation affects infants' cellular but not humoral immune response.* Mediators of Inflammation, 2011. **2011**: p. 493925.
11. Hauner, H., et al., *The impact of nutritional fatty acids during pregnancy and lactation on early human adipose tissue development. Rationale and design of the INFAT study.* Annals of Nutrition and Metabolism, 2009. **54**(2): p. 97-103.
12. Helland, I.B., et al., *Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women.* Pediatrics, 2001. **108**(5): p. E82-E82.
13. Hesselmar, B., et al., *Early fish introduction is associated with less eczema, but not sensitization, in infants.* Acta Paediatrica, International Journal of Paediatrics, 2010. **99**(12): p. 1861-7.
14. Innis, S.M. and R.W. Friesen. *Maternal DHA supplementation in pregnancy: a double blind randomized prospective trial of maternal N-3 fatty acid status, human milk fatty acids and infant development*. in *Pediatric Academic Societies Annual Meeting; 2007 May 5-8; Toronto, Canada*. 2007.
15. Judge, M.P., O. Harel, and C.J. Lammi-Keefe, *A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age.* Lipids, 2007. **42**(2): p. 117-22.
16. Karlsson, T., et al., *LC-PUFA supplemented to mothers during pregnancy and breast-feeding improves cognitive performance in their children four years later - an rct study*, in *9th Congress of the ISSFAL; 29 May-2 June* 2010: Maastricht, The Netherlands 2010:113
17. Kitz, R., et al., *Impact of early dietary gamma-linolenic acid supplementation on atopic eczema in infancy.* Pediatric Allergy and Immunology, 2006. **17**(2): p. 112-7.
18. Knudsen, V.K., et al., *Fish oil in various doses or flax oil in pregnancy and timing of spontaneous delivery: a randomised controlled trial.* BJOG: An International Journal of Obstetrics and Gynaecology, 2006. **113**(5): p. 536-43.
19. Krauss-Etschmann, S., et al., *Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial.* American Journal of Clinical Nutrition, 2007. **85**(5): p. 1392-400.

20. Mihrshahi, S., et al., *The childhood asthma prevention study (CAPS): design and research protocol of a randomized trial for the primary prevention of asthma.* Control Clinical Trials, 2001. **22**(3): p. 333-54.
21. Nwaru, B.I., et al., *Maternal intake of fatty acids during pregnancy and allergies in the offspring.* British Journal of Nutrition, 2012. **108**(4): p. 720-32.
22. Oien, T., O. Storrø, and R. Johnsen, *Do early intake of fish and fish oil protect against eczema and doctor-diagnosed asthma at 2 years of age? A cohort study.* Journal of Epidemiology and Community Health, 2010. **64**(2): p. 124-9.
23. Pena-Quintana, L., et al., *Consumption of a dairy product enriched with fish oil during pregnancy maintains the dha status of the mother and increase dha concentration in cord blood.* Journal of Pediatric Gastroenterology and Nutrition, 2011. **52**(Suppl 1): p. e84.
24. Ribeiro, P., et al., *Effect of fish oil supplementation in pregnancy on the fatty acid composition of erythrocyte phospholipids and breast milk lipids.* International Journal of Food Sciences and Nutrition, 2012. **63**(1): p. 36-40.
25. Romieu, I., et al., *Maternal fish intake during pregnancy and atopy and asthma in infancy.* Clinical and Experimental Allergy, 2007. **37**(4): p. 518-25.
26. Salam, M.T., et al., *Maternal Fish Consumption During Pregnancy and Risk of Early Childhood Asthma.* Journal of Asthma, 2005. **42**(6): p. 513-8.
27. Sausenthaler, S., et al., *Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age.* American Journal of Clinical Nutrition 2007. **85**(2): p. 530-7.

## Appendix 2.5 – Characteristics of on-going studies

### Table 2.5: Characteristics of on-going studies

**Bisgaard 2012 [1]**

| | |
|---|---|
| **Study name** | Fish oil supplementation during pregnancy for prevention of asthma, eczema and allergies in childhood: interventional trial in the COPSAC2010 |
| **Methods** | Randomized control trial |
| **Participants** | Pregnant women from week 24 |
| **Interventions** | n-3 fatty acid start from week 24 gestation to 1 week after delivery<br>olive oil start from week 26 gestation to 1 week after delivery |
| **Outcomes** | Primary Outcome Measures: Development of wheezy disorder from 0 to 3 years of age [ Time Frame: 3 years ]<br>Development of eczema from 0 to 3 years of age [ Time Frame: 3 years ]<br>Sensitization at 18 months of age [ Time Frame: 18 months ]<br><br>Secondary Outcome Measures: Development of Asthma exacerbations from 0 to 3 years of age [ Time Frame: 3 years ]<br>Infections from 0 to 3 years of age [ Time Frame: 3 ]<br>Growth [ Time Frame: 0 to 3 years of age ]<br>Cognitive, language and motor development [ Time Frame: 2½ years ] |
| **Starting date** | November 2008 |
| **Contact information** | Hans Bisgaard, MD, DMSc, Copenhagen University Hospital of Copenhagen, Gentofte, Denmark, 2820 |
| **Notes** | ClinicalTrials.gov identifier: NCT00798226 |

**Duchen 2012 [2]**

| | |
|---|---|
| **Study name** | Combined dietary supplementation with lactobacillus reuteri and omega-3 PUFA during pregnancy and postnatally in relation to development of IgE-associated disease during infancy |

| | |
|---|---|
| **Methods** | Randomized control trial |
| **Participants** | Pregnant women |
| **Interventions** | Dietary Supplement: Placebo<br>Dietary Supplement: Omega-3 fatty acids<br>Dietary Supplement: Refined coconut and peanut oil without L. reuteri<br>Dietary Supplement: L. reuteri |
| **Outcomes** | Primary Outcome Measures: IgE associated disease [ Time Frame: 2 years of age ]<br>A food reaction is defined as gastrointestinal symptoms, hives, aggravated eczema or wheezing following ingestion of a certain food with recovery after food elimination from the diet and reoccurrence of symptoms after ingestion of the particular food. Eczema is characterized as reoccurring, itching eczematous and lichenified or nummular dermatitis. Doctor diagnosed wheezing at least three times during the first two years is required for the diagnosis of asthma. If specific positive SPT or serum IgE antibodies is present, the food reaction, eczema in defined as IgE associated.<br>Secondary Outcome Measures: Maternal gastrointestinal function<br>[ Time Frame: 20th gestational week to 6 months post-partum ]<br>Maternal gastrointestinal function will be addressed by validated diary cards. The mothers will record every single stool, stool consistency, and corresponding defecatory symptoms (urgency, straining, and feeling of incomplete evacuation) for seven days at gestational week 25 and 35. Stool consistency will be defined by the Bristol Stool Form Scale. The mothers will also record every meal, and episodes (start and ending time) of abdominal pain and bloating. |
| **Starting date** | March 2012 |
| **Contact information** | Karel M Duchén, MD, PhD, Allergicentrum, Universitetssjukhuset, Linköping, Sweden, 58185. Tel +46-10-103 1355 |
| **Notes** | ClinicalTrials.gov Identifier:NCT01542970 |

**Ramakrishnan 2010 [3]**

| | |
|---|---|
| **Study name** | Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth |
| **Methods** | Randomized control trial |
| **Participants** | Mothers with pregnancy from week 18-22 |
| **Interventions** | 400mg of DHA and Placebo |
| **Outcomes** | Primary Outcome Measures:<br>Birth size and gestational age [ Time Frame: Birth ]<br>[ Designated as safety issue: No ]<br><br>Infant growth and development in the first 5 years of life [ Time Frame: first 5 years of life ] [ Designated as safety issue: No ]<br><br>Secondary Outcome Measures:<br>Immune function and morbidity [ Time Frame: first 6 mo of life ]<br>[ Designated as safety issue: No ] |
| **Starting date** | February 2005 |
| **Contact information** | Dr. Usha Ramakrishnan, Professor, Emory University |
| **Notes** | ClinicalTrials.gov identifier: NCT00646360<br><br>The main study was Ramakrishnan study which Romieu 2008 abstract was published related to allergy outcomes of the infants. The contact author of the abstract replied that requested data could not be given because the study was an ongoing study and there would issues with blinding. |


1. Bisgaard, H., *Fish oil supplementation during pregnancy for prevention of asthma, eczema and allergies in childhood: interventional trial in the COPSAC2010 (Copenhagen studies on asthma in childhood) birth cohort*, in http://clinicaltrials.gov/ct2/show/NCT007982262012.
2. Duchen, K., *Combined dietary supplementation with lactobacillus reuteri and omega-3 PUFA during pregnancy and postnatally in relation to development of IgE-associated disease during infancy*, in http://clinicaltrials.gov/ct2/show/NCT015429702012.
3. Ramakrishnan, U., et al., *Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico.* Food and Nutrition Bulletin, 2010. **31**(2 Suppl): p. S108-S116.

## Appendix 2. 6 - Forest plots for sub group comparisons

**Sub groups –Timing**

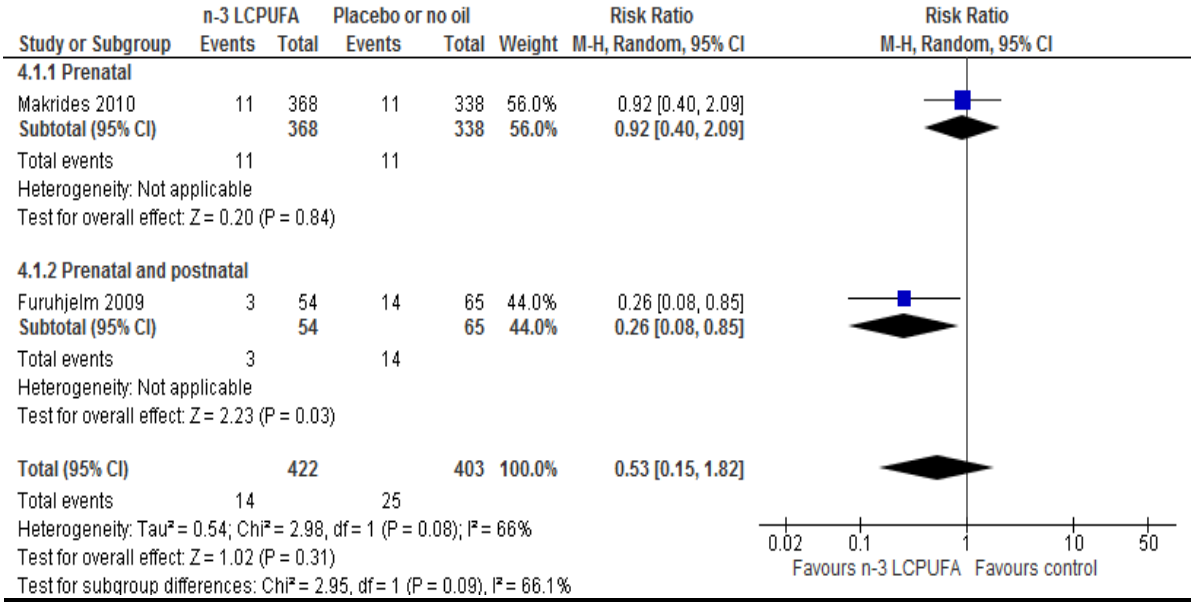


**Figure 2-11: Sub group comparison-Timing of supplementation, IgE mediated Food allergy at any age follow up**

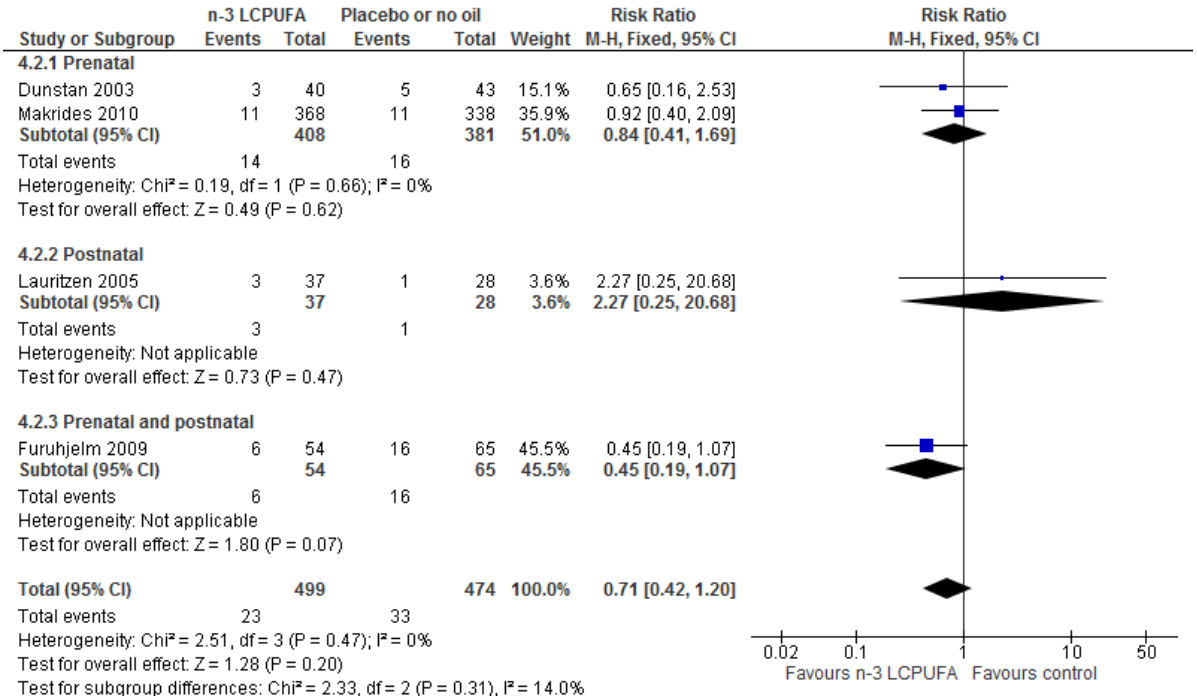


**Figure 2-12: Sub group comparison-Timing of supplementation, food allergy with and without IgE sensitivity at any age follow up**

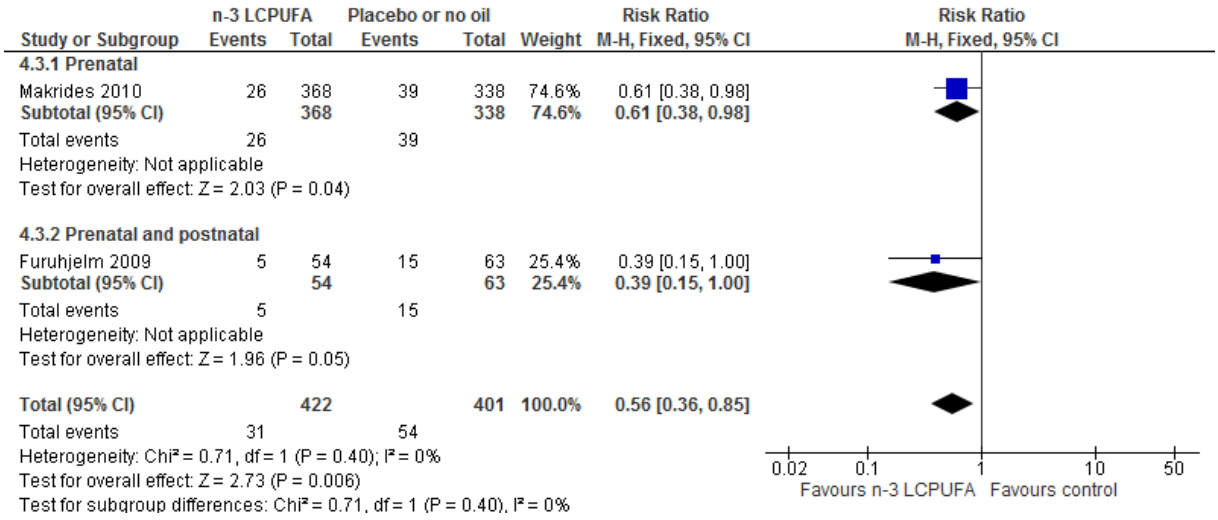


**Figure 2-13: Sub group comparison-Timing of supplementation, IgE mediated Eczema at any age follow up**

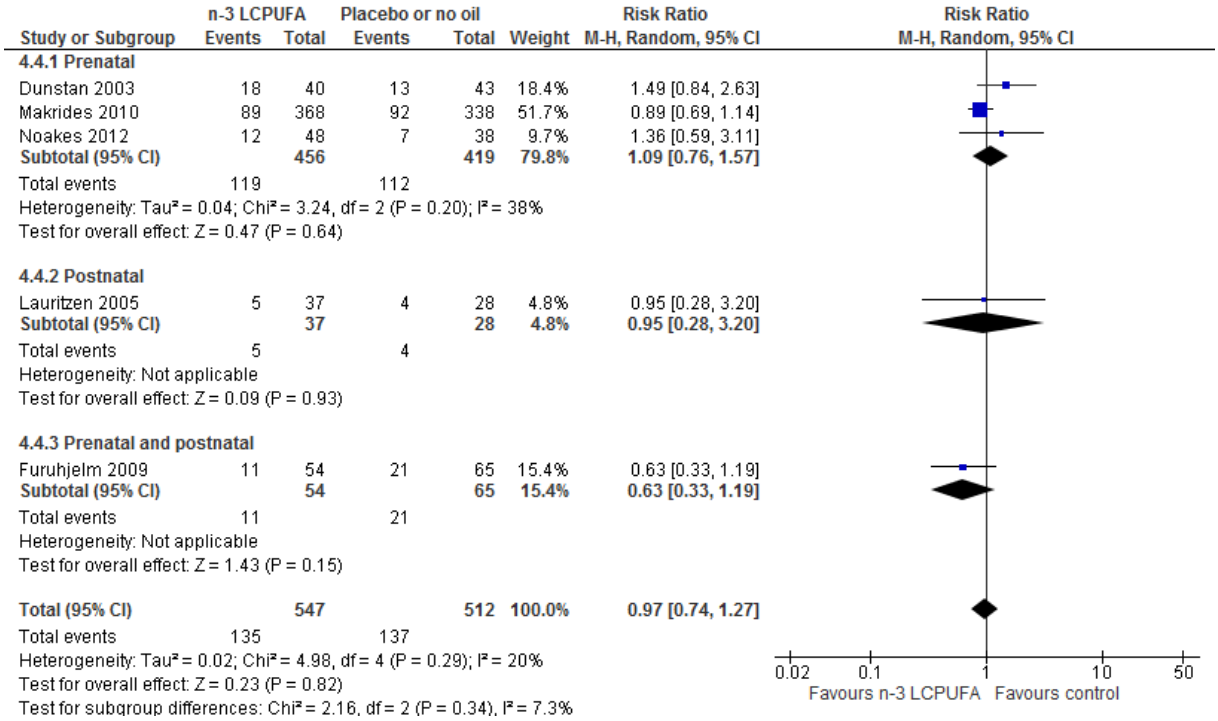


**Figure 2-14: Sub group comparison-Timing of supplementation, eczema with and without IgE sensitivity at any age follow up**

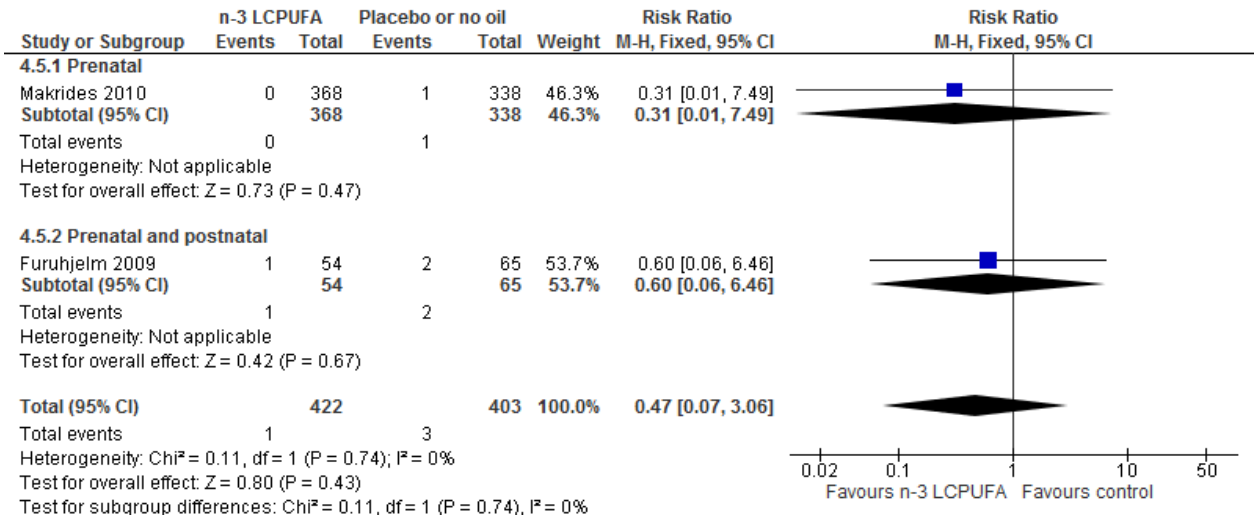


**Figure 2-15: Sub group comparison-Timing of supplementation, IgE mediated Allergic rhinitis at any age follow up**

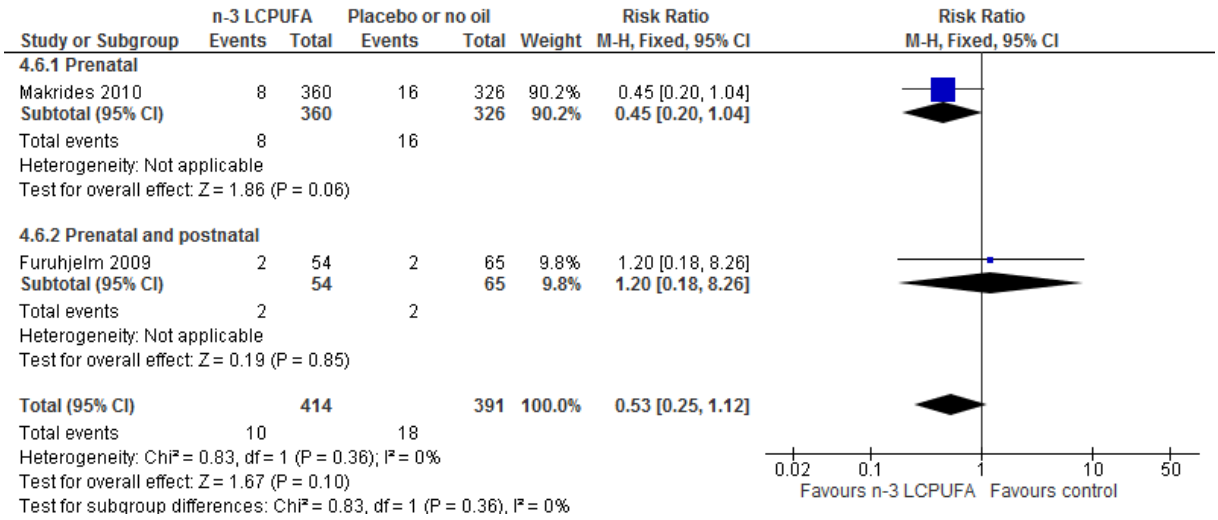


**Figure 2-16: Sub group comparison-Timing of supplementation, allergic rhinitis with and without IgE sensitivity at any age follow up**

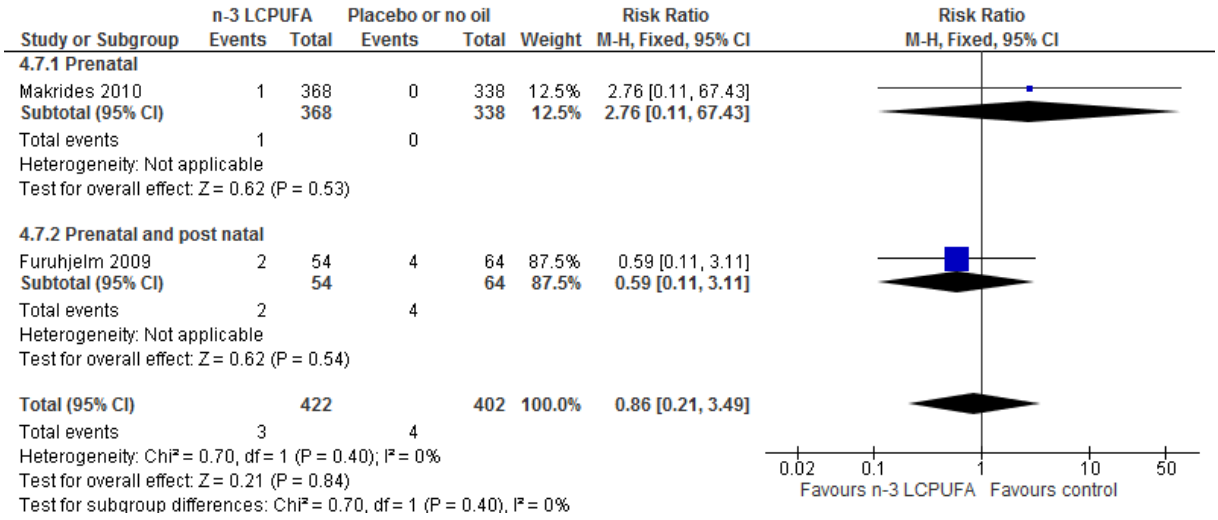


**Figure 2-17: Sub group comparison-Timing of supplementation, IgE mediated Asthma at any age follow up**

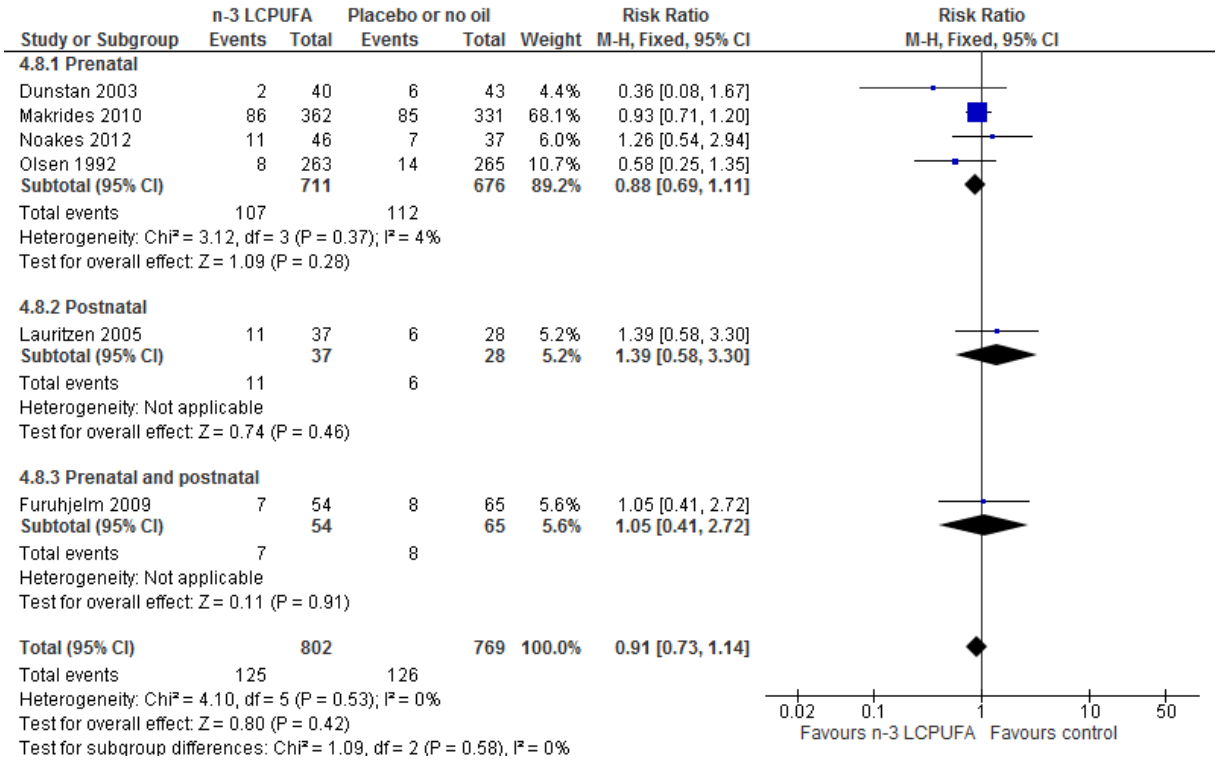


**Figure 2-18: Sub group comparison-Timing of supplementation, asthma with and without IgE sensitivity at any age follow up**

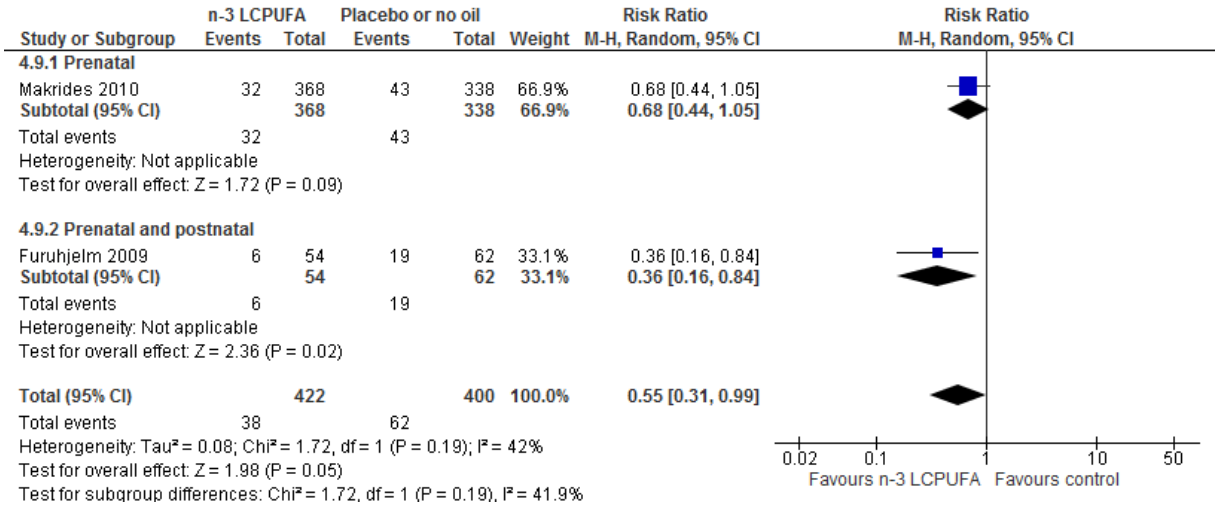


**Figure 2-19: Sub group comparison-Timing of supplementation, IgE mediated any allergies at any age follow up**

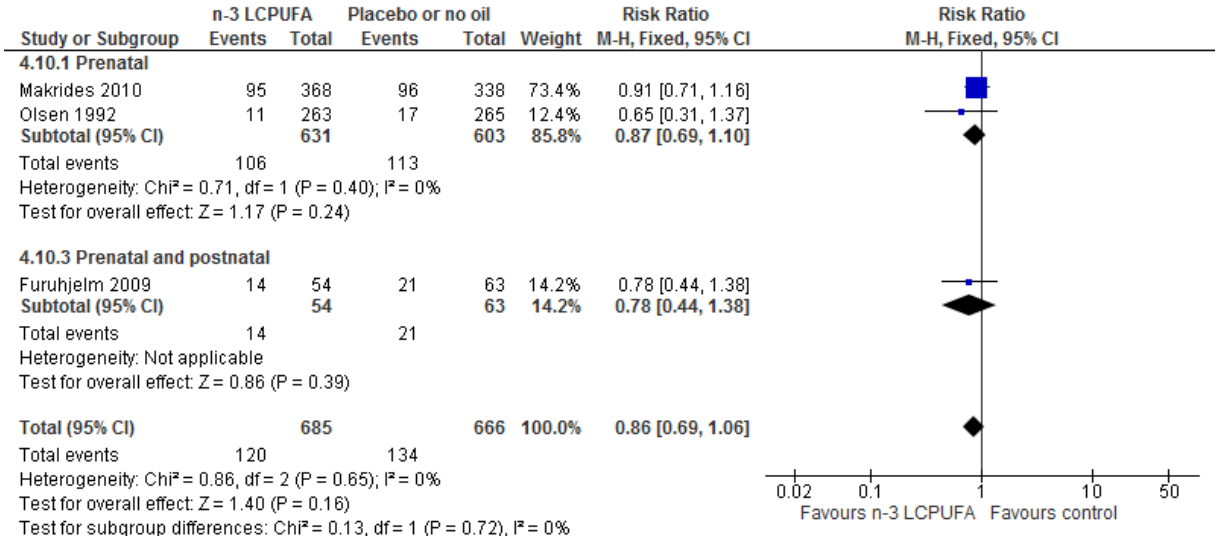


**Figure 2-20: Sub group comparison-Timing of supplementation, any allergies with and without IgE sensitivity at any age follow up**

## Sub groups –Allergy risk

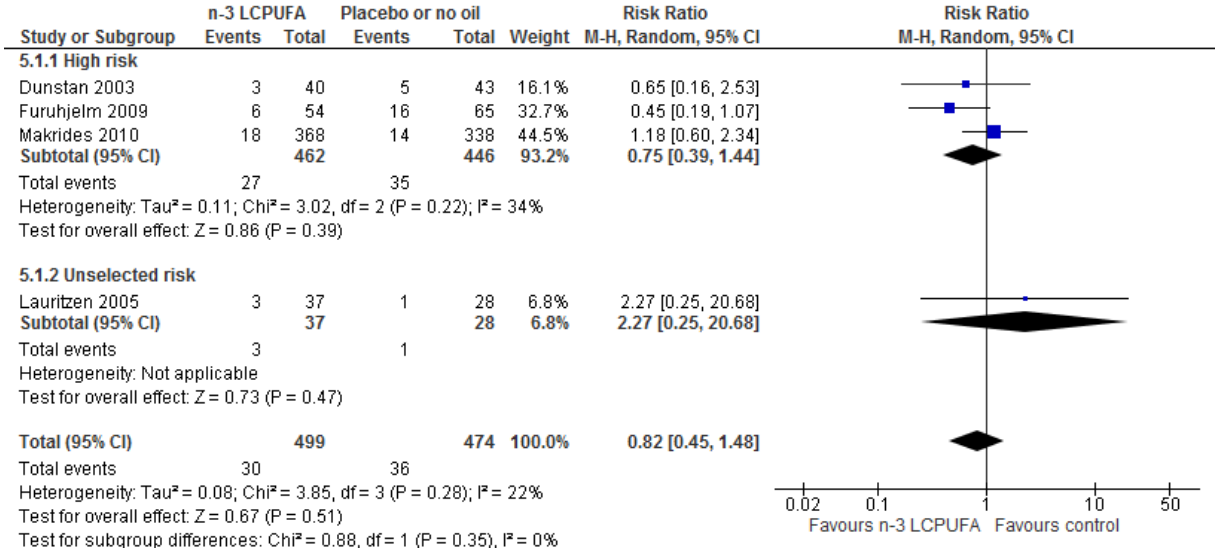


**Figure 2-21: Sub group comparison-high risk vs unselected risk on Food allergy with and without IgE sensitivity at any age follow up**

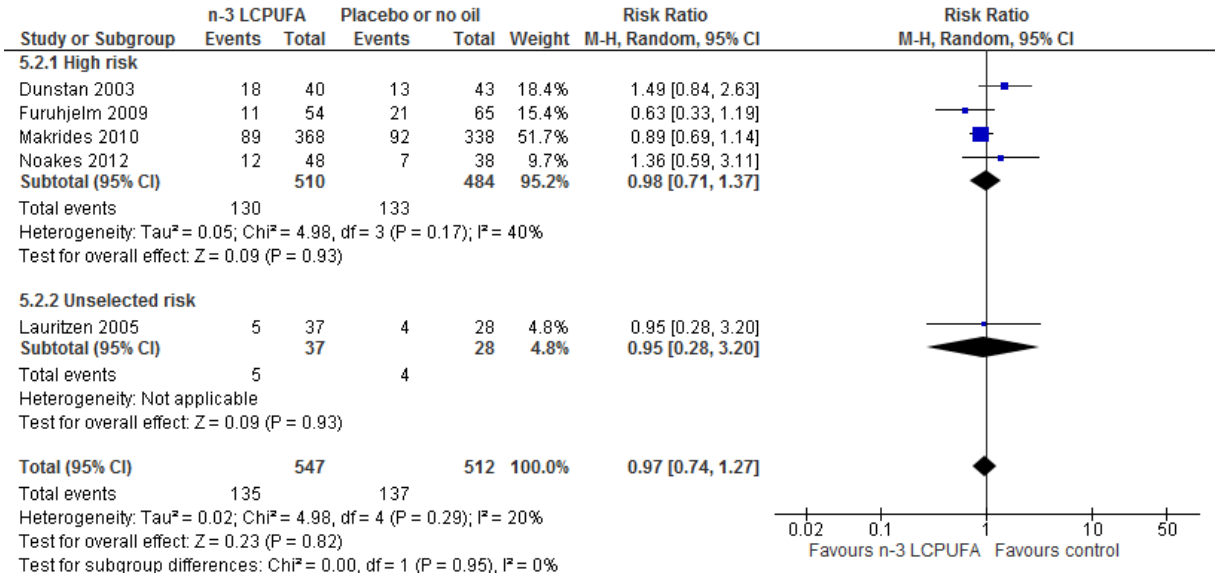


**Figure 2-22: Sub group comparison-high risk vs unselected risk on Eczema with and without IgE sensitivity at any age follow up (fish or fish oil)**

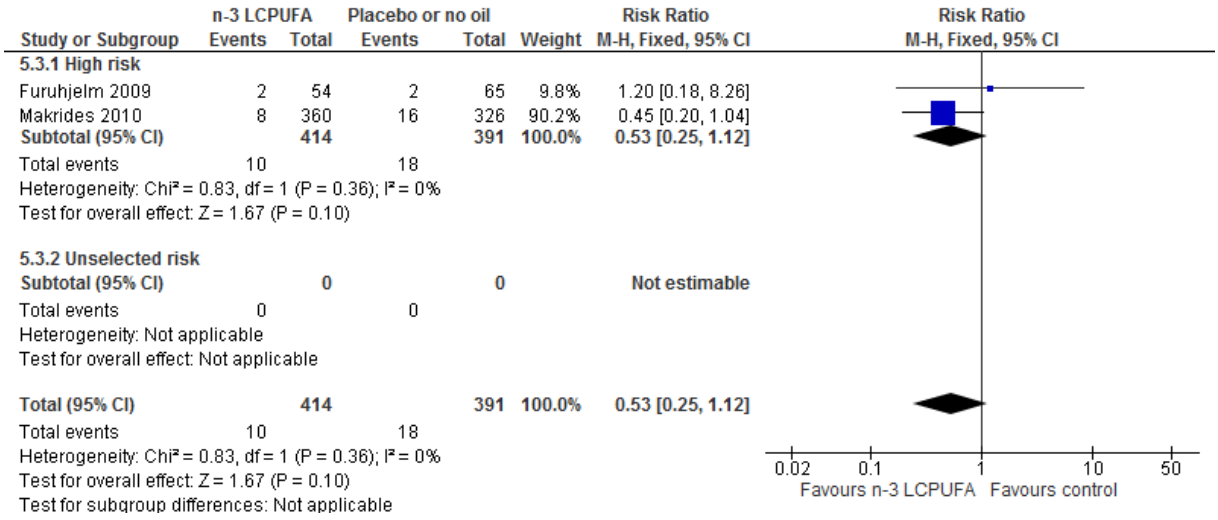


**Figure 2-23: Sub group comparison-high risk vs unselected risk on Allergic rhinitis with and without IgE sensitivity at any age follow up**

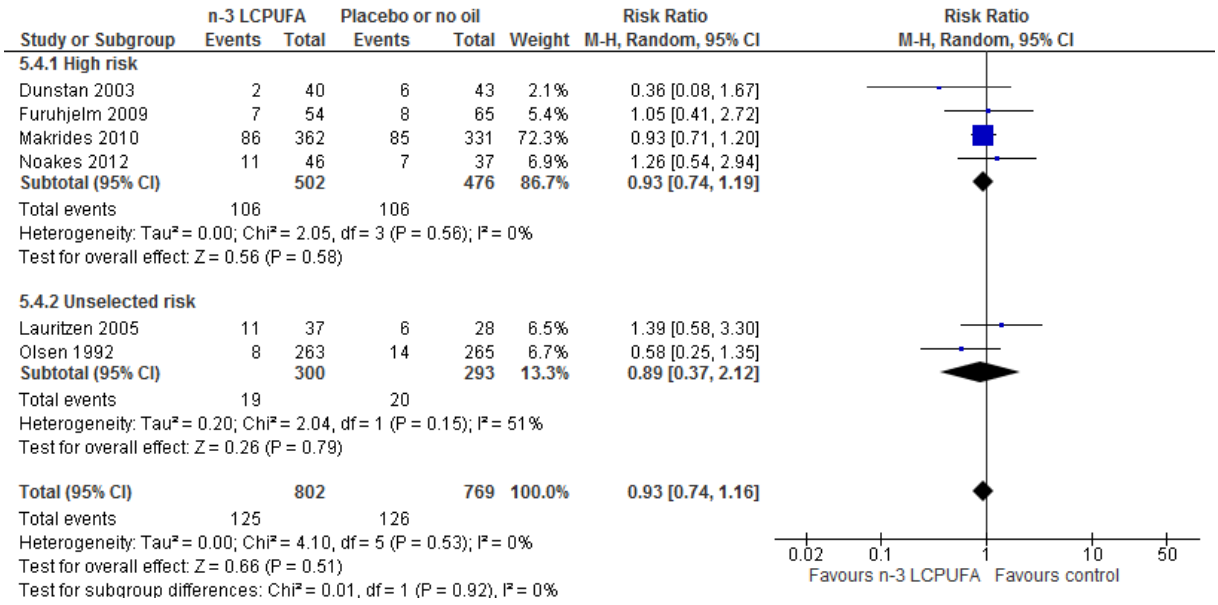


**Figure 2-24: Sub group comparison-high risk vs unselected risk on Asthma/Wheeze with and without IgE sensitivity at any age follow up (fish or fish oil)**

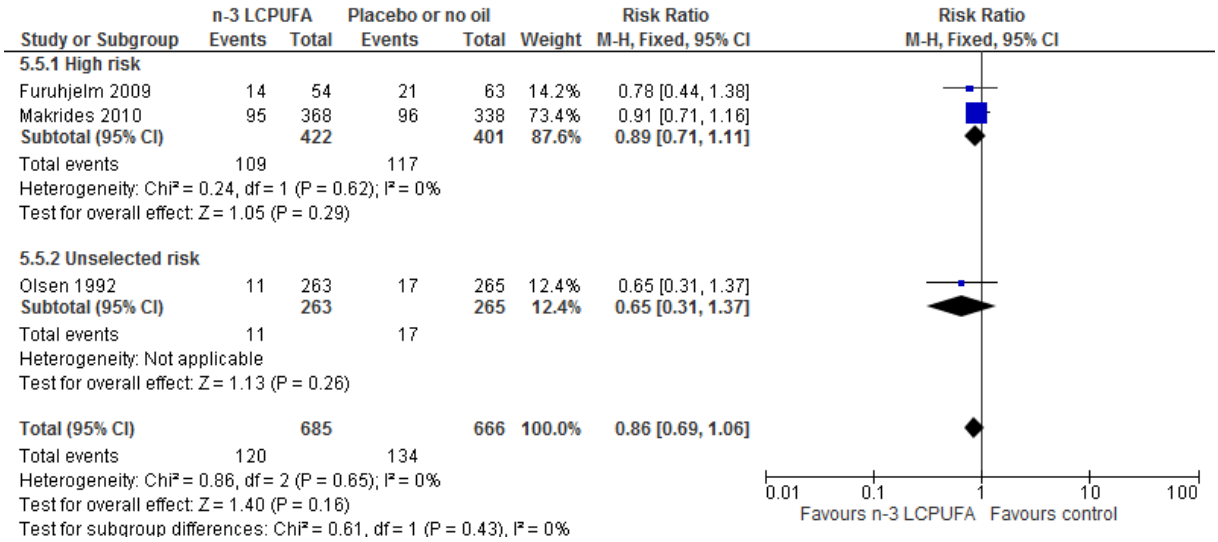


**Figure 2-25: Sub group comparison-high risk vs unselected risk sub group on Any allergy with and without IgE sensitivity at any age follow up**

# Appendices to chapter 3

## Appendix 3.1 –Questions used to collect parental reports of allergies

**The following questions were asked from parents at face to face interviews when children were at 12 and 36 months of age.**

Please record which of the following foods have been introduced and any reactions the baby may have experienced to the food;

1. Have any of these foods been introduced for the first time in the last 6 months?
   Dairy, Egg, Wheat, Fish and Nuts-at 12 months (Some of the foods dropped off the list as the child aged)
   Fish and Nuts - at 36 months
2. Did your baby have an allergic reaction to these foods?
   Dairy, Egg, Wheat, Fish and Nuts-at 12 months (Some of the foods dropped off the list as the child aged)
   Fish and Nuts- at 36 months of age
   (Allergic reactions (within 60 minutes) include: red skin rash on face or body, loose watery stools, hives, vomiting, wheeze or stridor, cough, blood stained stools, swelling of face or body, floppy unresponsive baby)
3. Has your baby experienced any allergic reactions to other solid foods or infant formula?
   (Allergic reactions (within 60 minutes) include: red skin rash on face or body, loose watery stools, hives, vomiting, wheeze or stridor, cough, blood stained stools, swelling of face or body, floppy unresponsive baby)
4. Has your child ever had an itchy rash?
5. Has this itchy rash affected any of the following places? (Cross all that apply)

   □ inside fold of elbows □ behind knees
   □ front of ankles □ behind ears
   □ around eyes □ around neck
   □ under the buttocks □ Other (please specify):

   _____

6. From birth, has a medical doctor ever diagnosed your child as having eczema or atopic dermatitis?
7. Has your child ever had wheezing or whistling in the chest at any time in the past?
8. Has your child ever had a night time cough that was not associated with a cold or chest infection which lasted for more than 3 days?
9. From birth, has a medical doctor ever diagnosed your child as having asthma?
10. Has your child ever had a problem with sneezing, a runny or blocked nose when he/she did not have a cold?

**Appendix 3.2 –Questions used to collect parental reports of allergies**

**The following questions were asked from parents at face to face interviews when children were at 12 and 36 months of age.**

Please record which of the following foods have been introduced and any reactions the baby may have experienced to the food;

1. Have any of these foods been introduced for the first time in the last 6 months?
   Dairy, Egg, Wheat, Fish and Nuts-at 12 months (Some of the foods dropped off the list as the child aged)
   Fish and Nuts - at 36 months
2. Did your baby have an allergic reaction to these foods?
   Dairy, Egg, Wheat, Fish and Nuts-at 12 months (Some of the foods dropped off the list as the child aged)
   Fish and Nuts- at 36 months of age
   (Allergic reactions (within 60 minutes) include: red skin rash on face or body, loose watery stools, hives, vomiting, wheeze or stridor, cough, blood stained stools, swelling of face or body, floppy unresponsive baby)
3. Has your baby experienced any allergic reactions to other solid foods or infant formula?
   (Allergic reactions (within 60 minutes) include: red skin rash on face or body, loose watery stools, hives, vomiting, wheeze or stridor, cough, blood stained stools, swelling of face or body, floppy unresponsive baby)
4. Has your child ever had an itchy rash?
5. Has this itchy rash affected any of the following places? (Cross all that apply)

   □ inside fold of elbows □ behind knees
   □ front of ankles □ behind ears
   □ around eyes □ around neck
   □ under the buttocks □ Other (please specify):

   _____

6. From birth, has a medical doctor ever diagnosed your child as having eczema or atopic dermatitis?
7. Has your child ever had wheezing or whistling in the chest at any time in the past?

8. Has your child ever had a night time cough that was not associated with a cold or chest infection which lasted for more than 3 days?
9. From birth, has a medical doctor ever diagnosed your child as having asthma?
10. Has your child ever had a problem with sneezing, a runny or blocked nose when he/she did not have a cold?

# Appendices to chapter 5

## Appendix 5.1 (a) – Details of ISAAC questions

Details of the ISAAC questions in the ISAAC questionnaire (Appendix 5.1) as follows:

Current eczema is defined as having an itchy rash affecting the characteristic skin areas such as the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes at any time in the past 12 months and the rash starts below 5 years of age in the children being studied. Severe eczema is defined as being kept awake at night by this itchy rash for one or more nights per week [1].

Wheezing, chest tightness, breathlessness and cough symptoms are all used for the self-reported asthma diagnosis, but wheezing is the most recognised symptom for the identification of asthma [2, 3].

Current rhinitis is defined as having a problem with sneezing or a runny or blocked nose, without having a cold or flu in the past 12 months. Current rhino-conjunctivitis is defined as when the sneezing or runny or blocked nose symptoms are accompanied by itchy-watery eyes in the past 12 months. Severe rhinitis is when these symptoms interfered with daily activities and a number of months are affected [4].

In the questionnaire the first 2 questions of each module (Q 1, Q 2, Q 9, Q 10, Q 15, and Q 16 in Appendix 5.1) were designed to obtain the prevalence of allergy symptoms at any time during the child's life and in the last 12 months [5]. Question 7 (Appendix 5.1) in the asthma module was developed to identify exercise induced asthma [5]. Nocturnal cough is known to be one of the main alternative symptoms present in asthma therefore question 8 (Appendix 5.1) was developed [5]. The severity of asthma, allergic rhinitis and eczema symptoms are evaluated using asthma questions 3, 4 and 5, allergic rhinitis questions 12 and 13, and eczema questions 19 and 20 (Appendix 5.1).

The ISAAC questionnaire collects the cumulative incidence of asthma symptoms, allergic rhinitis symptoms and eczema symptoms by using questions 1, 9 and 15 respectively (Appendix 5.1).

Question 6, question 14 and question 21 (Appendix 5.1) collected cumulative incidences of asthma, allergic rhinitis (hay fever) and eczema respectively from 0–7 years which was considered as prior medical diagnosis of these conditions [5, 6].


1. Williams, H.C., *Diagnostic criteria for atopic dermatitis.* Lancet, 1996. **348**(9038): p. 1391-1392.
2. Lee, D.A., et al., *Prevalence and spectrum of asthma in childhood.* British Medical Journal (Clinical research ed.), 1983. **286**(6373): p. 1256-8.
3. Gergen, P.J., D.I. Mullally, and R. Evans, *National survey of prevalence of asthma among children in the United States, 1976 to 1980.* Pediatrics, 1988. **81**(1): p. 1-7.
4. Strachan, D., et al., *Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International Study of Asthma and Allergies in Childhood (ISAAC).* Pediatric Allergy and Immunology, 1997. **8**(4): p. 161-76.
5. Asher, M.I. and S.K. Weiland, *The International Study of Asthma and Allergies in Childhood (ISAAC). ISAAC Steering Committee.* Clinical and Experimental Allergy, 1998. **28 Suppl 5**: p. 52-66; discussion 90-1.
6. Asher, M.I., et al., *International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods.* European Respiratory Journal, 1995. **8**(3): p. 483-91.

## Appendix 5.1 – ISAAC questionnaire

DINO7 ID: _ _ _ _ _ _ _ _ Date: __/__/____ Site: _____

Child sex: _____ Child DOB: __/__/____ Completed by: Mother/Father/Other .....

### International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire

This questionnaire contains a number of statements about symptoms of allergy and asthma. Please read each statement carefully and tick the answer that most applies to your child.

1. **Has your child <u>ever</u> had wheezing or whistling in the chest at any time in the past?**

   Yes ☐ No ☐ (If you have answered "No" please skip to question 6)

2. **Has your child had wheezing or whistling in the chest in the last 12 months?**

   Yes ☐ No ☐ (If you have answered "No" please skip to question 6)

3. **How many attacks of wheezing has your child had in the last 12 months?**

   None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12 ☐

4. **In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?**

   Never woken with wheezing ☐ Less than one night per week ☐ One or more nights per week ☐

5. **In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?**

   Yes ☐ No ☐

6. **Has your child ever had asthma?**

   Yes ☐ No ☐

7. **In the last 12 months, has your child's chest sounded wheezy during or after exercise?**

   Yes ☐ No ☐

8. **In the last 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection?**

   Yes ☐ No ☐

9. **Has you child ever had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or the flu?**

   Yes ☐ No ☐ (If you have answered "No" please skip to question 14)

10. **In the past 12 months, has your child had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or the flu?**

    Yes ☐ No ☐ (If you have answered "No" please skip to question 14)

11. **In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?**

    Yes ☐ No ☐

**12. In which of the past 12 months did this nose problem occur? (please tick any which apply)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| January | ☐ | February | ☐ | March | ☐ | April | ☐ |
| May | ☐ | June | ☐ | July | ☐ | August | ☐ |
| September | ☐ | October | ☐ | November | ☐ | December | ☐ |

**13. In the past 12 months, how much did this nose problem interfere with your child's daily activities?**

Not at all ☐ A little ☐ A moderate amount ☐ A lot ☐

**14. Has your child ever had hay fever?**

Yes ☐ No ☐

**15. Has your child ever had an itchy rash which was coming and going for at least 6 months?**

Yes ☐ No ☐ (If you have answered "No" please skip to question 21)

**16. Has your child had this itchy rash at any time in the last 12 months?**

Yes ☐ No ☐ (If you have answered "No" please skip to question 21)

**17. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?**

Yes ☐ No ☐

**18. At what age did this itchy rash first occur?**

Under 2 years ☐ Age 2–4 ☐ Age 5 or more ☐

**19. Has this rash cleared completely at any time during the last 12 months?**

Yes ☐ No ☐

**20. In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?**

Never in the last 12 months ☐ Less than one night per week ☐ One or more nights per week ☐

**21. Has you child ever had eczema?**

Yes ☐ No ☐

**Thank you very much for your help ☺**

**Appendix 5.2 – CRF Questions to collect medical diagnosis of eczema and asthma**

| | | | |
|---|---|---|---|
| **4** | **Medical conditions?** | ☐ Yes<br>☐ No | Within the last 5 years has your child been diagnosed by a doctor with any medical conditions such as eczema and asthma? |
| **4.1** | **If yes, specify** | **Eczema**<br>☐ Yes<br>☐ No | |
| **4.2** | | **Asthma**<br>☐ Yes<br>☐ No | |

**Appendix 5.3 – DINO7 CRF Questions to collect background, home environment, family health and child information**

***Family Structure***

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| **1.** | **Family Structure** | ☐ Adopted<br>☐ Fostered<br>☐ Intact family (Natural parents living together)<br>☐ New marriage /de facto relationship<br>☐ Other, specify<br>_____<br>_____<br>☐ Separated Parents (Divided Care)<br>☐ Sole Parent - Mother<br>☐ Sole Parent - Father | Which of the following statements best describes the structure of the child's family? That is the situation within the home in which the child spends the majority of time. |
| **2.** | **Number of Carers** | __ | Maximum two. Information regarding education and occupation will be collected for the carers.<br>In a traditional family arrangement, this will be the mother and father.<br>The primary carer is the person who has the major responsibility for the care of the child. In most |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | | cases the primary carer will be the mother of the child. If equally in the care of the mother and father, parents to identify primary carer, the default primary carer is the mother.<br><br>The secondary carer is the person who, after the primary carer, takes major responsibility for the care of the child. In the majority of cases the secondary carer will be the father of the child. The child may live with the secondary carer all the time or only some of the time.<br><br>If the child has no secondary carer (e.g. in a single parent family) then only enter one carer. |

**Primary Carer Education/Occupation**

- Information collected in this section relates to the environmental influences on the child's development.
- The primary carer is the person who has the major responsibility for the care of the child. In most cases the primary carer will be the mother of the child. If equal time with both mother and father, parents to identify primary carer, default is mother.

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | **Primary carer Yes** | | This section is automatically completed from earlier answer |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | **Other carer number** **__** | | – **do not change.** |
| **1** | **Relationship to the child** | ☐ Aunt<br>☐ Brother<br>☐ Father<br>☐ Godfather<br>☐ Godmother<br>☐ Grandfather<br>☐ Grandmother<br>☐ Mother<br>☐ Other<br>_____<br>☐ Sister<br>☐ Stepfather<br>☐ Stepmother<br>☐ Uncle | The primary carer is the person who has the major responsibility for the care of the child. In most cases the primary carer will be the mother of the child. If equal time with both mother and father, parents to identify primary carer, default is mother.<br>The primary carer has been identified and recorded on the MIS. We don't necessarily want to ask the same question again. But do need to check that the data we are collecting in this section relates to the primary carer already recorded. |
| **2** | **In full time care of Primary Carer?** | Fulltime ☐ Yes *Go to Q3*<br>☐ No *Go to Q2.1* | Is the child under fulltime care with the primary carer? Fulltime is defined as 7 days per week. |
| ***2.1*** | **Days in Care (per** | __ __ Days in care (per | On average, how many days is the child usually in the care of |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | **fortnight)** | fortnight) | the primary carer per fortnight? |
| **3** | **Date of birth of carer** | _ _/_ _ /_ _ _ _<br><br>☐ Unknown | Enter the carer's date of birth. Enter as DD/MM/YYYY<br>If date of birth of primary carer is unknown, leave the DD/MM/YYY at the default value and select 'Unknown'. |
| **4** | **Secondary School completion** | ☐ Yes Go to Q5<br>☐ No<br>☐ Unknown Go to Q5 | Completion of secondary school equates to the completion of the final year of schooling. |
| ***4.1*** | **If No, highest year level achieved** | __ __<br><br>☐ Unknown | If the highest available year of secondary school was not completed, enter the highest year level of secondary school that was completed. |
| **5** | **Completion of further study** | ☐ Yes<br>☐ No go to Q6<br>☐ Unknown Go to Q6 | Since leaving Secondary School, has any further study been completed? |
| ***5.1*** | **Years of further education** | __ __ Years<br><br>__ __ Months | How many years of fulltime, formal, further education have been completed? This includes any diploma, degrees, certificates obtained at a |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | ☐ Unknown go to Q5.2 | registered educational facility. If part time study was undertaken, estimate the full time equivalent. If completed an apprenticeship, count the full years of the apprenticeship e.g. 3 year apprenticeship is considered 3 years and 0 months. If 'unknown' go to 5.2 as may not know the number of years but know the level attained. |
| **5.2** | **Highest level attained** | ☐ Certificate/Diploma<br>☐ Degree<br>☐ Higher Degree<br>☐ Other, specify<br>_____<br>☐ Unknown | The education level specified should be the highest level completed. Please select only one. A trade or apprenticeship is classified as a certificate/diploma. |
| **6** | **Currently employed** | ☐ Yes<br>☐ No<br>☐ Unknown | Is the primary carer currently employed?<br>• if "yes", go to 6.1<br>• if "no", go to *'Usual occupation'* under 6.1<br>• if "unknown", go to next section 1.3 (*Other Carer*) |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| *6.1* | **Employer Name** | _____ | Enter the name of the employer? This can assist with classification of occupation; then complete *'Usual occupation'* |
| | **or Employer Unknown/ Self Employed** | ☐ Unknown<br>☐ Self Employed | Specify if employer is unknown or if self employed; then complete *'Usual occupation'* |
| | **Usual Occupation** | _____<br>☐ None | 1. If currently employed complete *'Usual occupation'*<br>2. If the answer to Q6 is 'No', ask length of time not in paid employment:<br>• If ≤12 months enter occupation prior to leaving workforce in 'Usual occupation'. Enter the occupation title including the industry employed in, for example: Sales Manager, Retail Clothing Outlet.<br>• If >12 months and student enter 'student' in *'Usual occupation'* and tick box |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | | *'none'*.<br>• If >12 months and 'home duties' enter 'Home duties' in *'Usual occupation'* and tick box *'none'.* To meet criteria for 'home duties' following must apply: not in paid employment, not a student, not actively looking for work and chooses to be at home.<br>• If >12 months and permanently unable to work (disability pension) enter same.<br>• If >12 months and not 'home duties' (according to definition above) or 'student' or 'permanently unable to work' enter 'Unemployed >12 months' in '*Usual occupation'* and tick box '*none'* |
| ***6.2*** | **Main tasks performed** | _____<br>_____<br>_____<br>_____<br>_____ | To enable classification of occupation, list main tasks performed in the occupation to describe the level of responsibility. |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | _____<br><br>☐ Unknown | For any of the above >12 months not in the labour force categories , do not complete A6.2 |

**Other Carer (2) Education/Occupation**

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | **Primary carer No**<br>**Other carer number 2** | | This section is automatically completed from earlier answer – **do not change.** |
| **1** | **Relationship to the child** | ☐ Aunt<br>☐ Brother<br>☐ Father<br>☐ Godfather<br>☐ Godmother<br>☐ Grandfather<br>☐ Grandmother<br>☐ Mother<br>☐ Other<br>_____<br>☐ Sister<br>☐ Stepfather<br>☐ Stepmother | The secondary carer of the child is the person who, after the primary carer, takes major responsibility for the care of the child. In the majority of cases the secondary carer will be the father of the child. The child may live with the secondary carer all the time (e.g. intact family living together) or only some of the time (e.g. separated parents).<br><br>If the child has no secondary carer (e.g. in a single parent family) then do not complete |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | ☐ Uncle | this section. |
| **2** | **Days in Care (fulltime)** | Fulltime to Q3 ☐ Yes Go<br>☐ No | Is the child under fulltime care of the 'other' carer? Fulltime is defined as 7 days per week. |
| ***2.1*** | **Days in Care (per fortnight)** | __ __ Days in care (per fortnight) | On average, how many days is the child usually in the care of the other carer per fortnight? |
| **3** | **Date of birth of carer** | _ _/_ _ /_ _ _ _<br>☐ Unknown | Enter the carer's date of birth. Enter as DD/MM/YYYY<br>If date of birth of Secondary carer is unknown, leave the DD/MM/YYY at the default value and select 'Unknown'. |
| **4** | **Secondary School completion** | ☐ Yes Go to Q5<br>☐ No<br>☐ Unknown Go to Q5 | Completion of secondary school equates to the completion of the final year of schooling. |
| ***4.1*** | **If No, highest year level achieved** | __ __<br>☐ Unknown | If the highest available year of secondary school was not completed, enter the highest year level of secondary school that was completed. |
| **5** | **Completion of** | ☐ Yes | Since leaving Secondary |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | **further study** | ☐ No Go to Q6<br>☐ Unknown Go to Q6 | School, has any further study been completed? |
| ***5.1*** | **Years of further education** | __ __ Years<br>__ __ Months<br>☐ Unknown go to Q5.2 | How many years of fulltime, formal, further education have been completed? This includes any diploma, degrees, certificates obtained at a registered educational facility. If part time study was undertaken, estimate the full time equivalent. If completed an apprenticeship, count the full years of the apprenticeship e.g. 3 year apprenticeship is considered 3 years and 0 months. If 'unknown' go to 5.2 as may not know the number of years but know the level attained. |
| **5.2** | **Highest level attained** | ☐ Certificate/Diploma<br>☐ Degree<br>☐ Higher Degree<br>☐ Other, specify<br>_____<br>☐ Unknown | The education level specified should be the highest level completed. Please select only one. A trade or apprenticeship is classified as a certificate/diploma. |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | | |
| **6** | **Currently employed** | ☐ Yes<br>☐ No<br>☐ Unknown | Is the secondary carer currently employed?<br>• if "yes", go to 6.1<br>• if "no", go to *'Usual occupation'* under 6.1<br>• if unknown, go to next section 1.4 (*Home Environment*) |
| ***6.1*** | **Employer Name** | _____<br>____ | Enter the name of the employer? This can assist with classification of occupation; then complete *'Usual occupation'* |
| | **or Employer Unknown/ Self Employed** | ☐ Unknown<br>☐ Self Employed | Specify if employer is unknown or if self employed; then complete *'Usual occupation'* |
| | **Usual Occupation** | _____<br>_____ | 3. If currently employed complete *'Usual occupation'*<br>4. If the answer to Q6 is 'No', ask length of time not in paid employment: |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | ☐ None | • If ≤12 months enter occupation prior to leaving workforce in 'Usual occupation'. Enter the occupation title including the industry employed in, for example: Sales Manager, Retail Clothing Outlet.<br>• If >12 months and student enter 'student' in *'Usual occupation'* and tick box *'none'*.<br>• If >12 months and 'home duties' enter 'Home duties' in *'Usual occupation'* and tick box *'none'*. To meet criteria for 'home duties' following must apply: not in paid employment, not a student, not actively looking for work and chooses to be at home.<br>• If >12 months and permanently unable to work (disability pension) enter same.<br>• If >12 months and not 'home duties' (according to |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | | definition above) or 'student' or 'permanently unable to work' enter 'Unemployed >12 months' in '*Usual occupation'* and tick box '*none'* |
| *6.2* | **Main tasks performed** | _____<br>_____<br>_____<br>_____<br>_____<br>☐ Unknown | To enable classification of occupation, list main tasks performed in the occupation to describe the level of responsibility.<br>• For any of the above >12 months not in the labour force categories , do not complete A6.2 |

**Home Environment**

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| **1** | **Is English the primary language spoken at home?** | ☐ Yes<br>☐ No<br>Specify<br>_____<br>_____ | Enter the primary language to which the child is exposed to the majority of the time. If two languages are spoken, enter the language spoken the majority of the time. |
| **2** | **Including parents, how many adults live in the home (≥16** | ___ | Include parents and siblings ≥16 years of age. Include any other adults living in the home. |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | **y.o.)?** | | |
| **3** | **How many other children live in the home?** | ___ ___ | Children must be <16 years of age.<br>Do not include THIS child.<br>Include siblings, step siblings extended family or friends that live the home ≥ 4 days per fortnight (on average).<br>Combine number of children from both homes if applicable. |
| **4** | **What is the position, by age, of the child in the family?** | ___ ___<br>☐ Unknown | Include all children that live in the home i.e. step children, foster children, and any other extended family children that live in the home ≥ 4 days per fortnight. For e.g., step child 1 is 14, step child 2, is 12, older sister 1, is 10, this child is 7 then this child's age position 4.<br>Multiple births to be recorded in the order delivered. |
| **5** | **Are there any pets in or around the home?** | ☐ Yes<br>☐ No Go to Q6 | Are there any pets currently living either inside or outdoors of the family home? Include both homes in the case of divided care. i.e. If there are pets in any home that the child |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | | spends ≥ 4 days per fortnight. |
| ***5.1*** | **If yes, specify** | ☐ Dog<br>☐ Cat<br>☐ Bird<br>☐ Fish<br>☐ Reptile (Lizard, turtle)<br>☐ Guinea Pig<br>☐ Mouse<br>☐ Rat<br>☐ Rabbit<br>☐ Other, specify<br>_____<br>____ | Enter all that apply.<br>1 Indicate which pets by crossing all that apply from the list, and specify any pets in the space provided if they are not on the list. |
| **6** | **Is there a free standing gas heater without a chimney in your home?** | ☐ Yes<br>☐ No<br>☐ Unknown | 2 Ask whether the family home is heated with a free-standing gas heater without a chimney or flue. The flue is a pipe which directs the flow of gases and water vapours from the burning of gas fuel to the outside air. These pipes are sometimes quite discreet.<br>3 Portable gas heaters do not have a flu.<br>Include any home that the child |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | | | spends ≥ 4 days per fortnight. |
| 7 | **Does anyone living in the family home smoke cigarettes?** | ☐ Yes<br>☐ No | 4 Indicate if anyone living in the family home smokes cigarettes.<br>Include any home that the child spends ≥ 4 days per fortnight. |
| *7.1* | **If yes, specify who.**<br>**Primary carer?** | ☐ Yes<br>☐ No | Indicate whether the primary carer of the child is a smoker. |
| | **How many other people smoke in the home?** | ☐ 1 person (other than primary carer)<br>☐ 2 people (other than primary carer)<br>☐ 3 or more people (other than primary carer)<br>☐ None<br>☐ Unknown | 5 Indicate how many other adults living in the family home smoke cigarettes.<br>Include any home that the child spends more than 4 days per fortnight. |

**Family Health Information**

| No. | Question | Response Options | Completion Instructions |
|---|---|---|---|
| 1 | **Has the mother of this child ever been medically diagnosed with asthma?** | ☐ Yes<br>☐ No<br>☐ Unknown | This relates to the biological mother.<br>Requires a medical diagnosis. Self diagnosis, or diagnosis by |

| No. | Question | Response Options | Completion Instructions |
|---|---|---|---|
| | | | alternative health practitioners is not recoded. Herbal remedies, would be recorded as a 'NO' response. |
| **2** | **Has the mother of this child ever been medically diagnosed with eczema?** | ☐ Yes<br>☐ No<br>☐ Unknown | This relates to the biological mother.<br>Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded as a 'NO' response. |
| **3** | **Has the mother of this child ever been medically diagnosed with hay fever?** | ☐ Yes<br>☐ No<br>☐ Unknown | This relates to the biological mother.<br>Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded as a 'NO' response. |
| **4** | **Has the father of this child ever been medically diagnosed with asthma?** | ☐ Yes<br>☐ No<br>☐ Unknown | This relates to the biological father.<br>Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded |

| No. | Question | Response Options | Completion Instructions |
|---|---|---|---|
| | | | as a ‘NO’ response. |
| **5** | **Has the father of this child ever been medically diagnosed with eczema?** | ☐ Yes<br>☐ No<br>☐ Unknown | This relates to the biological father.<br>Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded as a ‘NO’ response. |
| **6** | **Has the father of this child ever been medically diagnosed with hay fever?** | ☐ Yes<br>☐ No<br>☐Unknown | This relates to the biological father.<br>Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded as a ‘NO’ response. |
| **7** | **Are there any other siblings related to this child that have ever been medically diagnosed with asthma?** | ☐ Yes<br>☐ No<br>☐ Unknown<br>☐Not Applicable | Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded as a ‘NO’ response.<br>Include any biological children of the mother or the father.<br>If there are no siblings, enter |

| No. | Question | Response Options | Completion Instructions |
|---|---|---|---|
| | | | ‘not applicable’ |
| **8** | **Are there any other siblings related to this child that have ever been medically diagnosed with eczema?** | ☐ Yes<br>☐ No<br>☐ Unknown<br>☐Not Applicable | Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded as a ‘NO’ response.<br>Include any biological children of the mother or the father.<br>If there are no siblings, enter ‘not applicable’ |
| **9** | **Are there any other siblings related to this child that have ever been medically diagnosed with hay fever?** | ☐ Yes<br>☐ No<br>☐ Unknown<br>☐Not Applicable | Requires a medical diagnosis. Self diagnosis, or diagnosis by alternative health practitioners is not recoded. Herbal remedies, would be recorded as a ‘NO’ response.<br>Include any biological children of the mother or the father.<br>If there are no siblings, enter ‘not applicable’ |

**Child Information**

**Diet**

| No. | Question | Response options | Completion instructions |
|---|---|---|---|

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| 1 | **Fish meals within last month** | __ __ meals<br>☐ No Fish<br>☐ Unknown | Within the last month, how many fish meals did your child have?<br>A fish meal is defined as 60 to 80 grams of fish. This would equate to one small can of tuna or 4 fish fingers. |
| 2 | **DHA enriched foods within last month** | __ __ times<br>☐ No DHA enriched foods<br>☐ Unknown | Within the last month, how many times did your child consume DHA enriched foods?<br>The intent of the question is to determine the frequency of consumption, not the number of serves.<br>Examples:<br>1 or more slices of DHA enriched bread for breakfast every morning = 30 times /month<br>1 or more slices of bread and 1 or more DHA enriched eggs for breakfast once a week = 8 times/month.<br>Please refer to the list of DHA enriched foods. |
| 3 | **Vitamin/mineral** | ☐ Yes | Within the last month has |

| No. | Question | Response options | Completion instructions |
|---|---|---|---|
| | **supplement containing DHA within last month** | ☐ No<br>☐ Unknown | your child regularly (at least 3 days per week) taken a vitamin/mineral supplement containing DHA?<br>Please refer to the list of vitamins/Supplements containing fish oil. |

**Appendix 5.4 -DINO7 Statistical analysis plan**


# DINO7 STATISTICAL ANALYSIS PLAN

## CONFIDENTIAL

| | |
|---|---|
| Study name | DINO7 |
| NHMRC ID | 508003 |
| Analysis plan authors | Anoja W Gunaratne (PhD student) |
| | Thomas Sullivan (Statistician) |
| | Carmel Collins (Principal Investigator) |
| Version | 1 |
| Date | 06/08/2013 |
| Approved by | Name: Date: |

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## ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| SAP | Statistical analysis plan |
| DHA | Docosahexaenoic acid |
| CRF | Case report form |
| GA | Gestational age |
| ISAAC | International Study of Asthma and Allergies in Childhood |
| ITT | Intention to treat |
| BMI | Body mass index |
| GEE | Generalised estimating equation |
| WHO | World Health Organization |

## 1. PREFACE

This statistical analysis plan describes the planned analyses for the allergy outcomes in the DINO7 study. This component of the DINO7 study is being completed to assess whether high-dose dietary docosahexaenoic acid (DHA), given to infants born less than 33 weeks gestation from within 5 days of commencing enteral feeds up to term equivalent, impacts on allergy outcomes at seven years corrected age. The following documents were reviewed in preparation of this SAP:

DINO7 Trial Protocol (Version 6 10/08/2011).

DINO7 Case Report Form (CRF) and other data collection tools

Questions 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3 and 3.1, 3.2.

DINO Trial Protocol (15/04/2003)

DINO CRF

International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire.

ICH Guidance on Statistical Principles for Clinical Trials.

## 2. STUDY OBJECTIVES and OUTCOMES

### 2.1 Study Objectives

DINO7 is a follow-up of infants enrolled in the DINO Trial. DINO, which stands for *Docosahexaenoic Acid for the Improvement of Neurodevelopmental Outcome in Preterm Infants,* was designed to assess the effect of providing 1% of dietary fats as DHA to preterm infants born <33 weeks gestation on neurodevelopment at 18 months corrected age. The primary objective of the DINO7 allergy follow-up study is to assess allergy outcomes at seven years corrected age in these same children.

### 2.2 Outcome Variables

The primary outcomes are allergy prevalence at 1) 7 years of age and 2) birth to 7 years of age. This includes

- parental reports of asthma, allergic rhinitis, rhino-conjunctivitis, eczema

- and severity of asthma, allergic rhinitis, eczema.

## 3. STUDY METHODS

### 3.1 Overall Study Design and Plan

Seven year follow-up of participants enrolled in a randomised controlled, blinded five-centre trial.

### 3.2 Method of Treatment Assignment and Randomisation

Infants were assigned to receive a standard DHA diet or a high DHA diet using an independently generated computer-based telephone randomisation service. Centre, birth weight and infant sex, as defined in the table below, were used to stratify the randomisation. Infants from a multiple birth were treated as a single randomisation unit, with stratification performed according to the sex and weight of the firstborn infant.

| **Stratification Variable** | **Categories** |
|---|---|
| Centre | Women's and Children's Hospital<br>Flinders Medical Centre<br>King Edward Memorial Hospital<br>Royal Women's Hospital<br>Royal Brisbane Women's Hospital |
| Infant sex | Male<br>Female |
| Birth weight | <1250g<br>≥1250g |

### 3.3 Treatment Masking (Blinding)

Participants, clinicians and research staff in the original DINO trial were blinded to treatment group allocation. The standard DHA and high DHA capsules were identical in size, shape and colour. To facilitate blinding each treatment group was separately color coded into 2 groups. If preterm formula was required ready-to-feed high and standard DHA content formula was packaged according to the color codes. The blinding was broken by the statistician during the analysis of DINO results; however trial staff and investigators were not aware of individual group allocations and did not directly interact with participants or staff responsible for clinical and psychological examinations in DINO7. Participating mothers were able to request details of group allocation at any stage during follow-up. The number of mothers requesting to be unblinded will be reported as a post-randomisation characteristic (see Section 5.3).

## 4. General issues for statistical analysis

### 4.1 Analysis Software

All analyses will be performed using SAS® version 9.3 or later, SAS® Enterprise Guide version 5.1 or later, and Stata Release 12 or later.

### 4.2 Analysis Approach

Participants will be analysed according to the treatment they were randomised to receive.

### 4.3 Methods for Withdrawals, Missing Data and Outliers

Data collected on children and their families up until the time of withdrawal will be included in the statistical analysis. Following withdrawal, data will still be collected and used where permission has been obtained to do so.

Outliers will be queried during data collection and the statistical analysis. Unless confirmed as a data entry error, outliers will not be excluded from the primary analysis.

### 4.4 Protocol Violations and Deviations

No subjects will be excluded from the analyses due to protocol deviations.

## 4.5 Data Transformations

No data transformations are planned. The statistical analyses detailed in Section 6 are based on assumptions about the distribution of the outcomes. Should these assumptions turn out to be invalid, data transformations may be required. Data transformations are not planned to correct for departures from normality, since the sample size is sufficient for the central limit theorem to apply (Lumley et al., 2002).

## 4.6 Potential Confounders

In order to address each hypothesis, both unadjusted and adjusted analyses will be performed. The adjusted analyses will be used to draw conclusions about the effect of treatment, with unadjusted analyses performed for completeness and to confirm the results of the adjusted analyses.

The Committee for Proprietary Medicinal Products (CPMP, 2004) state that stratification variables should generally be adjusted for in the primary analysis, regardless of their effect on the outcome. Recently it has been shown that stratification leads to positive correlation between treatment groups; failure to adjust for stratification variables in the analysis biases standard error estimates for the treatment effect upwards (Kahan and Morris, 2012). We therefore chose to adjust for stratification variables and to draw conclusions about the effect of treatment based on the adjusted analyses.

Since centre, infant sex and birth weight were used to stratify the randomisation, where appropriate analyses will be adjusted for these variables (with categories as defined in Section 0). The baseline variables to be adjusted for in the analysis of each outcome are detailed in Section 6.

If convergence is an issue, covariates may need to be excluded from the adjusted analysis. Any deviation from the planned adjustment for potential confounders will be clearly identified in the final report.

## 4.7 Planned Treatment by Covariate Interactions

Statistical analysis of all primary and secondary outcome variables will be performed

to determine the effect of treatment on the outcome (see Section 6 for details). Secondary analyses will also be performed to test for evidence of effect modification by the sex and birth-weight (<1250g, ≥1250g) of the child. Effect modification by these two factors will be assessed separately by including interaction effects between treatment and sex and between treatment and birth weight in the statistical models. Separate estimates of treatment effect will be obtained for males and females and for children born <1250g and ≥1250g, independent of whether the interaction effect is statistically significant, since this is a priori of interest.

Any unplanned treatment by covariate interactions are to be considered exploratory and will be clearly identified in the final report.

### 4.8 Multiple Comparisons and Multiplicity

Multiple hypothesis tests will be performed to assess the effectiveness of high-dose DHA due to multiple secondary outcomes, unadjusted and adjusted analyses based on raw data and planned treatment by covariate interactions. No adjustment will be made for the number of secondary analyses performed or any planned treatment by covariate interactions as these analyses are of less importance and less emphasis will be placed on the results. Since conclusions will be drawn based on the adjusted results using data, no adjustment will be made for the fact that both adjusted and unadjusted analyses are being performed on both raw and imputed datasets for each outcome.

## 5. Descriptive statistics

### 5.1 Disposition of Subjects and Withdrawals

Information will be presented by treatment group (where appropriate) on:

- The number of children whose families were not approached to participate in DINO7 by reason.
- The number of children whose families were approached to participate in DINO7.
- The number of children whose families did not consent to DINO7 by reason.
- The number of children whose families consented to DINO7.
- The number of children who did not complete any of the allergy related

assessments at 7 years by reason.

- The number of children who completed any of the allergy related assessments at 7 years.

5.2 Baseline Characteristics

| A descriptive comparison of the randomised groups will be conducted on the baseline characteristics presented in the following table. **Baseline characteristic (CRF Reference)** | **Categories** |
|---|---|
| Centre | Women's and Children's Hospital<br>Flinders Medical Centre<br>King Edward Memorial Hospital<br>Royal Women's Hospital<br>Royal Brisbane Women's Hospital |
| Sex (DINO A2.2) | Male<br>Female |
| Birth weight in grams (DINO A2.1) | <1250g<br>≥1250g |
| Birth weight in grams (DINO A2.1) | - |
| Birth length in centimetres (DINO F3.2) | - |
| Birth head circumference in centimetres (DINO F3.2) | - |
| Gestational age (GA) at birth in weeks (DINO F2) | - |
| Small for GA (DINO A2.1 & F2, the British 1990 growth reference) | Yes<br>No |
| Infant from multiple pregnancy (DINO A2.3) | Yes<br>No |
| Birth order of pregnancy (DINO F1) | Singleton<br>First of a multiple birth<br>Second of a multiple birth |

| | |
|---|---|
| | Third of a multiple birth |
| Mother's age at trial entry (DINO C2) | - |
| Mother completed secondary education (DINO D3) | Yes<br>No |
| Father completed secondary education (DINO D3) | Yes<br>No |
| Mother smoked during pregnancy (DINO D4) | Yes<br>No |
| Infants receiving breast milk at trial entry (DINO J4) | Yes<br>No |

Means and standard deviations, or medians and interquartile ranges, will be reported for continuous variables. Frequencies and percentages will be reported for categorical variables.

Due to the inclusion of infants from multiple births in the DINO trial, infants were nested within mothers/families. Some characteristics were measured at the infant level (e.g. infant sex), others were measured on the mother or family level (e.g. mother completed secondary education). For the purpose of summarising baseline and post-randomisation characteristics, variables measured on the mother or family level will be presented on this level.

## 5.3 Post Randomisation Characteristics

A descriptive comparison of the randomised groups will be conducted on the 7 year post randomisation characteristics presented in the following table.

| **Post randomisation characteristic (Assessment or CRF Reference)** | **Categories** |
|---|---|
| Child's corrected age at the time of the primary outcome assessment | - |
| Mother's highest level of education (DINO7 1.2) | Secondary school incomplete<br>Secondary school complete<br>Certificate or diploma<br>Degree or higher degree |
| Father's highest level of education (DINO7 1.2) | Secondary school incomplete<br>Secondary school complete<br>Certificate or diploma<br>Degree or higher degree |
| Years of full time schooling of child (DINO7 2.1.3) | 0<br>1<br>2<br>3+ |
| Mother employment (DINO7 1.2.6) | Professional/managerial (ANZSCO categories 1-2)<br>Semi-skilled/trade/unskilled (ANZSCO categories 3-8)<br>Other |

| | |
|---|---|
| Father employment (DINO7 1.2.6) | Professional/managerial (ANZSCO categories 1-2)<br>Semi-skilled/trade/unskilled (ANZSCO categories 3-8)<br>Other |
| Child's family structure (DINO7 1.1) | Intact family<br>New marriage/de facto relationship<br>Adopted<br>Fostered<br>Separated parents (divided care)<br>Sole parent mother<br>Sole parent father<br>Other |
| English the primary language spoken at home (DINO7 1.4.1) | Yes<br>No |
| Position by age of child in family (DINO7 1.4.4) | First<br>Second or later |
| Smoker in household (DINO7 1.4.7) | Yes<br>No |
| Dog or cat as pet (DINO7 1.4.5) | Yes<br>No |
| First degree relative with medical diagnosis of eczema, asthma or hay fever (DINO7 1.5.1) | Yes<br>No |
| DHA enriched foods in last month (DINO7 2.3.2) | - |
| Fish oil supplementation during last month (DINO7 2.3.3) | - |

| | |
|---|---|
| Number of children in the house hold (DINO7 1.4.3) | - |
| Presence a gas heater without a chimney (DINO7 1.4.6) | Yes<br>No |
| Weight at 7 year of age (DINO7 3.1) | |
| Height at 7 year of age (DINO7 3.2) | |
| BMI=Weight (kg)/Height$^{2}_{(m)}$ | |
| Child or family unblinded before the 7 year assessment | Yes<br>No |

Post randomisation characteristics will be compared statistically between groups using t-tests for continuous outcomes and Chi-square tests for categorical outcomes. Characteristics measured on the mother or family level will be analysed on this level.

5.4 Missing Data

Missing data will be assessed descriptively by treatment group for each outcome variable specified in Section 6 and for each post randomisation characteristic specified in Section 5.3.

## 6. Statistical analyses

In this section, the following details are provided for each outcome variable:

Outcome - A detailed description of the outcome variable, including the type of variable, the relevant assessments and/or sections(s) of the ISAAC questionnaire and CRF and how it will be calculated.

Effect – The measure of treatment effect to be reported.

Analysis - The type of statistical analysis to be performed.

Adjustment – The baseline covariates (stratification variables and potential confounders) to adjust for in the adjusted analysis of the randomised groups.

For each outcome variable, statistical significance will be assessed at the 0.05 level using a two-sided comparative test of treatment effect, unless otherwise specified.

For binary outcomes, the number and percentage of subjects experiencing the outcome of interest will be examined. If the number of subjects experiencing the outcome is considered too small for the planned analysis to be sensible, a Fisher's exact test on unimputed data will be performed instead.

## 7. Primary Outcome Variable

Binary outcome based on parent responses to the International Study of Asthma and Allergies in Childhood (ISAAC) questionniare.

### 7.1.1 Incidence of Allergy

Outcome: Binary outcomes based on parent responses to the International Study of Asthma and Allergies in Childhood (ISAAC) questionniare. Outcomes include:

*Asthma/wheeze*

- Prevalence previous 12 months
    - Child had wheeze (ISAAC Q2)
    - Child had wheeze after exercise (ISAAC Q7)
    - Child had dry cough at night (ISAAC Q8)
- Severity previous 12 months
    - Child had more 4 or more attacks of wheezing (ISAAC Q3)
    - Child had sleep disturbed on one or more nights per week (ISAAC Q4)
    - Child's wheezing limited speech (ISAAC Q5)
    - Child had severe asthma symptoms (ISAAC Q3 4 or more attacks, Q4 yes, Q5 yes)
- Cumulative incidence 0-7 years corrected age
    - Child ever had wheeze (ISAAC Q1)

    - Child ever had asthma (ISAAC Q6)
- Parent report medically diagnosed asthma within last 5 years
    - Child diagnosed by doctor (CRF 4.1)

*Allergic rhinitis*

- Prevalence previous 12 months
    - Child had allergic rhinitis (ISAAC Q10)
    - Child had rhino-conjunctivitis (yes to ISAAC Q10 and Q11)
    - Child had seasonal allergic rhinitis (ISAAC Q10 yes, Q11 no and ISAAC Q12 months occurred September and/or October and/or November)

- Severity previous 12 months
    - Child had severe allergic rhinitis symptoms (ISAAC Q10 yes, ISAAC Q13 'a lot')

- Cumulative incidence 0-7 years corrected age
    - Child ever had allergic rhinitis symptoms (ISAAC Q9)
    - Child ever had hay fever (ISAAC Q14)

*Eczema*

- Prevalence previous 12 months
    - Child had itchy rash symptoms (ISAAC Q16)
    - Itchy rash in typical locations (ISAAC Q17)
    - Eczema (ISAAC Q17 yes and Q18 age under 5)
- Severity previous 12 months
    - Child rash cleared (ISAAC Q19)
    - Child had severe eczema symptoms – kept awake >one or more nights per week (ISAAC Q20)

- Child had severe eczema symptoms – rash has not cleared (ISAAC Q19) and child kept awake >one or more nights per week (ISAAC Q20)

- Cumulative incidence 0-7 years corrected age
  - Child ever had itchy rash (ISAAC Q15)
  - Child ever had eczema (ISAAC Q21)
- Parent report medically diagnosed eczema within last 5 years
  - Child diagnosed by doctor (CRF 4.1)

Effect: Relative risk of allergy (high DHA vs. standard DHA).

Analysis: Test for a treatment effect using a log binomial GEEs. The models will use an independence working correlation matrix to account for the clustering of multiple infants within mother.

Adjustment: Centre, birth weight, sex.

***Sub group analysis***

**Breastfeeding mothers only**

Association between DHA supplementation and symptoms of parental reported asthma, allergic rhinitis and eczema and severity of asthma, allergic rhinitis and eczema at 7 years of age

Association between DHA supplementation and parental reported asthma, allergic rhinitis and eczema from birth to 7 years of age

Association between DHA supplementation and parental report medically diagnosed eczema and asthma within last 5 years at 7 years of age

## 8. References to the data analysis plan

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# Appendices to chapter 6

**Appendix 6 –Published Systematic Review (pages 373-467)**

# Statement of Authorship

| | |
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| Title of Paper | Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood (Review) |
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## Principal Author

| | |
|---|---|
| Name of Principal Author (Candidate) | Anoja Wickrama Gunaratne |
| Contribution to the Paper | Thesis Chapter 2 and Chapter 6 nominated to save as this paper to the Cochrane Library.<br>Conceived the idea, designed and developed the review, conducted searches, undertook selection of studies meeting inclusion and exclusion criteria, undertook data extraction, critical appraisal and entering data, analysed and interpreted data, wrote the manuscript. Coordinates the review. |
| Signature | Date 19/08/15 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

| | |
|---|---|
| Name of Co-Author | Maria Makrides |
| Contribution to the Paper | Conceived the idea, contributed to the design, and development of the review, provided expert advice and supervision on a methodological perspective and a clinical perspective, provided general advice and editing the review. |
| Signature | Date 19/08/15 |

| | |
|---|---|
| Name of Co-Author | Catherine T Collins |
| Contribution to the Paper | Contributed to the design and development of the review, supervised selection of studies meeting inclusion criteria, undertook data extraction and critical appraisal (Cochrane reviews require this to be done independently by two authors), provided expert advice and supervision on a methodological perspective and a clinical perspective and editing the review and is the senior and corresponding author. |
| Signature | Date 19/08/2015 |

Please cut and paste additional co-author panels here as required.

# Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood (Review)

**Gunaratne AW, Makrides M, Collins CT**

THE COCHRANE COLLABORATION®

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2015, Issue 7

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[Intervention Review]

# Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

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## ABSTRACT

### Background

Allergies have become more prevalent globally over the last 20 years. Dietary consumption of n-3 (or omega 3) long chain polyunsaturated fatty acids (LCPUFA) has declined over the same period of time. This, together with the known role of n-3 LCPUFA in inhibiting inflammation, has resulted in speculation that n-3 LCPUFA may prevent allergy development. Dietary n-3 fatty acids supplements may change the developing immune system of the newborn before allergic responses are established, particularly for those with a genetic predisposition to the production of the immunoglobulin E (IgE) antibody. Individuals with IgE-mediated allergies have both the signs and symptoms of the allergic disease and a positive skin prick test (SPT) to the allergen.

### Objectives

To assess the effect of n-3 LCPUFA supplementation in pregnant and/or breastfeeding women on allergy outcomes (food allergy, atopic dermatitis (eczema), allergic rhinitis (hay fever) and asthma/wheeze) in their children.

### Search methods

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (6 August 2014), PubMed (1966 to 01 August 2014), CINAHL via EBSCOhost (1984 to 01 August 2014), Scopus (1995 to 01 August 2014), Web of Knowledge (1864 to 01 August 2014) and ClinicalTrials.gov (01 August 2014) and reference lists of retrieved studies.

### Selection criteria

We included randomised controlled trials (RCTs) evaluating the effect of n-3 LCPUFA supplementation of pregnant and/or lactating women (compared with placebo or no treatment) on allergy outcomes of the infants or children. Trials using a cross-over design and trials examining biochemical outcomes only were not eligible for inclusion.

**Data collection and analysis**

Two review authors independently assessed eligibility and trial quality and performed data extraction. Where the review authors were also investigators on trials selected, an independent reviewer assessed trial quality and performed data extraction.

**Main results**

Eight trials involving 3366 women and their 3175 children were included in the review. In these trials, women were supplemented with n-3 LCPUFA during pregnancy (five trials), lactation (two trials) or both pregnancy and lactation (one trial). All trials randomly allocated women to either a n-3 LCPUFA supplement or a control group. The risk of bias varied across the eight included trials in this review with only two trials with a low risk of selection, performance and attrition bias.

N-3 LCPUFA supplementation showed a clear reduction in the primary outcome of any allergy (medically diagnosed IgE mediated) in children aged 12 to 36 months (risk ratio (RR) 0.66, 95% confidence interval (CI) 0.44 to 0.98; two RCTs; 823 children), but not beyond 36 months (RR 0.86, 95% CI 0.61 to 1.20; one RCT, 706 children). For any allergy (medically diagnosed IgE mediated and/or parental report), no clear differences were seen in children either at 12 to 36 months (RR 0.89, 95% CI 0.71 to 1.11; two RCTs, 823 children) or beyond 36 months of age (RR 0.96, 95% CI 0.84 to 1.09; three RCTs, 1765 children).

For the secondary outcomes of specific allergies there were no clear differences for food allergies at 12 to 36 months and beyond 36 months, but a clear reduction was seen for children in their first 12 months with n-3 LCPUFA (both for medically diagnosed IgE mediated and medically diagnosed IgE mediated and/or parental report). There was a clear reduction in medically diagnosed IgE-mediated eczema with n-3 LCPUFA for children 12 to 36 months of age, but not at any other time point for both medically diagnosed IgE mediated and medically diagnosed IgE mediated and/or parental report. No clear differences for allergic rhinitis or asthma/wheeze were seen at any time point for both medically diagnosed IgE mediated, and medically diagnosed IgE mediated and/or parental report.

There was a clear reduction in children's sensitisation to egg and sensitisation to any allergen between 12 to 36 months of age when mothers were supplemented with n-3 LCPUFA.

In terms of safety for the mother and child, n-3 LCPUFA supplementation during pregnancy did not show increased risk of postpartum haemorrhage or early childhood infections.

**Authors' conclusions**

Overall, there is limited evidence to support maternal n-3 LCPUFA supplementation during pregnancy and/or lactation for reducing allergic disease in children. Few differences in childhood allergic disease were seen between women who were supplemented with n-3 LCPUFA and those who were not.

## PLAIN LANGUAGE SUMMARY

**Fish oil (n-3 or omega-3) for pregnant mothers or breastfeeding mothers to prevent allergies in their young children**

Fish and fish oil are the major sources of omega-3 long chain fatty acids. Dietary marine omega-3 fatty acid supplements during pregnancy may change the immune system of the newborn before allergic responses are established, particularly for those with a genetic predisposition to the production of the immunoglobulin E (IgE) antibody. Individuals with IgE-mediated allergies have both the signs and symptoms of the allergic disease and a positive skin prick test (SPT) to the allergen.

Allergy is an important public health problem that places a burden on individuals, society and healthcare costs. Allergic diseases include food allergies, eczema (atopic dermatitis), asthma or wheeze and hay fever (allergic rhinitis). Many childhood allergies continue into adulthood.

Pregnant women, especially those from Western countries, are not eating as much fish and allergic diseases have been increasing over the time that pregnant women have been eating less fish. The unborn baby gets nutrition from his or her mother and so the mother's diet is important. Supplementing women with omega-3 fatty acids from marine origin may be important in preventing their children from developing allergies.

In this review of randomised controlled studies, we evaluated the effects of adding marine omega-3 fatty acids to women's diets during pregnancy or lactation on allergic diseases in their children. We analysed eight trials that involved 3366 women and 3175 children. The women were randomly assigned to receive a marine omega-3 supplement (as fish oil capsules, or added to foods) or no treatment during

pregnancy (five trials), during breast feeding (two trials) or both pregnancy and breast feeding (one trial). Overall, the methodological quality of the trials varied, with only two trials being at low risk of bias.

Overall, the results showed little effect of maternal marine omega-3 supplementation during pregnancy and/or breast feeding for the reduction of allergic disease in the children. However there were reductions in some outcomes such as food allergy during the baby's first year and eczema with marine omega-3 supplementation in women with a baby at high risk of allergy. Currently, there is not enough evidence to say that omega-3 supplements from marine origin during pregnancy and/or breast feeding for mothers will reduce allergies in their children.

In terms of safety for the mother and child, omega-3 fatty acids supplementation from marine origin during pregnancy did not show increased risk of excessive bleeding after the baby was born (postpartum haemorrhage) or early childhood infections.

# BACKGROUND

## Description of the condition

Over the past 20 years the prevalence of allergies in industrialised countries has increased fivefold, from approximately 4% to an estimated 20% (Asher 2006; Pawanker 2011). Allergic diseases include food allergies, atopic dermatitis (eczema), asthma and allergic rhinitis (hay fever) (Arkwright 2008). The pattern of allergy expression differs with age (Spergel 2003). Food allergies and eczema are common in children under three years of age, while asthma and allergic rhinitis are more common between the ages of three and 15 years (Saarinen 1995). Regardless of these changing patterns of allergic disease in childhood, many childhood allergies persist, with 50% of childhood asthma sufferers and 80% of allergic rhinitis sufferers continuing to experience allergic symptoms into adulthood (Asher 2006; Barbee 1998; Greisner 1998; Spergel 2010).

Atopy is defined as a genetic predisposition to the production of specific immunoglobulin E (IgE) antibodies to allergens and atopic people are more liable to have immune reactions that lead to allergies (Sears 1996). One of the principal methods of determining specific IgE sensitisation to an allergen is the skin prick test (SPT). To perform a SPT, a drop of allergen extract is placed on the skin and a small prick is made through the drop. This allows a small amount of allergen to enter the skin. If allergic to the tested allergen, a small lump (wheal) will appear at the site of testing within 15 to 20 minutes. A test is positive if there is a mean wheal diameter of 3 mm or greater (Bousquet 2012). A positive SPT to an allergen, along with clinical allergy symptoms, forms the basis for IgE-mediated allergy diagnosis (Johansson 2001; Johansson 2004).

The risk of allergy is 30% greater if one first degree relative (parent or sibling) is atopic, but if both parents are atopic then the allergy risk increases to 70% (Sears 1996). Allergic responses are reactions to an extrinsic substance (allergen) that is mediated by an immunological response (Arkwright 2008). This immunological response may cause mild to severe reactions in different individuals and can be life threatening (Arkwright 2008). Thus allergy is an important public health problem which places a burden on individuals, society and the healthcare system (Gupta 2004).

Environmental factors seem to have had an important influence on the increasing incidence of allergies. Possible contributing factors include lack of breastfeeding, higher socio-economic conditions with higher standards of hygiene, fewer respiratory infections, greater use of antibiotics early in life, fewer older siblings in the household, less contact with farm animals, general lack of microbial exposure and changes in dietary patterns (Gupta 2004; Strong 2005).

Among the changes in dietary patterns, it has been hypothesised that the balance of long chain polyunsaturated fatty acids (LCPUFA), specifically the n-3 (omega 3) to n-6 (omega 6) ratio, may be a factor in the increased incidence of childhood allergies (Calder 2000; Calder 2010b; Prescott 2004). Furthermore, maternal fish consumption during pregnancy has also reduced due to precautionary public health advice regarding the consumption of specific fish which may contain methyl mercury (Oken 2003).

## Description of the intervention

Fish and fish oil are the major sources of n-3 LCPUFA, while vegetable oils are the major source of n-6 LCPUFA. Recent data suggest that dietary consumption of n-3 LCPUFA has declined in Western diets to favour the intake of n-6 fatty acids (Meyer 2003). Epidemiological data suggest that a higher fish intake during pregnancy is associated with fewer symptoms of allergic diseases in the offspring in early childhood (Calvani 2006; Romieu 2007; Sausenthaler 2007; Willers 2007). Thus, supplementing maternal diets with n-3 LCPUFA may be an important factor in reducing the incidence of allergic diseases.

### How the intervention might work

Dietary n-3 LCPUFA supplementation during pregnancy and lactation has been suggested to modulate the immune system of the fetus, neonate or infant before allergic responses are established (Denburg 2005). The early programming of fetal immune responses to allergens possibly begins in the epithelial tissue where antigen (allergen) proteins first encounter antigen-presenting cells (Prescott 2007). The pattern of cytokine production by antigen-presenting cells determines the pattern of T-helper (Th) cells differentiation (Prescott 2007). T cells producing Type 1 T cells (Th1), develop under the influence of interleukin (IL)-12 and IL-2, whereas T cells producing Type 2 T cells (Th2), develop in the relative absence of pro-Th2 factors such as IL-4 (Snijdewint 1993). The differences in T cell phenotypes also determine the pattern of B-cell antibody production with Th2 cytokines (IL-4, IL-5, and IL-13), prompting IgE production and allergic inflammation, whereas Th1 cytokines (IFN-$\gamma$) largely inhibit this in favour of low level IgG production (Calder 2006; Calder 2010a). Th2 cytokines are also important in determining whether these immune responses result in clinically relevant diseases such as asthma, allergic rhinitis or allergic dermatitis (Calder 2003; Georas 2005; Prescott 2007). A well regulated placental balance between the Th1 and Th2 responses is important for developing a robust immune system during pregnancy (Wilczynski 2005).

Maternal n-3 LCPUFA intervention studies support this immune programming hypothesis (Krauss-Etschmann 2007; Lauritzen 2005; Lee 2013; Romero 2013). Two studies investigated Th1/Th2 related molecules in cord blood (Krauss-Etschmann 2008; Romero 2013), and the other investigated cytokine production in children at two and a half years of age (Lauritzen 2005). These studies showed that allergy-related immune parameters were lower in the offspring of women who had n-3 LCPUFA supplementation during pregnancy or lactation (Krauss-Etschmann 2008; Lauritzen 2005; Romero 2013). The other study also showed that maternal n-3 LCPUFA supplementation during pregnancy was associated with balancing Th1/Th2 and modulating IFN$\gamma$ and IL13 in infants (Lee 2013).

Supporting evidence from mechanistic (Denburg 2005; Prescott 2007) and small scale intervention studies show that allergy markers and allergy mechanisms are influenced by n-3 LCPUFA (Dunstan 2003; Prescott 2007a). Also there were studies in which maternal or postnatal consumption of n-3 LCPUFA through oily fish or fish oil had an effect on allergy outcomes in children (Hodge 1996; Romieu 2007), as well as a transient effect on allergy outcomes (Mihrshahi 2004; Oddy 2004).

### Why it is important to do this review

Allergy is an important public health problem that places a burden on individuals, society and healthcare costs (Gupta 2004; Kemp 1999; Pawanker 2011). Consequently, allergy prevention is a major global challenge (Strong 2005) and the World Allergy Organization (WAO) recommends that allergy preventative strategies are urgently needed (Asher 2004). It is uncertain if n-3 LCPUFA supplementation during pregnancy or lactation reduces allergic disease in children. For these reasons, we need clear evidence from intervention studies. Therefore, in this systematic review we aim to evaluate the effects of maternal n-3 LCPUFA supplementation during pregnancy and/or lactation on allergy outcomes in children.The safety aspects of n-3 LCPUFA also need to be considered; it is postulated that high doses of n-3 LCPUFA may have antithrombotic antiplatelet properties which may lead to bleeding (Simopoulos 1991), as well as immune cell alterations which may have an effect on infections (Calder 2007).

## OBJECTIVES

To assess the effect of maternal n-3 LCPUFA supplementation during pregnancy and/or lactation on the allergy outcomes of their children.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

We included randomised controlled trials (RCTs) focusing on n-3 LCPUFA supplementation of pregnant and/or lactating women (compared with placebo or no treatment) and assessed allergy outcomes of the infants or children. Quasi-RCTs and RCTs using a cluster-randomised design were eligible for inclusion but none were identified. Trials published in abstract form only were not identified for inclusion. In future updates we will only consider abstracts for inclusion if unpublished data can be obtained from the trails. Trials using a cross-over design and trials examining biochemical outcomes only were not eligible for inclusion.

#### Types of participants

Women and their children, with either a normal or high risk of developing allergic disease, were included. A fetus or a child with a first degree relative with medically diagnosed allergies, or a positive a SPT, or a positive radioallergosorbent test (RAST) was defined as being at high risk of allergies. Infants were also considered at high risk of allergies if their cord blood IgE level was above 0.70 IU/mL.

### Types of interventions

We considered all randomised comparisons of n-3 LCPUFA supplementation given to pregnant or lactating women (either with or without arachidonic acid), with placebo or no supplementation as a control, regardless of dose regimens and duration of intervention. Trials in which fish was the intervention were included if appropriately controlled, for example, if the diet was appropriately adjusted to match the protein contribution of fish.

### Types of outcome measures

Primary outcome measures included children with allergy, including food allergy, atopic dermatitis (eczema), asthma/wheeze, allergic rhinitis (hay fever) or any allergies (children with one or more of the allergy types). Outcomes were assessed as short term (occurring at less than 12 months of age), medium term (occurring from 12 to less than 36 months of age) and long term (36 months of age and older). Outcomes were also assessed by combining short-term, medium-term and long-term results to assess the cumulative incidence.

#### Primary outcomes

1. Medically diagnosed any allergy with sensitisation, i.e. IgE-mediated allergies where both the signs and symptoms of the allergic disease and a positive SPT and/or RAST test are present.
2. Medical diagnosis or parental report (using validated questionnaire) of any allergy, +/- IgE sensitisation.

#### Secondary outcomes

Secondary outcome measures included children with specific forms of allergy, including food allergy, atopic dermatitis (eczema), asthma/wheeze, allergic rhinitis (hay fever) with IgE sensitisation and +/- IgE sensitisation, SPT results, and parent-reported allergies using non-validated questionnaires. Secondary safety outcomes included infant safety (e.g. infections) and maternal safety (e.g. postpartum haemorrhage or infection) due to the theoretical risk associated with higher doses of n-3 LCPUFA.

## Search methods for identification of studies

The following methods section of this review is based on a standard template used by the Cochrane Pregnancy and Childbirth Group.

### Electronic searches

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register by contacting the Trials Search Co-ordinator (6 August 2014).

The Cochrane Pregnancy and Childbirth Group's Trials Register is maintained by the Trials Search Co-ordinator and contains trials identified from:

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE (Ovid);
3. weekly searches of Embase (Ovid);
4. handsearches of 30 journals and the proceedings of major conferences;
5. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Details of the search strategies for CENTRAL, MEDLINE and Embase, the list of handsearched journals and conference proceedings, and the list of journals reviewed via the current awareness service can be found in the 'Specialized Register' section within the editorial information about the Cochrane Pregnancy and Childbirth Group.

Trials identified through the searching activities described above are each assigned to a review topic (or topics). The Trials Search Co-ordinator searches the register for each review using the topic list rather than keywords.

In addition, we searched PubMed (1966 to 01 August 2014) ( Appendix 1), CINAHL via EBSCOhost (1984 to 01 August 2014) (Appendix 2), Scopus (1995 to 01 August 2014) (Appendix 3), Web of Knowledge (1864 to 01 August 2014) (Appendix 4) and ClinicalTrials.gov (01 August 2014) (Appendix 5).

### Searching other resources

We searched reference lists of retrieved studies.

We did not apply any language or date restrictions.

## Data collection and analysis

### Selection of studies

Two review authors Anoja W Gunaratne (AWG) and Carmel T Collins (CTC) independently assessed the eligibility of trials identified by the search. Disagreements were resolved through discussion or, if required, by consultation with the third review author Maria Makrides (MM).

### Data extraction and management

Two review authors (AWG, CTC) independently extracted the data from eligible trials using the agreed form. Discrepancies were resolved through discussion or through consultation with the third author (MM) if required. When information was unclear or incomplete, we attempted to contact authors of the original reports to provide further details. We entered data into Review Manager software 5.3 (RevMan 2014) and checked them for accuracy.

### Assessment of risk of bias in included studies

Two review authors (AWG, CTC) independently assessed the risk of bias for each trial using the criteria outlined in*the Cochrane Handbook for Systematic Reviews of Interventions (Handbook) (*Higgins 2011*)*. We resolved any disagreement by discussion or by involving a third assessor (MM). MM and CTC are Investigators on two trials, Makrides 2009 and Makrides 2010 included in the review. These trials were independently assessed for risk of bias and data extracted by AWG and an independent researcher third party Karen Best (KB).

#### (1) Random sequence generation (checking for possible selection bias)

For each included trial, we described the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it would produce comparable groups.
We assessed the method as:
low risk of bias (any truly random process, e.g. random number table, computer random number generator); high risk of bias (any non-random process, e.g. odd or even date of birth, hospital or clinic record number); unclear risk of bias.

#### (2) Allocation concealment (checking for possible selection bias)

For each included trial, we described the method used to conceal allocation to interventions prior to assignment and assessed whether intervention allocation could have been foreseen before recruitment, during recruitment or changed after assignment.
We assessed the methods as:
low risk of bias (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes); high risk of bias (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); unclear risk of bias.

#### (3.1) Blinding of participants and personnel (checking for possible performance bias)

We described the methods used, if any, to blind trial participants and personnel from knowledge of which intervention a participant received for each trial. We considered that trials were at low risk of bias if they were blinded, or if we judged that the lack of blinding would be unlikely to affect results. We assessed blinding separately for different outcomes or classes of outcomes.
We assessed the methods as:
low, high or unclear risk of bias for participants; low, high or unclear risk of bias for personnel.

#### (3.2) Blinding of outcome assessment (checking for possible detection bias)

We described the methods used, if any, to blind outcome assessors from knowledge of which intervention a participant received for each included trial. We assessed blinding separately for different outcomes or classes of outcomes.
We assessed the methods used to blind outcome assessment as:
low, high or unclear risk of bias.

#### (4) Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)

We described the completeness of data, including attrition and exclusions from the analysis, for each included trial and for each outcome or class of outcomes. We stated whether attrition and exclusions were reported and the numbers included in the analysis at each stage (compared with the total randomised participants). We also outlined the reasons for attrition or exclusion, where reported, and whether missing data were balanced across groups or related to outcomes. We included missing data in the analyses where sufficient information was reported or could be supplied by the trial authors.
We assessed methods as:
low risk of bias (e.g. no missing outcome data; missing outcome data balanced across groups); high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups; 'as treated' analysis done with substantial departure of intervention received from that assigned at randomisation); unclear risk of bias.

#### (5) Selective reporting (checking for reporting bias)

We described our investigation of possible selective outcome reporting bias and our results for each included trial.
We assessed the methods as having a:
low risk of bias - where it is clear that all of the trial's pre-specified outcomes and all expected outcomes of interest to the review were reported; high risk of bias - where not all the trial's pre-specified outcomes had been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest were reported incompletely and so could not be used; or trial failed to include results of a key outcome that would have been expected to have been reported; unclear risk of bias.

#### (6) Other bias (checking for bias due to problems not covered by (1) to (5) above)

We described any important concerns we had about other possible sources of bias for each included trial.
We assessed whether each trial was free of other problems that could put it at risk of bias, as low, high or unclear risk of other bias.

#### (7) Overall risk of bias

We made explicit judgements about whether trials were at high risk of bias according to the criteria given in the *Handbook* (Higgins 2011). With reference to (1) to (6) above, we assessed the likely magnitude and direction of the bias and whether we considered it was likely to impact on the findings. We explored the impact of the level of bias through undertaking sensitivity analyses - *see* Sensitivity analysis.

### Measures of treatment effect

#### Dichotomous data

Results are presented as summary risk ratio with 95% confidence intervals.

#### Continuous data

We planned to present the results of continuous data as the mean difference, if outcomes were measured in the same way between trials. We would have used the standardised mean difference to combine trials that measured the same outcome but used different methods.

### Unit of analysis issues

#### Cluster-randomised trials

We did not identify any cluster-randomised trials for inclusion in this review. If we identify cluster-randomised trials in future updates of this review, we will include them in the analyses along with individually-randomised trials. We will adjust their sample size using the methods described in the *Handbook* (Higgins 2011) using an estimate of the intracluster correlation co-efficient (ICC) derived from the trial (if possible), from a similar trial or from a trial of a similar population. If we use ICCs from other sources, we will report this and conduct sensitivity analyses to investigate the effect of variation in the ICC. If we identify both cluster-randomised trials and individually-randomised trials, we will synthesis the relevant information. We will consider it reasonable to combine the results from both if there is little heterogeneity between the trial designs and the interaction between the effect of intervention and the choice of randomisation unit is considered to be unlikely.

We will also acknowledge heterogeneity in the randomisation unit and perform a sensitivity analysis to investigate the effects of the randomisation unit.

#### Cross-over trials

Cross-over trials are not an appropriate design for this review.

#### Other unit of analysis issues

#### Trials with more than two treatment groups

Trials using one or more treatment groups (multi-arm trials) were combined to create a single pair-wise comparison where appropriate. We used the methods described in the *Handbook* (Higgins 2011) to ensure that we did not double count participants.

### Dealing with missing data

We noted levels of attrition within the included trials. We used sensitivity analyses to explore the impact of including trials with high levels of missing data on the overall assessment of treatment effect. For all outcomes, we carried out analyses (as far as possible) on an intention-to-treat basis. This meant that we attempted to include all participants randomised to each group in the analyses with all participants analysed in the group to which they were allocated, regardless of whether or not they received the allocated intervention. The denominator used for each outcome was the number randomised minus the number with missing outcomes. In studies where there were missing data, we imputed results, if the imputed results differed little from the raw data.

### Assessment of heterogeneity

We assessed statistical heterogeneity in each meta-analysis using the $T^2$, $I^2$ and $Chi^2$ statistics. We regarded heterogeneity as substantial if an $I^2$ was greater than 30% and either the $T^2$ was greater than zero or there was a low P value (less than 0.10) in the $Chi^2$ test for heterogeneity.

### Assessment of reporting biases

In future updates, if there are 10 or more studies in the meta-analysis, we will investigate reporting biases (such as publication bias) using funnel plots. We will assess funnel plot asymmetry visually. If asymmetry is suggested by a visual assessment, we will perform exploratory analyses to investigate it.

### Data synthesis

We carried out statistical analyses using the Review Manager software 5.2 (RevMan 2014). We used fixed-effect meta-analysis for combining data where it was reasonable to assume that trials were estimating the same underlying treatment effect: that is, where trials were examining the same intervention and the trials' populations and methods were judged sufficiently similar. If there was

clinical heterogeneity sufficient to expect that the underlying treatment effects differed between trials, or if substantial statistical heterogeneity was detected, we used random-effects meta-analysis to produce an overall summary, if an average treatment effect across trials was considered clinically meaningful. The random-effects summary was treated as the average range of possible treatment effects and we discussed the clinical implications of treatment effects differing between trials. We did not combine trials if the average treatment effect was not clinically meaningful.

Where we used random-effects analyses, the results are presented as the average treatment effect with 95% confidence intervals and estimates of $T^2$ and $I^2$.

### Subgroup analysis and investigation of heterogeneity

Substantial heterogeneity was investigated using subgroup analyses and sensitivity analyses. We considered whether an overall summary was meaningful, and if it was, used random-effects analyses. We planned to carry out the following subgroup analyses.

1. Timing of supplementation
   i) n-3 LCPUFA supplementation during pregnancy versus placebo or no supplementation during pregnancy
   ii) n-3 LCPUFA supplementation during lactation versus placebo or no supplementation during lactation
   iii) n-3 LCPUFA supplementation during pregnancy and lactation versus placebo or no supplementation during pregnancy and lactation
2. Allergy risk
   i) Maternal n-3 LCPUFA supplementation in women whose infants were at high risk of allergic disease versus placebo or no supplementation
   ii) Maternal n-3 LCPUFA supplementation in women whose infants were not considered as at high risk of allergic disease versus placebo or no supplementation
3. Infant maturity
   i) Maternal n-3 LCPUFA supplementation in term born infants versus placebo or no supplementation
   ii) Maternal n-3 LCPUFA supplementation in preterm born infants versus placebo or no supplementation

We restricted subgroup analyses to the primary outcomes.

### Sensitivity analysis

We planned to carry out sensitivity analyses for the review's primary outcomes to investigate the effect of trial quality by removing those trials rated as 'high' or 'unclear' risk of selection, performance or attrition bias to establish whether it was likely to impact on the findings.

## RESULTS

### Description of studies

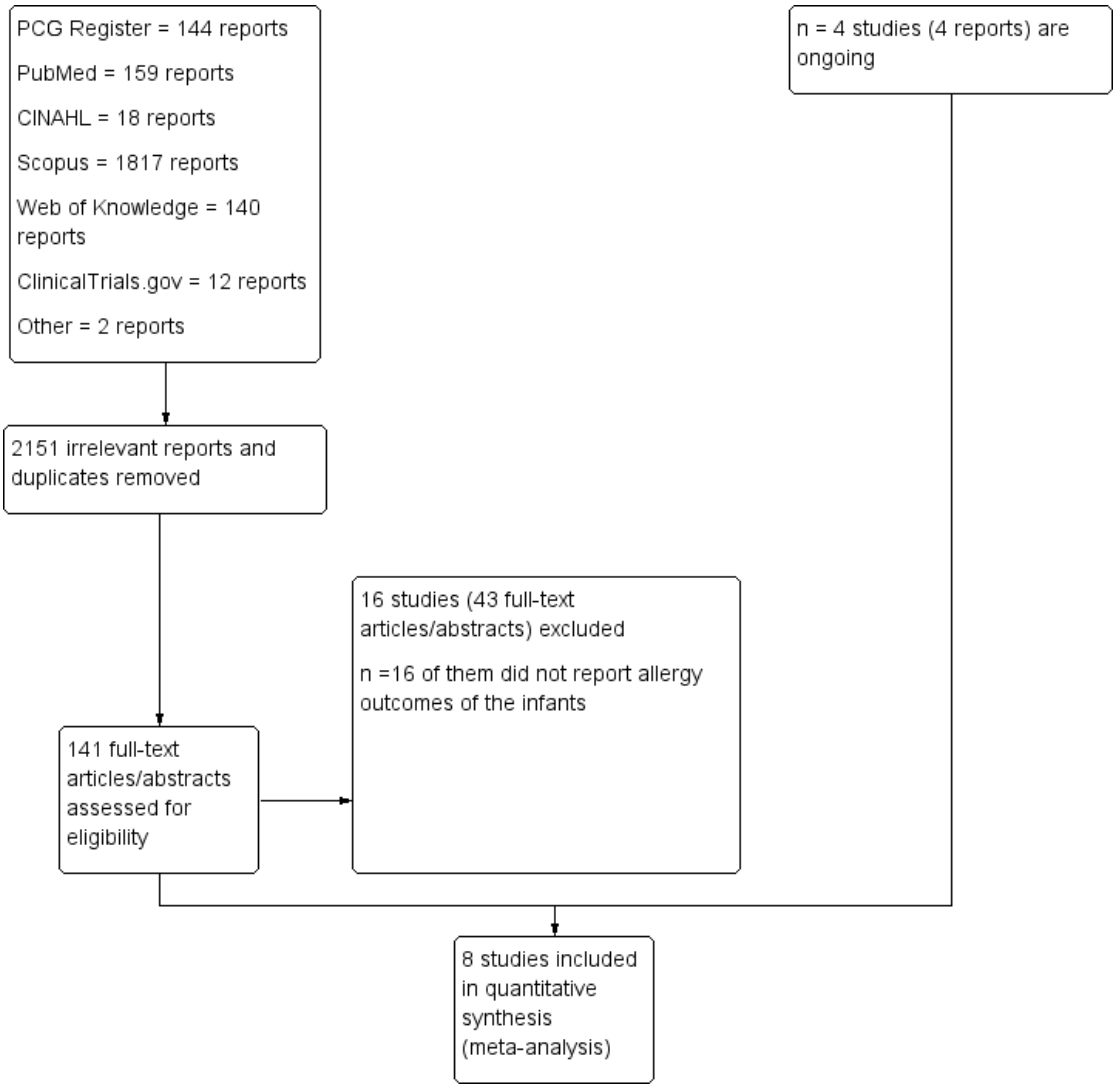
See: Characteristics of included studies; Characteristics of excluded studies.

### Results of the search

Electronic searches yielded 2290 records. The Cochrane Pregnancy and Childbirth Trials Register search (06 August 2014), retrieved 144 reports. Other electronic databases, PubMed (1966 to 01 August 2014), CINAHL via EBSCOhost (1984 to 01 August 2014), Scopus (1995 to 01 August 2014), Web of Knowledge (1864 to 01 August 2014) and ClinicalTrials.gov (01 August 2014) retrieved 159, 18,1817, 140 and 12 reports respectively. Two additional reports included the doctoral thesis (submitted) of AWG and an unpublished honours dissertation supervised by MM and CTC.

Of the 2292 records, we removed duplicates and irrelevant reports and identified 141 titles and abstracts by considering the inclusion criteria. Sixteen trials (with 43 reports) were excluded, leaving eight trials (with 94 reports) for inclusion in this review. Four trials (with four reports) are ongoing (Bisgaard 2012; Duchen 2012; Laitinen 2013; Liu 2013.) See Figure 1.

**Figure 1. Study flow diagram.**




## Included studies

See: Characteristics of included studies.

We identified eight randomised controlled trials with 3366 women (3175 children) who were supplemented with oily fish, or n-3 LCPUFA supplements during pregnancy and/or during lactation and assessed allergic outcomes in their children.

### Study design

All eight trials were parallel randomised controlled trials published in English. Six trials had two parallel groups (Dunstan 2003; Furuhjelm 2009; Makrides 2009; Noakes 2012; Makrides 2010; Ramakrishnan 2010), one trial included two parallel groups and a high fish intake non-randomised reference group (Lauritzen 2005), and one trial had three parallel groups including intervention, placebo and no oil group (Olsen 1992).

Full details of the included trials are provided in the Characteristics of included studies table.

### Participants

Of the 3366 women included in the review, 667 were supplemented during the postnatal period only (Lauritzen 2005;

Makrides 2009), 145 received supplementation in both the prenatal and postnatal period (Furuhjelm 2009) and the remaining 2554 women were only supplemented in the prenatal period (Dunstan 2003; Noakes 2012; Olsen 1992; Makrides 2010; Ramakrishnan 2010). Women with a fetus at high risk of allergies (n = 1072) were included in four trials (Dunstan 2003; Furuhjelm 2009; Noakes 2012; Makrides 2010) while the remainder included women with a fetus at both high and normal risk of allergies (Lauritzen 2005; Makrides 2009; Olsen 1992; Ramakrishnan 2010), see 'types of participants for definition of high risk'. In one trial, only preterm infants were included (Makrides 2009).

#### Sample sizes

The sample sizes of the included trials ranged from 98 (Dunstan 2003) to 1094 (Ramakrishnan 2010). Four trials had approximately 100 to 150 mothers (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Noakes 2012) and four trials had > 500 participants (Makrides 2009; Makrides 2010; Olsen 1992; Ramakrishnan 2010).

#### Study location

Three trials (Dunstan 2003; Makrides 2009; Makrides 2010) were undertaken in Australia, two (Lauritzen 2005; Olsen 1992) in Denmark, one (Furuhjelm 2009) in Sweden, one (Ramakrishnan 2010) in Mexico, and one (Noakes 2012) in the UK. Most of the trials were conducted in high-income, well-developed industrialised countries except one trial (Ramakrishnan 2010) which was conducted in a upper-middle income country.

#### Intervention

Six trials used n-3 LCPUFA capsules (Dunstan 2003; Furuhjelm 2009; Makrides 2009; Makrides 2010; Olsen 1992; Ramakrishnan 2010), one used muesli bars containing microencapsulated fish oil as a source of n-3 LCPUFA (Lauritzen 2005), and the remaining trial used oily fish (Noakes 2012). The daily dosage of n-3 LCPUFA varied between 400 mg and 4500 mg; providing between 331 mg and 2070 mg of docosahexaenoic acid (DHA) and 100 mg and 1600 mg of eicosapentaenoic acid (EPA). Control groups received olive oil in three trials (Dunstan 2003; Lauritzen 2005; Olsen 1992), soy oil in two trials (Furuhjelm 2009; Makrides 2009), a blend of vegetable oils (rapeseed, sunflower, and palm in equal proportions) in one trial, (Makrides 2010), or a blend of corn and soy oil in one trial (Ramakrishnan 2010). Olsen 1992 included a third randomised group who did not receive any supplementation and the control group in Noakes 2012 also received no supplementation.

The prenatal supplementation trials commenced supplementation between 18 to 20 weeks (Ramakrishnan 2010), at 20 (Dunstan 2003; Makrides 2010; Noakes 2012) and 30 (Olsen 1992) weeks of gestation and continued until delivery. One trial supplemented in both the prenatal and postnatal period, commencing from 25 weeks' gestation and continuing until the infant reached four months of age (Furuhjelm 2009). In the two postnatal supplementation trials, supplementation commenced within one week after delivery (Lauritzen 2005; Makrides 2009). The duration of supplementation was four months in Lauritzen 2005 while in Makrides 2009 trial in preterm infants, supplementation continued until the infant reached 40 weeks postmenstrual age (a median duration of 9.4 weeks supplementation).

#### Outcome measures

The allergy outcomes were determined by parental reports of doctor diagnosed allergy in one trial (Lauritzen 2005), parental reports of allergy symptoms in one trial (Ramakrishnan 2010) and parental reports of allergy symptoms and parental reports of doctor diagnosed allergy in one trial (Makrides 2009). Allergy was medically diagnosed in the remaining trials (Dunstan 2003; Furuhjelm 2009; Noakes 2012; Olsen 1992; Makrides 2010). In Olsen 1992, the medical diagnosis of allergy was obtained from the Danish Medical registries. Parent reports of allergy outcome data were collected using a non-validated questionnaire and validated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire in Makrides 2009 trial. Parent reports of allergy outcome data were collected using a non-validated questionnaire in Ramakrishnan 2010 trial. Additionally, Makrides 2010 trial also had parent reports of allergy outcome data using a non-validated questionnaire. The data that were collected using non-validated questionnaires were only included in the secondary outcomes.

The age of assessments differed in the trials. Assessments were conducted at one month and three months (Ramakrishnan 2010), six months (Furuhjelm 2009; Noakes 2012; Makrides 2010; Ramakrishnan 2010), 12 months (Dunstan 2003; Furuhjelm 2009; Makrides 2009; Makrides 2010), 18 months corrected age (Makrides 2009; Ramakrishnan 2010), 24 months (Furuhjelm 2009), 30 months (Lauritzen 2005), three to five years corrected age (Makrides 2009) (subgroup), 36 months (Makrides 2010), seven years of age (Makrides 2009), and 16 years of age (Olsen 1992). Of the five trials that used medical diagnosis of allergies (Dunstan 2003; Furuhjelm 2009; Noakes 2012; Olsen 1992; Makrides 2010), four performed skin prick tests (SPT) (Dunstan 2003; Furuhjelm 2009; Noakes 2012; Makrides 2010) and included children at high risk of allergy. Two trials (Furuhjelm 2009; Noakes 2012) reported SPT results for children under 12 months of age, three trials (Dunstan 2003; Furuhjelm 2009; Makrides 2010), reported SPT between 12 to 36 months of age, and one trial (Makrides 2010) reported SPT in children at 36 months of age.

IgE-mediated allergies were reported in Furuhjelm 2009 and Makrides 2010. Two trials (Furuhjelm 2009; Noakes 2012) used blood samples for IgE detection in infants and one trial (Noakes 2012) reported the results at birth and six months of age while

the other trial (Furuhjelm 2009) analysed the serum IgE levels at three and 12 months of age, but results were not reported.

The type of allergies reported in the trials differed. Five trials (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2009; Makrides 2010) reported food allergy. Six trials (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2009; Noakes 2012; Makrides 2010) reported eczema. All trials (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2009; Noakes 2012; Olsen 1992; Makrides 2010; Ramakrishnan 2010) reported asthma or wheeze. Three trials (Furuhjelm 2009; Makrides 2009; Makrides 2010) reported allergic rhinitis and four trials (Furuhjelm 2009; Makrides 2009; Olsen 1992; Makrides 2010) reported any allergy.

Two trials (Olsen 1992; Makrides 2010) reported postpartum haemorrhage. Makrides 2010 (n = 2399) was the primary trial from which the Palmer 2012 participants were recruited (see table of included studies); the incidence of postpartum haemorrhage is reported for the primary trial. Four trials (Noakes 2012; Makrides 2009; Makrides 2010; Ramakrishnan 2010) reported early childhood infections. Infection outcomes (in-hospital proven late onset sepsis) for Makrides 2009 are reported for the whole sample (n = 657). Ramakrishnan 2010 also reported fever in infants.

### Excluded studies

See: Characteristics of excluded studies.

We excluded 16 randomised controlled trials where the intervention was n-3 LCPUFA supplementation but allergy outcomes of the infants and/or children were not reported (Bergmann 2008; Campos-Martinez 2012; Carlson 2013 : Colombo 2004; Courville 2011; Granot 2011; Hauner 2009; Helland 2001; Innis 2007; Judge 2007; Karlsson 2010; Knudsen 2006; Krauss-Etschmann 2007; Martin-Alvarez 2012: Pena-Quintana 2011; Ribeiro 2012). Many of these trials are included in the Cochrane Systematic Review currently being updated (Makrides 2006).

### Characteristics of ongoing studies

See: Characteristics of ongoing studies.

There are four ongoing trials (Bisgaard 2012; Duchen 2012; Laitinen 2013; Liu 2013).

### Risk of bias in included studies

Overall, the eight included studies had various levels of risk of bias for methodological quality. See Figure 2 and Figure 3 for details.

**Figure 2. 'Risk of bias' graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.**

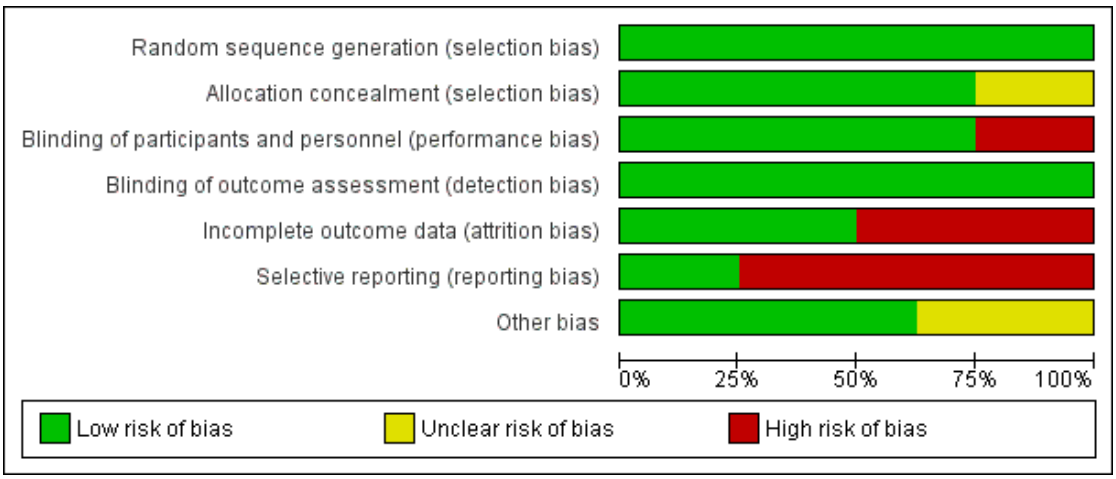
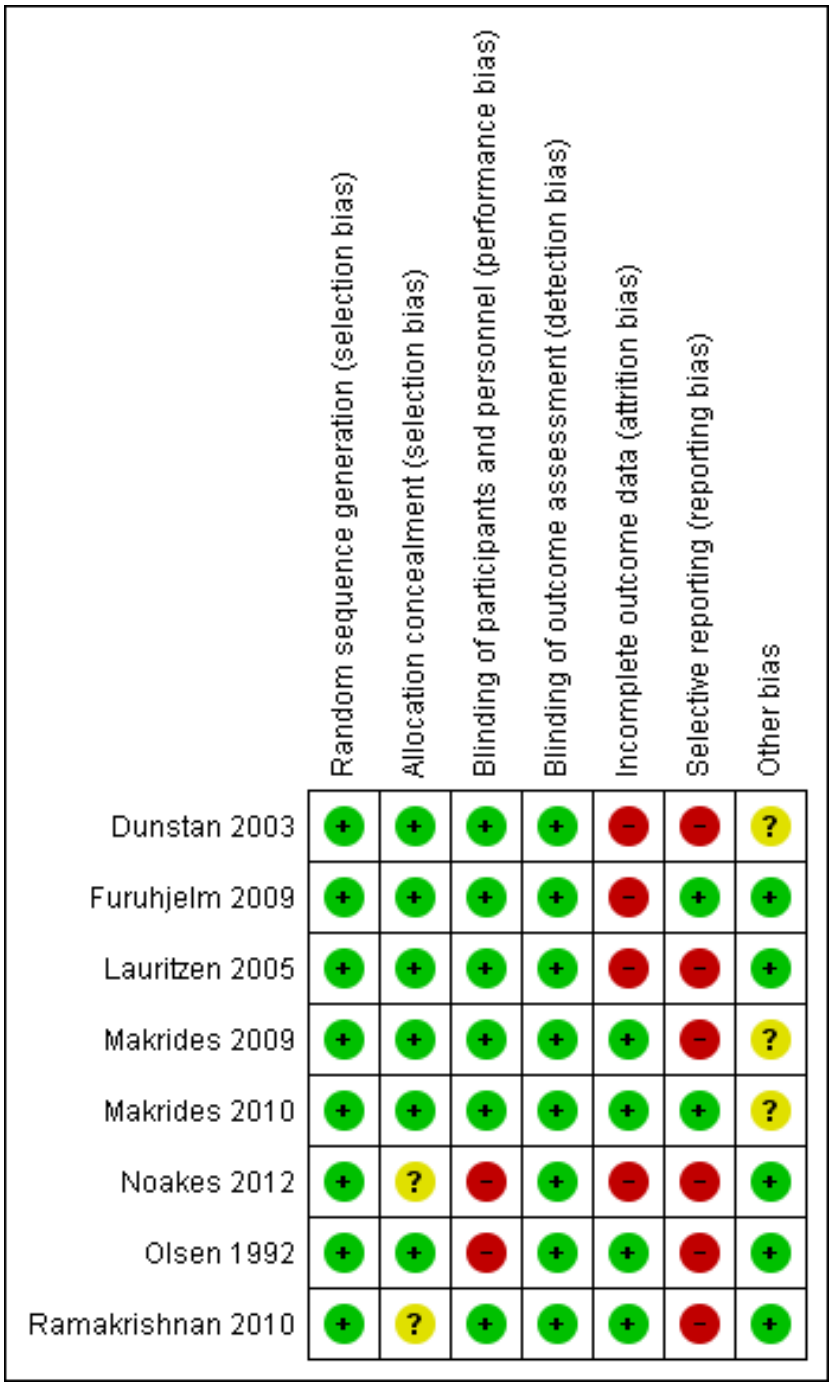


**Figure 3. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.**



### Allocation

All of the eight trials were at low risk of bias for sequence generation.

Six trials reported adequate allocation concealment methods (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2009; Makrides 2010: Olsen 1992) and two trials (Noakes 2012; Ramakrishnan 2010) had an unclear risk of bias as the method of allocation concealment was not described.

### Blinding

Women, care providers and research personnel were blinded in six trials (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2009; Makrides 2010; Ramakrishnan 2010). In two trials (Noakes 2012; Olsen 1992), women who were randomised to the 'no supplement' group could not be blinded.

Outcome assessments were performed by assessors who were blinded to the trial supplementations in all eight trials (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2009; Makrides 2010: Noakes 2012; Olsen 1992; Ramakrishnan 2010).

### Incomplete outcome data

All trials reported withdrawals and dropouts. We assessed four trials (Makrides 2009; Makrides 2010: Olsen 1992; Ramakrishnan 2010) as having a low risk of attrition bias. Makrides 2009 reported 6% of participant losses to follow-up at 18 months, 8% at three to five years of age and 10.5% at seven years of age. Olsen 1992 reported 1% of participant losses to follow-up at 16 years of age. Makrides 2010 reported participant follow-up losses of 3.5% and 9.6% at one and three years of age respectively. Ramakrishnan 2010 reported 25% participants losses to follow-up at six months, but balanced between group, and reasons for withdrawals were reported (intervention (23%) and control (24%)). Dunstan 2003, Furuhjelm 2009, Lauritzen 2005 and Noakes 2012 were assessed as having a high risk of attrition bias. Dunstan 2003 reported a loss of 15% to follow-up and participant exclusion after randomisation, which differed between intervention (n = 12, 23%) and control (n = 3, 6.5%). More women in the intervention group discontinued treatment because of nausea (n = 7, 13.5%) than in the control (n = 1, 2%). Furuhjelm 2009 reported a 19% loss of participants to follow-up (n = 16), with 23% in the treatment group (n = 9) and 12% in the placebo group excluded from the analysis because they did not complete the 15-week intervention. Lauritzen 2005 had large follow-up losses of more than 47%, with no reasons reported for the withdrawals (treatment n = 25, 40%; control n = 32, 53%). Noakes 2012 also had large follow-up losses (30%) at six months with no reasons reported (treatment n = 16, 23%; control n = 23, 37%).

### Selective reporting

All trials (Dunstan 2003; Lauritzen 2005; Makrides 2009; Noakes 2012; Olsen 1992; Ramakrishnan 2010) except two (Furuhjelm 2009; Makrides 2010) were assessed as having a high risk of reporting bias, as expected outcomes of interest to this review were not reported or were not reported completely.

### Other potential sources of bias

There were no obvious other potential sources of bias identified in four trials (Furuhjelm 2009; Lauritzen 2005; Noakes 2012; Ramakrishnan 2010). There was an unclear risk of bias in the two trials that included subgroups of their original trials (Makrides 2009; Makrides 2010). In Olsen 1992 the placebo group and no oil group were combined in this review. We conducted analyses with the n-3 LCPUFA supplementation group compared to olive oil control group separately to n-3 LCPUFA supplementation group compared to no supplement control group, and found that although the direction of effect differed it made little difference to the meta-analysis therefore for this review the control groups were combined. Dunstan 2003 was rated as having an unclear risk of bias because preterm infants were excluded from their analysis after randomisation.

## Effects of interventions

### N-3 LCPUFA supplementation versus placebo or no supplementation

#### Primary outcomes - any allergy

See: Table 1; Table 2.

We considered any allergy that included children with one or more allergy types including food allergy, atopic dermatitis (eczema), asthma/wheeze, allergic rhinitis (hay fever) as primary outcomes of the review. Allergic disease was considered in two ways. Firstly, the effect of n-3 LCPUFA supplementation on IgE-mediated allergic disease, then the effect of supplementation on all allergic disease (+/- IgE sensitivity) was analysed, for each of the allergic diseases under study. The effect of supplementation was analysed at various points in the child's life - short term (up to 12 months of age), medium term (12 to 36 months), and long term (36 months and beyond). We were unable to report cumulative incidences due to variation in reporting between studies.

### Any allergies (Analysis 1.1; Analysis 1.2)

N-3 LCPUFA supplementation showed a clear reduction in IgE-mediated allergies in 12 to 36 months of age children when compared with the control group (Analysis 1.1; Furuhjelm 2009; Makrides 2010, 823 children, risk ratio (RR) 0.66, 95% confidence interval (CI) 0.44 to 0.98). No clear differences were found in IgE-mediated allergies between treatments in 36 months of age or older children (Makrides 2010, 706 children, RR 0.86, 95% CI 0.0.61 to 1.20). No included trials reported on combined IgE-mediated allergies in infants under 12 months of age.

When all allergies (+/- IgE sensitivity) were considered, n-3 LCPUFA supplementation did not show clear differences in allergies in children at 12 to 36 months (Furuhjelm 2009, Makrides 2010, RR 0.89, 95% CI 0.71 to 1.11, 823 children) or 36 months and beyond (Makrides 2009, Makrides 2010, Olsen 1992, RR 0.96, 95% CI 0.84 to 1.09, 1765 children) (Analysis 1.2). No included trials reported on combined +/- IgE-mediated allergies in infants under 12 months of age.

## Secondary outcomes

See: Table 1; Table 2.

As secondary outcomes, we considered specific forms of allergy including food allergy, atopic dermatitis (eczema), asthma/wheeze and allergic rhinitis (hay fever) (IgE-mediated and +/- IgE sensitivity).

### Food allergy (Analysis 2.1; Analysis 2.2)

N-3 LCPUFA supplementation reduced the incidence of IgE-mediated food allergies in children up to 12 months of age (Furuhjelm 2009, 117 infants, RR 0.13, 95% 0.02 to 0.95; Analysis 2.1), but there were no clear differences found between the intervention and control groups at any other age (12 to 36 months Furuhjelm 2009; Makrides 2010, 825 children, average RR 0.58, 95% CI 0.18 to 1.88; > 36 months of age Makrides 2010, 706 children, RR 1.43, 95% CI 0.63 to 3.26). A random-effects analysis was used as substantial heterogeneity was noted at the 12- to 36-month time point ($Tau^2$ = 0.39; P = 0.15; $I^2$ = 51%) (Analysis 2.1). The heterogeneity may be due to the duration of the intervention, the dose used and the difference in assessment ages.

When food allergies +/- IgE sensitivity were considered (Analysis 2.2), results showed few differences from those for IgE-mediated allergies (Analysis 2.1) with no differences in the direction of findings from those for IgE-mediated allergies (up to 12 months of age, Furuhjelm 2009, 117 infants, RR 0.13, 95% CI 0.02 to 0.95; between 12 to 36 months, Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2010, 973 children, RR 0.72, 95% CI 0.40 to 1.30; > 36 months of age Makrides 2010, 706 children, RR 1.43, 95% CI 0.63 to 3.26).

### Eczema (Analysis 2.3; Analysis 2.4)

N-3 LCPUFA supplementation reduced the incidence of IgE-mediated eczema in children at 12 to 36 months of age (Furuhjelm 2009; Makrides 2010; 823 children, RR 0.61, 95% CI 0.39 to 0.95, Analysis 2.3). There were no clear differences between groups at the other time points (< 12 months Furuhjelm 2009; 117 children, RR 0.38, 95% CI 0.13 to 1.11 or > 36 months of age Makrides 2010, 706 children, RR 0.84, 95% CI 0.57 to 1.23)

When eczema outcomes +/- IgE sensitivity were considered (Analysis 2.4), results showed few differences from those for IgE-mediated eczema (Analysis 2.3), however the direction of effect was reversed for the 12- to 36-month age group (up to 12 months of age (Furuhjelm 2009; Noakes 2012, 203 infants, average RR 0.76, 95% CI 0.22 to 2.62; between 12 to 36 months, Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Makrides 2010, 973 children, average RR 0.96, 95% CI 0.69 to 1.33; > 36 months of age, Makrides 2009; Makrides 2010, 1237 children, average RR 0.88, 95% CI 0.68 to 1.13). A random-effects analysis was used all time points as substantial heterogeneity was noted at the 12-month time point ($Tau^2$ = 0.57; P = 0.06; $I^2$ = 71%) (Analysis 2.4).

### Allergic rhinitis (Analysis 2.5; Analysis 2.6)

No clear difference was seen between n-3 LCPUFA and control groups in either IgE-mediated allergic rhinitis (Analysis 2.5: 12 to 36 months, Furuhjelm 2009; Makrides 2010, 825 children, RR 0.47, 95% CI 0.07 to 3.06; > 36 months of age, Makrides 2010, 706 children, RR 0.83, 95% CI 0.44 to 1.54 or allergic rhinitis +/- IgE sensitivity (Analysis 2.6:12 to 36 months, Furuhjelm 2009, Makrides 2010, 805 children, RR 0.53, 95% CI 0.25 to 1.12; > 36 months of age, Makrides 2009, Makrides 2010, 1169 children, RR 1.03, 95% CI 0.81 to 1.30) across any age group. No included trials reported allergic rhinitis outcomes in infants under 12 months of age.

### Asthma (Analysis 2.7; Analysis 2.8)

No clear differences were found between n-3 LCPUFA and control groups in children with either IgE-mediated asthma (Analysis 2.7; 12 to 36 months, Furuhjelm 2009; Makrides 2010, 824 children, RR 0.86, 95% CI 0.21 to 3.49; > 36 months of age, Makrides 2010, 706 children, RR 1.10, 95% CI 0.34 to 3.58), or asthma +/- IgE sensitivity (Analysis 2.8; < 12 months, Noakes 2012, 83 infants, RR 1.26, 95% CI 0.54 to 2.94; 12 to 36 months, Dunstan 2003, Furuhjelm 2009, Lauritzen 2005, Makrides 2010, 955 children, RR 0.93, 95% CI 0.73 to 1.18; > 36 months of age, Makrides 2009, Makrides 2010, Olsen 1992, 1697 children, RR 0.94, 95% CI 0.78 to 1.13) across any age group. No included trials reported IgE-mediated asthma outcomes in infants under 12 months of age.

**Maternal safety (Analysis 3.1)**

There was no clear difference in postpartum haemorrhage (defined as > 500 mL of blood loss post delivery) in women supplemented with n-3 LCPUFA compared with those in the control group (Analysis 3.1; Makrides 2010; Olsen 1992, n = 2932, average RR 0.73, 95% CI 0.49 to 1.10). Given the substantial heterogeneity between trials, a random-effects model was used ($Tau^2$ = 0.05, P = 0.11; $I^2$ = 60%; Analysis 3.1). Postpartum infection was not reported in any of the included trials.

**Infant safety (Analysis 3.2)**

Infant safety was assessed using early childhood infections. Four trials reported this outcome (Makrides 2009; Makrides 2010; Noakes 2012; Ramakrishnan 2010, 2280 infants) with no clear difference between the n-3 LCPUFA and control group (RR 0.99; 95% CI 0.87 to 1.12). Ramakrishnan 2010 reported fever in 834 infants and found no differences between groups (RR 0.99; 95% CI 0.74 to 1.31; Analysis 3.2).

**Sensitisation to allergens (Analysis 4.1 to Analysis 4.9)**

See: Table 3.

Sensitisation is the strongest predictor of IgE-mediated allergy and was defined by a positive skin prick test to an allergen (de Jong 2011). **Sensitisation to egg** (Analysis 4.1) was reduced in the n-3 LCPUFA group compared with the control in 12- to 36 month-old children (Dunstan 2003; Furuhjelm 2009; Makrides 2010, 893 children, RR 0.55; 95% CI 0.39 to 0.77). No clear differences between groups were seen in egg sensitisation in children up to 12 months of age (Furuhjelm 2009; Noakes 2012) or in children 36 months or older (Makrides 2010).

**Sensitisation to cows' milk** was not different between the treatment groups at any age of assessment (Analysis 4.2), although no trials contained data for children aged 36 months or older. Given the substantial heterogeneity at 12 to 36 months, a random-effects model was used ($Tau^2$ = 0.48; P = 0.19; $I^2$ = 40%).

There were no clear differences between groups in **peanut sensitisation** at any time point (Analysis 4.3; no trials in infants under 12 months of age),

The effect of n-3 LCPUFA supplementation on **wheat sensitisation** was not different from the control group at any age (Analysis 4.4) and similar results were seen with **sensitisation to fish** (Analysis 4.5; no trials up to 12 months of age), **inhalant allergens (pollens)** (Analysis 4.6; no trials up to 12 months of age), **dust mites** (Analysis 4.7; no trials up to 12 months of age) and **cats** (Analysis 4.8).

When **all allergens** were considered (Analysis 4.9), no clear differences were found between treatments for infants up to 12 months and beyond 36 months. However, n-3 LCPUFA showed a clear reduction in sensitisation in 12 to 36 months of age children (Dunstan 2003; Furuhjelm 2009; Makrides 2010, 892 children, RR 0.70; 95% CI 0.53 to 0.94).

**Parent's report of allergies from non-validated questionnaires (Analysis 5.1 to Analysis 5.5)**

Three trials (Makrides 2009; Makrides 2010; Ramakrishnan 2010), included parents' reports of allergy collected using non-validated questionnaires. No clear differences were found between n-3 LCPUFA supplementation and control at any age in the incidence of parental reports of food allergies (Analysis 5.1), eczema (Analysis 5.2), allergic rhinitis (Analysis 5.3), asthma/wheeze (Analysis 5.4), or any allergies (Analysis 5.5).

### Subgroup analysis

Data were reported at last time point only in the subgroup analysis comparisons.

**Timing of supplementation (Analysis 6.1 to Analysis 6.2)**

Five trials included in the review confined supplementation with n-3 LCPUFA to the prenatal period only (Dunstan 2003; Noakes 2012; Olsen 1992; Makrides 2010; Ramakrishnan 2010). Ramakrishnan 2010 collected allergy outcome data using a non-validated questionnaire, therefore these data did not meet the inclusion criteria for the primary outcome. Two of the included trials supplemented women with n-3 LCPUFA in the postnatal period only (Lauritzen 2005; Makrides 2009), and only one trial (Furuhjelm 2009) supplemented women with n-3 LCPUFA through both prenatal and postnatal periods.

There were no significant subgroup differences for any of the outcomes (Analysis 6.1; Analysis 6.2).

**Allergy risk of the offspring (Analysis 5.1 to Analysis 5.5)**

Four trials (Dunstan 2003; Furuhjelm 2009; Noakes 2012; Makrides 2010) provided n-3 LCPUFA supplements to women whose fetuses were at high risk of allergy development with two reporting only IgE-mediated allergies (Furuhjelm 2009; Makrides 2010). Three trials studied the effect of n-3 LCPUFA supplementation on allergy in women with fetuses or women with infants who were not selected on the basis of allergy risk (Lauritzen 2005; Makrides 2009; Olsen 1992). There were no significant subgroup differences for any outcome (Analysis 7.1).

**Infant maturity**

Subgroup analyses based on infant maturity were not able to be conducted. Although data from preterm children were available for Makrides 2009, it was not possible to separate out children according to their gestational age in the remaining included trials (Furuhjelm 2009; Lauritzen 2005; Noakes 2012; Olsen 1992; Makrides 2010). Dunstan 2003 excluded preterm infants after randomisation.

### Sensitivity analysis

Sensitivity analyses were conducted for the primary outcome removing trials with high or unclear risk of selection, performance or attrition bias; Makrides 2009 and Makrides 2010 were the only trials with low risk of bias across these parameters. Removing trials with high or unclear risk of bias changed the direction for IgE-mediated any allergy at 12 to 36 months time point, but not beyond the 36 months time point, or for medically diagnosed IgE mediated and/or parental report any allergy outcome at any time points (Analysis 8.1; Analysis 8.2).

## DISCUSSION

### Summary of main results

Eight trials involving 3366 women with 3175 children were included in this review. Supplementation occurred during pregnancy, lactation or both pregnancy and lactation.

Overall, the available evidence shows that maternal n-3 LCPUFA (long chain polyunsaturated fatty acid) supplementation showed little benefit in the reduction of childhood allergic disease. There was no clear overall effect of maternal n-3 LCPUFA supplementation on the incidence of medically diagnosed or parental reports of allergy (+/- IgE sensitisation) including food allergy, eczema, allergic rhinitis, asthma/wheeze or any allergy. No reduction was observed with maternal n-3 LCPUFA supplementation on IgE-mediated allergic rhinitis or IgE-mediated asthma.

However, there were reductions in some outcomes such as IgE-mediated food allergy up to 12 months of age, IgE-mediated eczema between one and three years of age and the risk of developing any IgE-mediated allergic disease between one and three years of age. However, this needs to be interpreted with caution as IgE-mediated allergies were reported in only two trials, which only focused on high-risk populations of allergy.

There was also a reduction in the incidence of sensitisation to egg and any allergen in children in the n-3 LCPUFA group.

### Overall completeness and applicability of evidence

The majority of the evidence came from children of women supplemented with n-3 LCPUFA during pregnancy and/or lactation and from women with a fetus at high risk of allergy (who were supplemented during pregnancy). Most of the trials included in this review were conducted in high-income industrialised countries and the findings are therefore applicable to the most affluent societies where the burden of allergy is known to be high.

IgE-mediated allergies, where both the signs and symptoms of the allergic disease and a positive skin prick test (SPT) and/or radioallergosorbent test (RAST) were present, were reported in two trials (Furuhjelm 2009; Makrides 2010 and all trials except three (Lauritzen 2005; Makrides 2009; Ramakrishnan 2010), used medical diagnosis of allergy for the analyses (with IgE status not tested or unknown).

Most allergic responses are mediated by IgE antibodies, specific to the trigger allergen (Johansson 2001; Sicherer 2012). However, although the presence of IgE antibodies indicates a sensitised state, the most reliable diagnosis of allergic disease should take into account clinical history as well (Sicherer 2012). Thus, diagnoses involving laboratory tests (RAST), SPT and assessment of clinical symptoms are more reliable than clinical presentation or parental reports (using validated questionnaires), or laboratory reports alone.

As asthma is difficult to diagnose in young children, authors often report 'wheeze' (Lauritzen 2005; Makrides 2010; Noakes 2012; Ramakrishnan 2010); we therefore included reports of wheeze in asthma outcomes.

The selection of women differed with respect to fish intake, with three trials targeting women with a low fish intake (Dunstan 2003; Lauritzen 2005; Noakes 2012), while fish intake was not related to inclusion criteria in other trials (Furuhjelm 2009; Makrides 2009; Makrides 2010; Olsen 1992; Ramakrishnan 2010). One of the reasons for some of the mixed results in this review may be due to the mothers baseline intake of n-3 LCPUFA not being considered in some studies.

In the two trials where there was not an assigned control and women continued their 'usual diet' (Noakes 2012; Olsen 1992), the women in these groups may have had a high intake of dietary n-3 LCPUFA as the benefits of dietary n-3 LCPUFA in pregnancy was promoted in the countries in which these two trials were conducted.

In relation to diet and supplementation, some studies excluded women who were known to be allergic to fish (Dunstan 2003; Olsen 1992), as a safety precaution in case they may have reacted to fish oil capsules. Consequently, not many studies looked at fish allergy and hence data in this area may be limited (Dunstan 2003; Olsen 1992).

The evidence is incomplete for subgroup comparisons on the timing of supplementation, allergy risk of infants and maturity of infants. The trial in which n-3 LCPUFA supplementation started during pregnancy and continued through to lactation was limited by small sample size and high risk of attrition bias (Furuhjelm 2009).

### Quality of the evidence

The majority of the comparisons in this review were based on data from two trials (Furuhjelm 2009; Makrides 2010). The quality of the evidence for each of the comparisons greatly depends on the quality of these two trials. However, other primary outcome data regardless of IgE mediation were based on all included trials

except Ramakrishnan 2010. The risk of bias varied across the eight included trials with only two trials (Makrides 2009; Makrides 2010) with a low risk of selection, performance and attrition bias. Women's adherence to the supplementation may also impact on the outcomes. Blood analysis was used to check adherence to the supplementation in most trials (Dunstan 2003; Furuhjelm 2009; Lauritzen 2005; Noakes 2012; Olsen 1992; Makrides 2010), and all reported a significant n-3 LCPUFA increase in the intervention group. The use of fish, rather than fish oil as a supplement also needs consideration as it may contribute to the energy and protein content in the maternal diet and influence outcomes in a different way. In this review we included one trial (Noakes 2012) which supplemented with fish, however this trial had been designed to overcome any additional effect of the diet by using it as a replacement for white fish, chicken and some red meat; thus minimising any additional energy and protein contributions (personal communication Noakes 2012).

### Potential biases in the review process

Our search strategy was comprehensive and was not limited by language or publication status. We searched the major international and local bibliographic databases, handsearched major journals and the proceedings of major conferences in the field and set weekly current awareness alerts for a further 44 journals as well as monthly BioMed Central email alerts. We used a clear inclusion criteria and thorough quality assessment methodology to appraise each trial. Two review authors (AWG, CTC) independently screened and appraised the trials, and used pre-designed data extraction forms to extract data. Therefore, biases in the review process are unlikely. Review authors MM and CTC were investigators on two trials (Makrides 2009; Makrides 2010) included in the review. These trials were independently assessed for inclusion, risk of bias and data extracted by AWG and an independent researcher Karen Best (KB).

### Agreements and disagreements with other studies or reviews

Two systematic reviews (Klemens 2011; Kremmyda 2011), have been recently published on this topic. Kremmyda 2011 aimed to determine the effect of n-3 LCPUFA supplementation during the perinatal period on allergies (irrespective of IgE status) in children, but did not conduct a meta-analysis. Klemens 2011 conducted a meta-analysis and reported that n-3 LCPUFA supplementation during pregnancy reduced the incidence of childhood asthma but had no effect on atopic dermatitis. Their analysis did not include the most recent trials (Makrides 2009; Makrides 2010; Noakes 2012; Ramakrishnan 2010). Neither of the Klemens 2011 or Kremmyda 2011 reviews separated IgE-mediated allergies from allergies with or without IgE mediation.

## AUTHORS' CONCLUSIONS

### Implications for practice

Overall, there is limited evidence to support maternal n-3 LCPUFA supplementation during pregnancy and/or lactation for the reduction of allergic disease in the children with few differences seen in allergic disease in children between women who were supplemented with n-3 LCPUFA and those who were not. However, at some time points there were reductions in some outcomes such as IgE-mediated food allergy, IgE-mediated eczema and IgE-mediated any allergy, with n-3 LCPUFA supplementation in women with a fetus at high risk of allergy; therefore, further research is warranted.

### Implications for research

As the studies included in this review used differing doses, docosahexaenoic acid (DHA) to eicosapentaenoic acid (EPA) ratios and duration of n-3 LCPUFA supplementation, and did not take into account the baseline n-3 long chain polyunsaturated fatty acid (LCPUFA) status of the women, further research is needed to investigate the influence of these factors on childhood allergic outcomes. Trials should clearly differentiate between children at high risk of allergy and children at low risk of allergy given the suggestion of benefit to children who were at high risk of allergy. Trials should report both IgE-mediated allergy and allergy +/- IgE mediation and should include follow-up into the school years. Studies should also be conducted in other than high-income countries to determine the generalisability of findings.

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* *Indicates the major publication for the study*

# CHARACTERISTICS OF STUDIES

## Characteristics of included studies *[ordered by study ID]*

### Dunstan 2003

| | |
|---|---|
| Methods | Randomised controlled trial.<br>Dunstan 2003 was the main trial with 12 publications at different times with different outcomes. All 12 publications are included in the references to included studies |
| Participants | Setting: Australia.<br>98 pregnant atopic women whose fetus was considered to be at high risk of allergic disease. All women had a history of physician-diagnosed allergic rhinitis and/or asthma and 1 or more positive SPT to common allergens<br>Exclusions: women who smoked, had other medical problems, complicated pregnancies, seafood allergy or if normal dietary intake exceeded 2 meals of fish per week |
| Interventions | Intervention: 4 (1 g) n-3 LCPUFA capsules per day comprising a total of 3.7 g of n-3 LCPUFAs with 56.0% as DHA and 27.7% as EPA to give 2.07 g of DHA and 1.03 g of EPA (n = 52)<br>Control: 4 (1 g) capsules of olive oil per day containing 66.6% n-9 oleic acid and < 1% n-3 LCPUFAs (n = 46)<br>Duration of intervention: 20th week of gestation until delivery |
| Outcomes | Dunstan 2003 reported.<br>Primary outcomes: allergen-specific T-cell responses in cord blood<br>Secondary outcomes: medically-diagnosed allergies including incidence of asthma, atopic eczema, and food allergy at 1 year of age. The diagnosis of asthma was made in children with recurrent wheezing; i.e. 3 or more episodes with at least 1 episode confirmed by a paediatrician or general practitioner. Atopic eczema diagnosis was made in infants exhibiting typical skin lesions or physician-diagnosed eczema response to topical steroids. The severity was scored according to the modified assessment clinical tool called SCORing Atopic Dermatitis (SCORAD)<br>The SPT was performed using a standardised technique and allergen extracts (egg, milk, peanut, house dust mite, cat), the positive control was histamine and negative control glycerin, a wheel diameter of ≥ 2 mm was considered positive |
| Notes | Contacted authors to determine if they diagnosed IgE-mediated allergies - no response received to date<br>Supported by grants from the National Health and Medical Research Council and Raine Medical Research Foundation, Australia |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "The groups were block-randomised according to parity, prepregnancy BMI, age and maternal allergy" |

**Dunstan 2003** (*Continued*)

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Quote: "Randomization and allocation of capsules occurred at a different centre separate from the recruitment of participants. Capsules were administered to the participants by someone separate from those doing the allocation" |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | The capsules in the 2 groups were image matched, and the participants, research scientists, and paediatrician remained blinded to the groups for the duration of the trial |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Participants, research scientists, and paediatrician remained blinded to the groups for the duration of the trial |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Randomised n = 98 (52 intervention, 46 control).<br>At birth 15 (15%) excluded (12 treatment, 3 control): 8 discontinued intervention due to nausea (7 treatment, 1 control), 1 cord blood not collected (control), 4 gestation < 36 weeks (3 treatment, 1 control), 2 unrelated infant disease (treatment), (85% follow-up rate; intervention 77%, control 94%)<br>Outcomes were reported on n = 83 (85%) at 1 year of age (fish oil group n = 40, 77% and control group n = 43, 94%). Of the 83, telephone interviewed n = 11, clinic visit and SPT n = 72 |
| Selective reporting (reporting bias) | High risk | Trial registered at anzctr.org.au Identifier: ACTRN12611000041954<br>Prespecified outcomes were reported in this trial according to their protocol<br>Unable to determine IgE-mediated allergy from results reported although both medically diagnosed allergy and SPT results reported separately (authors contacted for information) |
| Other bias | Unclear risk | Preterm infants were excluded from their analysis after randomisation |

**Furuhjelm 2009**

| | |
|---|---|
| Methods | Randomised controlled trial.<br>Furuhjelm 2009 was the main trial with 6 reports. All 6 publications are included in the references to included studies |
| Participants | Setting: Sweden.<br>145 pregnant women who were at high risk of having a baby with atopy were recruited through antenatal care clinics during a 2-year period in 2003-2005. Women were considered at high risk if they, or their husband or an older child had current or previous allergic symptoms, i.e. bronchial asthma diagnosed by a doctor, atopic eczema, allergic food reactions, itching and running eyes and nose on exposure to pollen, pets or other known allergens |
| Interventions | Intervention: Women took 9 500 mg capsules a day containing 35% EPA, and 25% DHA, to provide 1.6 g of EPA and 1.1 g of DHA, n = 70<br>Control: 9 soy oil capsules a day, containing 58% LA to provide 2.5 g LA/day and 6% ALA to provide 0.28 g ALA/day, (n.=.75)<br>Duration of intervention: 25th week of gestation to delivery, encouraged to continue during lactation (average 3 to 4 months) |
| Outcomes | 1. Furuhjelm 2009 reported<br>Medically diagnosed allergy outcomes at 3, 6 and 12 months of age including: IgE antibody analysis, food allergy and eczema<br>Food allergy was defined as gastrointestinal symptoms, hives, aggravated eczema or wheeze following ingestion of egg or milk in the presence of detectable IgE antibodies or a positive SPT towards the particular food. Recovery from symptoms after elimination of the particular food from the diet and reoccurrence after ingestion of the food was required for the diagnosis. IgE-associated eczema was characterised as reoccurring and itching eczematous, lichenified or nummular dermatitis according to the criteria modified by Oranje 1995 in the presence of detectable IgE antibodies or positive SPT towards egg, milk or wheat<br>2. Furuhjelm 2011 reported<br>Medically diagnosed allergy outcomes at 2 years of age including: food allergy, eczema, allergic rhinitis, asthma and any allergies with or without IgE associated |
| Notes | The trial was supported financially by the Medical Research Council of Southeast Sweden (FORSS), The Östergötland County Council, The Ekhaga Foundation, Swedish Asthma and Allergy Association, The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), The Swedish Society of Medicine and Glaxo Smith Kline, Sweden |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "The mothers were randomly allocated to dietary supplementation either with ω-3 fatty acids or placebo" |

**Furuhjelm 2009** (*Continued*)

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Quote: "Producer performed the block randomisation". We interpreted this as central allocation |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote: "Active and placebo capsules could not be distinguished from each other" |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: "The research nurses, the paediatricians and the person performing the laboratory analyses were blinded during the intervention and follow-up" |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Randomised n = 145 (70 intervention, 75 control).<br>25 did not complete the requested 15 week intervention period (16, 23% treatment and 9, 12% placebo) and were excluded from the analysis, 1 withdrew post delivery, 2 not followed as moved before 6/12 follow up (group not stated). Total 28 (19%) not included in analysis, 117 included (81%)<br>SPT 117 (81%) at 6 months, 115 (79%) at 12 months.<br>Medically diagnosed allergy outcomes were reported on n = 117 (81%) (fish oil group n = 52 (74%) and control group n = 65 (87%), at 6 months and at 1 year of age<br>Medically diagnosed allergy outcomes were reported on n = 119 (82%) (fish oil group n = 54 (77%) and control group n = 65 (87%), at 2 years of age |
| Selective reporting (reporting bias) | Low risk | Trial registered at ClinicalTrials.gov identifier: NCT00892684<br>Prespecified outcomes were reported in this trial according to their protocol. Outcomes of interest to the review are reported |
| Other bias | Low risk | No obvious risk of other bias. |

**Lauritzen 2005**

| | |
|---|---|
| Methods | Randomised controlled trial.<br>Lauritzen 2004 was the main trial with 9 publications at different times with different outcomes. All 9 publications are included in the references to included studies |

| | |
|---|---|
| Participants | Setting: Denmark.<br>Recruited from among pregnant women recruited for the Danish National Birth Cohort study based on their intake of n-3 LCPUFA<br>Inclusion criteria included: an uncomplicated pregnancy, pre-pregnancy BMI < 30 kg/m², no metabolic disorders, and the intention to breastfeed for at least 4 months. Infants had to be healthy, term and singleton with normal weight for gestation and Apgar score > 7, women with a fish intake below the population median (< 0.4 g n-3 LCPUFA/day) were recruited for the randomised intervention trial (n = 122) and women with a fish intake in the upper quartile (> 0.8 g n-3 LC-PUFA/day) as a high-fish-intake reference group |
| Interventions | Intervention: microencapsulated fish oil given in muesli bars. The fish oil supplement provided 1.5 g/day of n-3 LCPUFA (equivalent to 4.5 g/day of fish oil) with 22.8% as DHA and 10% as EPA to provide 0.342 g per day of DHA and 0.15 g per day of EPA. As an alternative, the supplements were offered in homemade cookies or oil capsules (n = 62)<br>Control: microencapsulated olive oil given in muesli bars . As an alternative, the olive oil supplements were offered in homemade cookies or oil capsules (n = 60)<br>Referance group: 64 women with high fish intake (> 0.8 g n-3 LC-PUFA/day)<br>Duration of intervention: 4 months postpartum. |
| Outcomes | 1. Lauritzen 2004 reported<br>Primary outcomes - DHA content of breast milk and infant red blood cell membranes at 2 and 4 months of age, and infant visual acuity at 2 and 4 months of age<br>2. Lauritzen 2005 reported<br>Pirmary outcome - immune function assessed by interferon gamma and interleukin 10 production and plasma immunoglobulin E (IgE)<br>Secondary outcomes - parent report of doctor diagnosed allergy: food allergy, wheeze, eczema at 2.5 years of age |
| Notes | The high fish intake reference group participants were not included in our meta-analysis as this group was not randomised<br>The trial was funded by FOTEK-The Danish Research and Development program for Food Technology and BASF Aktiengesellschaft |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "After birth, the women with fish intakes below the 50th percentile were randomly assigned to a supplementation group by a randomisation schedule prepared by a person uninvolved in the study" |
| Allocation concealment (selection bias) | Low risk | Quote: "Owing to the non-identical appearance of the capsules for the two groups, a person who was not otherwise involved |

| | | |
|---|---|---|
| | | in the project handled the capsules in order to avoid breaking the blinding of the investigators" |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote: "Investigators and families were blinded to the randomisation" |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: "A person not otherwise involved in the study handled the capsules in order to avoid breaking the blinding of the investigators" |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Randomised: trial entry n = 122 (intervention 62, control 60)<br>At 2.5 years of age: n = 65 (53%) (fish oil group n = 37 (60%) and control group n = 28 (47%))<br>Large losses to follow-up, all noted as 'withdrawals' or no reason given |
| Selective reporting (reporting bias) | High risk | Trial registered at ClinicalTrials.gov identifier: NCT00266305<br>Most of the prespecified review outcomes were not reported in this trial |
| Other bias | Low risk | No obvious risk of other bias. |

**Makrides 2009**

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|---|---|
| Methods | Randomised controlled trial - the DINO trial.<br>Unpublished data reporting outcomes for subgroup of infants whose mothers were providing breast milk at trial entry from Makrides 2009 are included in this systematic review. There are 11 publications, 1 thesis and 1 dissertation from this trial. All publications, the thesis and dissertation details are included in the references to included studies |
| Participants | Setting: 5 Australian perinatal centres.<br>Infants born before 33 weeks' gestation and within 5 days of any enteral feeds were eligible to participate (included n = 657 infants and 545 women)<br>Excluded were infants 1) with major congenital or chromosomal abnormalities, 2) from a multiple birth in which not all live-born infants were eligible, or 3) were in other trials of fatty acid supplementation. Lactating women in whom tuna oil was contraindicated (for example, because of bleeding disorders or therapy with anticoagulants) were also excluded<br>To meet the inclusion criteria for this systematic review only infants whose mothers were supplying breast milk at trial entry were included (n = 603 infants; 92% of the 657 randomised to the trial; 297/322, 92% high-DHA and 306/335, 91%, control).<br>A follow-up of the first 143 infants who participated in the pilot phase was conducted |

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| | at 3 to 5 years corrected age (Simmonds 2007), again only infants whose mothers were providing breast milk at trial entry are included in this systematic review (n = 125)<br>A follow-up of was conducted at 7 years corrected age and n = 569 children completed ISAAC questionnaires, again only infants whose mothers were providing breast milk at trial entry are included in this systematic review (n = 531) |
| Interventions | Intervention: lactating women whose infants were randomly assigned to the high-DHA group consumed 6 x 500 mg DHA-rich tuna oil capsules per day which provided 900 mg DHA and 195 mg EPA. The intent was to achieve a breast milk DHA concentration that was1% of total fatty acids without altering the naturally occurring concentration of AA in breast milk. If supplementary formula was required, infants were given a high-DHA preterm formula (1% DHA and 0.6% AA)<br>Control: lactating women with infants allocated to the standard-DHA group consumed 6 500 mg placebo soy oil capsules with no n-3 LCPUFA. If supplementary formula was required in this group, a standard preterm infant formula was used (0.35% DHA and 0.6% AA)<br>Duration of the intervention: within 5 days from the infant receiving any enteral feeds until infants reached their expected date of delivery |
| Outcomes | 1. Makrides 2009 reported<br>Primary outcome: neurodevelopment at 18 months corrected age<br>Secondary outcomes: infection outcomes (in-hospital proven late onset sepsis).<br>2. Manley 2011 reported<br>Secondary outcomes: parental reported food allergy, eczema, asthma and allergic rhinitis at 18 months corrected age.<br>3. Simmonds 2007 reported<br>ISAAC questionnaire was used to collect parent report of allergy diagnosis and parent report of doctor diagnosis eczema, allergic rhinitis and asthma at 3 to 5 years corrected age. (The ISAAC questionnaire is not validated for this age group).<br>4. Gunaratne 2014 reported<br>ISAAC questionnaire was used to collect parent report of allergy outcomes at 7 years of corrected age and parent report of allergy outcomes from birth to 7 years of corrected age for eczema, allergic rhinitis and asthma. (The ISAAC questionnaire is validated for this age group.) |
| Notes | This study was supported by a grant from the Australian National Health and Medical Research Council (grant 250322) and by the Channel 7 Children's Research Foundation of South Australia Inc. Treatment and placebo capsules were donated by Clover Corp and infant formula was donated by Mead Johnson Nutritionals and Nutricia Australasia |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote "Mother-infant pairs were randomly assigned a unique study number through a computer-driven telephone randomisation service according to an independently generated randomisation sched- |

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| | | ule. Stratification was by centre, birth weight (1250 grams vs 1250 grams), and infant sex. Multiple births were considered a single randomisation unit and randomisation of twins or triplets was according to the sex and birth weight of the first born infant" |
| Allocation concealment (selection bias) | Low risk | Central allocation by a computer-driven telephone randomisation service |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "Parents, clinicians, and all research personnel were blinded to participant study group" |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote "Parents, clinicians, and all research personnel were blinded to participant study group" |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | A total of 603 infants whose mothers were providing breast milk at trial entry were enrolled in the trial (297 were randomised to the high DHA group and 306 to the control)<br>18 months corrected age: 565 (94%) completed the allergy follow up (274, 92% in the intervention and 291, 95% in the control)<br>3 to 5 years corrected age: 120 infants whose mothers in the pilot phase of the study were providing breast milk at trial entry (55 in intervention and 65 in control) , at 3 to 5 years corrected age 110 (92%) completed the allergy follow-up (49, 89% in the intervention and 61, 94% in the control)<br>7 years corrected age: 531 (88%) completed ISAAC questionnaire (260, 87.5% in the intervention and 271, 88.6% in the control) |
| Selective reporting (reporting bias) | High risk | Trial registered at anzctr.org.au Identifier: ACTRN12606000327583<br>Most of the prespecified review outcomes were not reported in this trial |
| Other bias | Unclear risk | The children included in this review were a subgroup of the primary DINO trial and included children of mothers who were |

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| | | providing breast milk at trial entry, 92% of the infants randomised to the primary trial met this criteria |

**Makrides 2010**

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|---|---|
| Methods | Randomised controlled trial.<br>Makrides 2010 was the main trial with 10 publications. All 10 publications and 1 thesis are included in the references to included studies |
| Participants | Setting: Australian maternity hospitals.<br>Recruitment to primary 'DOMInO' trial (Makrides 2010): women with singleton pregnancies at less than 21 weeks' gestation<br>Excluded were women already taking a prenatal supplement with DHA, with a bleeding disorder in which tuna oil was contraindicated, were taking anticoagulant therapy, had a documented history of drug or alcohol abuse, were participating in another fatty acid trial, fetus had a known major abnormality, or were unable to give written informed consent or if English was not the main language spoken at home (n = 2399)<br>Pregnant women were approached to enter the allergy follow-up (Palmer 2012) after randomisation into the DOMInO trial. Only Adelaide-based women were eligible for the allergy follow-up. Women were eligible if the unborn baby had a mother, father, or sibling with a history of any medically diagnosed allergic disease (asthma, allergic rhinitis, eczema) (n = 706) |
| Interventions | Intervention: 3 x 500 mg capsules daily of DHA rich fish oil concentrate, providing 800 mg of DHA and 100 mg of EPA per day<br>Control: 3 500 mg vegetable oil capsules daily without DHA. |
| Outcomes | 1. Makrides 2010 reported<br>Primary outcome - maternal postnatal depression at 6 weeks and 6 months; child neurodevelopment at 18 months of age<br>Secondary outcomes included a range of clinical outcomes including postpartum haemorrhage<br>2. Palmer 2012 reported<br>Primary outcome: At 1 year of age<br>1) IgE-associated allergic diseases including:<br>food allergy, eczema, asthma, allergic rhinitis and any allergy with sensitisation<br>2) Medically diagnosed allergic diseases with or without IgE-mediated allergic diseases including:<br>food allergy, eczema, asthma/wheeze, allergic rhinitis and any allergy with or without sensitisation based on medically diagnosed allergy at the age of 1 year<br>Secondary outcomes included IgE sensitisation - SPT at 1 year of age. Infants with respiratory tract infections between birth and 1 year<br>3. Palmer 2013 reported<br>the 3-year follow-up of the same children in Palmer 2012 to evaluate medically diagnosed allergy.<br>Reported IgE-associated allergic diseases including food allergy, eczema, asthma, allergic rhinitis and any allergy with sensitisation at 3 years of age<br>Obtained medically diagnosed allergic diseases with or without IgE-mediated allergic |

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| | diseases including: food allergy, eczema, asthma/wheeze, allergic rhinitis and any allergies with or without sensitisation at the age of 3 years<br>4. Gunaratne 2014 reported<br>parental reports of doctor diagnosed allergy outcomes including food allergy, eczema, asthma, allergic rhinitis and any allergy below 36 months of age.<br>Modified ISAAC questions were used to collect parent report of doctor diagnosed eczema, allergic rhinitis and asthma from birth to 3 years of age. (The ISAAC questions are not validated for this age group.) |
| Notes | Supported by grants from the Australian National Health and Medical Research council and Australian Egg Corporation Limited. Treatment and placebo capsules were donated by Efamol, UK |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " Women were randomly allocated to a unique number that corresponded to treatment or control through a computer driven telephone randomisation service according to an independently generated randomisation schedule, with balanced variable sized blocks. Stratification was by centre and parity" |
| Allocation concealment (selection bias) | Low risk | Computer-driven telephone randomisation service. |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | To maintain the blind, both active and placebo capsules were identical in appearance |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Neither the parents nor the research staff were aware of the treatment allocated. Described as "double-blind" |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | 681/706 (96.5%) infants (intervention: 357/368, 97%,placebo: 324/338, 96%) attended their 1-year medical review and 666/706 (94.3%) infants had SPT results. Missing data were imputed for all IgE-mediated allergy outcomes and medially diagnosed food allergy, eczema and any allergy but not imputed for medically diagnosed asthma (wheeze) and allergic rhinitis. In secondary outcomes missing data were imputed for egg, cow's milk. peanut and any SPT sensitisation but not for wheat, fish, |

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| | | pollen, house dust mite and cat allergens<br>638/706 (90.4%) infants (intervention: 333/368, 90.5%placebo: 305/338, 90.2%) attended their 3-year medical review and 587/706 (83.1%) infants had SPT results; missing data were imputed for all IgE-mediated allergy outcomes and medically diagnosed food allergy, eczema and any allergy but not imputed for medically diagnosed asthma (wheeze) and allergic rhinitis. In secondary outcomes missing data were imputed for egg, wheat, peanut, fish, cat and any allergens but not for pollens and house dust mite allergens. Parent reported allergy: below 12 months of age - 695/706 (98.4%) (intervention: 362/368, 98.4%, placebo: 335/338, 99%), between 12- 36 months of age - 698/706 (99%) , (intervention: 365/368, 99%, placebo: 333/338, 98.5%) and at 36 months of age - 638/706 (90%) (intervention: 333/368, 90.5%, placebo: 305/338, 90%) were available. Missing data were not imputed for any parent reported allergy outcomes<br>Results using imputed data were reported to differ little from raw data (Palmer 2012; Palmer 2013). |
| Selective reporting (reporting bias) | Low risk | Trial registered at anzctr.org.au Identifier: ACTRN12605000569606<br>Prespecified outcomes were reported in this trial according to their protocol. Most of the outcomes of interest to the review are reported |
| Other bias | Unclear risk | Of the original DOMInO trial (n = 2399), a subgroup of mothers whose unborn child had a family history of allergies were included |

**Noakes 2012**

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|---|---|
| Methods | Randomised controlled trial.<br>Miles 2011 was the main trial with 13 reports. All 13 publications are included in the references to included studies |
| Participants | Setting: United Kingdom.<br>Women aged 18 to 40 years who were at 19 weeks' gestation with a healthy uncomplicated pregnancy who reported low habitual consumption of oily fish (2 portions/month |

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| | excluding canned tuna and no use of fish oil supplements in previous 3 months) and had a family history of atopy (1 or more first-degree relatives of the infant affected by atopy, asthma, or allergy) were recruited to the trial (n = 123). Excluded were those participating in another research study, known diabetes, autoimmune disease, learning disability, terminal illness or mental health problems |
| Interventions | Intervention: women in the salmon group (n = 62) were asked to incorporate 2 portions of salmon (300 g) which contained 7.12 g total n-3 LCPUFAs (1.14 g EPA/week and 2. 32 g DHA/week) into their diet from week 20 (trial entry) until they gave birth. Women and their partners were provided with 2 portions of salmon per week to incorporate into their diet. They also received a cookbook that provided recipes for preparing and cooking salmon. Each portion of salmon was to replace a serve of protein (e.g. white fish, chicken or red meat)<br>Control: women in the control group were asked to continue their habitual diets; these women received the information sheet that described the possible health benefits of consuming oily fish during pregnancy and the government recommendation that pregnant women consume 1 or 2 oily fish meals/week. They also received a cookbook providing recipes for healthy eating during pregnancy |
| Outcomes | 1. Miles 2011 reported<br>Primary outcome - determine the effect on maternal and umbilical cord plasma n-3 LCPUFA content<br>2. Noakes 2012 reported<br>Effect on neonatal immune responses and diagnosis, by research nurse, of eczema and wheeze at 6 months of age<br>Chest infections and SPT at 6 months of age. |
| Notes | Supported by the European Commission under Framework 6: Sustainable aqua feeds to maximize the health benefits of farmed fish for consumers (Aquamax; FOOD-CT-2006-16249). 2 researches were supported by the Southampton NIHR Biomedical Research Unit in Nutrition, Diet & Lifestyle. Salmon was donated by the University of Bergen, Norway |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote "The women were allocated to one of two groups according to a previously generated random number table" |
| Allocation concealment (selection bias) | Unclear risk | Not described. |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | Participants were not blinded. |

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| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Researchers responsible for assessing outcome measures (both laboratory and clinical) remained blinded |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Enrolled 123 (62 salmon, 61 control).<br>At delivery: 107 (87%): 53 (85%) salmon, 54 (89%) control.<br>Birth samples: 101 (82%): 51 (82%) salmon, 50 (82%) control.<br>6-month clinic visit: 86 (70%): 48 (77%) salmon, 38 (62%) control |
| Selective reporting (reporting bias) | High risk | Trial registered at clinicaltrials.gov as NCT00801502.<br>Only limited data were reported on some of the prespecified review outcomes |
| Other bias | Low risk | There is no obvious risk of other bias. |

**Olsen 1992**

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|---|---|
| Methods | Randomised controlled trial.<br>Olsen 1992 was the main trial with 16 publications. All 16 publications are included in the references to included studies |
| Participants | Setting: Denmark.<br>Pregnant women were recruited from the midwife clinic at 30 weeks' gestation. Exluded were women with a history of placental abruption in a previous pregnancy or a serious bleeding episode in the present pregnancy, women who used prostaglandin inhibitors regularly, multiple pregnancies, allergy to fish, and regular intake of fish oil (n = 533) |
| Interventions | Intervention: 4 x 1 g fish oil capsules daily containing 32% EPA and 23% DHA to provide 1.28 g EPA and 0.92 g DHA<br>Control group 1: 4 x 1 g capsules of olive oil daily.<br>Control group 2: no supplement.<br>Duration of intervention: 30th week of gestation until delivery |
| Outcomes | 1. Olsen 1992 reported<br>Primary outcome - pregnancy duration.<br>Secondary outcomes included side effects and complications including postpartum bleeding<br>2. Olsen 2008 reported<br>Primary outcome - asthma at 16 years of age.<br>Secondary outcomes - combined outcome of asthma, atopic dermatitis and allergic rhinitis at 16 years of age<br>Medically diagnosed (from a mandatory registry that recorded diagnoses from hospitals in Denmark) allergy outcomes |

**Olsen 1992** (*Continued*)

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| Notes | For this systematic review control groups 1 and 2 were combined to compare with the intervention group (see 'Risk of bias' table) |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote "Women were randomly assigned to the three groups in the ratio 2/1/1. Randomisation was stratified by parity and arranged in balanced blocks of between 8 and 12" |
| Allocation concealment (selection bias) | Low risk | Quote "Sealed, opaque envelope for that study number contained a randomisation number that either identified a particular package of oil capsules or showed that the woman should receive no oil supplement" |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | While group 1 and 2 were blinded, group 3 received no supplement and were therefore unblinded |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Independant evaluation, registry based diagnosis. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Randomised 533 (intervention 266; olive oil 136; and no oil 131)<br>Assessed at 16 years 528 (99%) (intervention 263; olive oil 136; and no oil 129) |
| Selective reporting (reporting bias) | High risk | Trial registered at ClinicalTrials.gov identifier: NCT01353807<br>Most of the prespecified outcomes were reported in this trial according to their protocol. However, outcomes of interest to the review are not reported completely. Only limited data were reported on some of the prespecified review outcomes |
| Other bias | Low risk | Placebo group and no oil group were combined in the review. Analyses were conducted with the n-3 LCPUFA supplementation group compared to olive oil control group separately to n-3 LCPUFA supplementation group compared to no supplement control group, although the direction |

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| | | of effect differed it made little difference to the meta-analysis |

**Ramakrishnan 2010**

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| Methods | Randomised controlled trial.<br>Ramakrishnan 2010 was the main trial with 14 publications. All 14 publications are included in the references to included studies |
| Participants | Setting: Mexico.<br>Women were recruited at the Mexican Institute of Social Security (Instituto Mexicano del Seguro Social [IMSS]) General Hospital in Cuernavaca, Mexico, and 3 small health clinics within the IMSS system in Cuernavaca during routine prenatal care visits. Women were recruited for inclusion in the study if they were in gestation week 18 to 22, were aged 18 to 35 years, planned to deliver at the IMSS General Hospital in Cuernavaca, planned to predominantly breastfeed for at least 3 months, and planned to live in the area for 2 years after delivery. Women were excluded, if they had a (1) high-risk pregnancy, (2) lipid metabolism/ absorption disorders, (3) regular intake of fish oil or DHA supplements, or (4) chronic use of certain medications. (n = 1094) |
| Interventions | Intervention: 2 200 mg capsules daily of DHA rich algal oil concentrate, providing 400 mg of DHA per day<br>Control: 2 200 mg corn oil and soy oil capsules daily without DHA |
| Outcomes | Ramakrishnan 2010 reported<br>Primary outcome measures: birth size and gestational age.<br>Imhoff-Kunsch 2011<br>Outcome measures: immune function and morbidity.<br>Parental reports of wheeze were reported at 1 month, 3 months and 6 months of age . A non-validated questionnaire was used to collect data<br>Upper respiratory tract infections and fever were reported at 1 month, 3 months and 6 months of age<br>Escamilla-Nunez 2014<br>Outcome measures: respiratory symptoms in children at 18 months of age (reported as incidence rate according to atopy) |
| Notes | Contacted authors to obtain incidence of 18 month allergy outcomes<br>The research was supported by NIH (HD-043099) and the March of Dimes foundation |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Ouote "All eligible women were randomly assigned to either the treatment or the control group by use of a computer generated list created by the study biostatistician at Emory University. We used block random- |

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| | | ization to create balanced replicates of 4 treatments (2 colors for DHA and 2 for control) using a block size of 8" |
| Allocation concealment (selection bias) | Unclear risk | It is likely that participants and investigators enrolling participants could not foresee assignment because the assignment codes were placed in sealed envelopes and kept in a sealed location administered by a faculty member of the university who was not involved in the study. It is not stated if they were sequentially numbered nor if they were opaque |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | All participants and members of the study team were blinded to the treatment scheme throughout the intervention period of the study<br>Capsules were similar in appearance and taste. |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote "Data were unblinded for the analytical study team after the last infant in the study was born and had reached the age of 6 months at which time the participants were no longer taking supplements" |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | 1094 randomised, 834 assessed at 6 months - 76% follow-up rate, similar between intervention (77%) and control (76%)<br>At 18 months, 869 assessed according to mother atopy and treatment group - 82% follow-up rate, similar between intervention (80%) and control (78%)<br>54 randomised but did not begin treatment, 67 withdrew after beginning treatment (lack of family support, moved from area, disliked flavour, heartburn, nausea) |
| Selective reporting (reporting bias) | High risk | ClinicalTrials.gov Identifier: NCT00646360.<br>Most of the prespecified outcomes were reported in this trial according to their protocol. However, outcomes of interest to the review are reported incompletely and so cannot be used |
| Other bias | Low risk | No obvious risk of other bias. |

AA: arachidonic acid
ALA: alpha-linolenic acid
BMI: body mass index
DHA: docosahexaenoic acid
EPA: eicosapentaenoic acid
g: gram
IgE: immunoglobulin E
LA: linolenic acid
PUFA: polyunsaturated fatty acids
SPT: skin prick test
vs: versus

## Characteristics of excluded studies *[ordered by study ID]*

| Study | Reason for exclusion |
|---|---|
| Bergmann 2008 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Campos-Martinez 2012 | Excluded because this trial published as an abstract and did not report allergy outcome of the infants and/or children |
| Carlson 2013 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Colombo 2004 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Courville 2011 | Excluded because this trial did not report allergy outcome of the infants and/or children |
| Granot 2011 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Hauner 2009 | Excluded because the trial does not report allergy outcome of the infants and/or children |
| Helland 2001 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Innis 2007 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Judge 2007 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Karlsson 2010 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Knudsen 2006 | Excluded because the trial reported only timing of spontaneous delivery |
| Krauss-Etschmann 2007 | Excluded because the trial did not report allergy outcome of the infants and/or children |
| Martin-Alvarez 2012 | Excluded because the trial published as an abstract and did not report allergy outcome of the infants and/or children |
| Pena-Quintana 2011 | Excluded because this trial published as an abstract and did not report allergy outcome of the infants and/or children |

(*Continued*)

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|---|---|
| Ribeiro 2012 | Excluded because the trial did not report allergy outcome of the infants and/or children |

## Characteristics of ongoing studies *[ordered by study ID]*

**Bisgaard 2012**

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| Trial name or title | Fish oil supplementation during pregnancy for prevention of asthma, eczema and allergies in childhood: interventional trial in the COPSAC2010 |
| Methods | Randomised controlled trial. |
| Participants | Pregnant women from week 24. |
| Interventions | n-3 fatty acid start from week 24 gestation to 1 week after delivery.<br>Olive oil start from week 26 gestation to 1 week after delivery |
| Outcomes | Primary outcome measures: development af wheezy disorder from 0 to 3 years of age [time frame: 3 years]<br>Development of eczema from 0 to 3 years of age [time frame: 3 years]<br>Sensitisation at 18 months of age [time frame: 18 months]<br>Secondary outcome measures: development of asthma exacerbations from 0 to 3 years of age [time frame: 3 years].<br>Infections from 0 to 3 years of age [time frame: 3 years].<br>Growth [time frame: 0 to 3 years of age].<br>Cognitive, language and motor development [time frame: 2½ years] |
| Starting date | November 2008. |
| Contact information | Hans Bisgaard, MD, DMSc, Copenhagen University Hospital of Copenhagen, Gentofte, Denmark, 2820 |
| Notes | ClinicalTrials.gov identifier: NCT00798226. |

**Duchen 2012**

| | |
|---|---|
| Trial name or title | Combined dietary supplementation with *Lactobacillus reuteri* and omega-3 PUFA during pregnancy and postnatally in relation to development of IgE-associated disease during infancy |
| Methods | Randomised controlled trial. |
| Participants | Pregnant women. |
| Interventions | Dietary supplement: placebo.<br>Dietary supplement: omega-3 fatty acids.<br>Dietary supplement: refined coconut and peanut oil without *L. reuteri*.<br>Dietary supplement: *L. reuteri*. |

**Duchen 2012** (*Continued*)

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|---|---|
| Outcomes | Primary outcome measures: IgE-associated disease [time frame: 2 years of age]<br>A food reaction is defined as gastrointestinal symptoms, hives, aggravated eczema or wheezing following ingestion of a certain food with recovery after food elimination from the diet and reoccurrence of symptoms after ingestion of the particular food. Eczema is characterised as reoccurring, itching eczematous and lichenified or nummular dermatitis. Doctor diagnosed wheezing at least 3 times during the first 2 years is required for the diagnosis of asthma. If specific positive SPT or serum IgE antibodies is present, the food reaction, eczema in defined as IgE associated.<br>Secondary outcome measures: maternal gastrointestinal function [time frame: 20th gestational week to 6 months postpartum]<br>Maternal gastrointestinal function will be addressed by validated diary cards. The mothers will record every single stool, stool consistency, and corresponding defecatory symptoms (urgency, straining, and feeling of incomplete evacuation) for 7 days at gestational week 25 and 35. Stool consistency will be defined by the Bristol Stool Form Scale. The mothers will also record every meal, and episodes (start and ending time) of abdominal pain and bloating |
| Starting date | March 2012. |
| Contact information | Karel M Duchén, MD, PhD, Allergicentrum, Universitetssjukhuset, Linköping, Sweden, 58185. Tel +46-10-103 1355 |
| Notes | ClinicalTrials.gov Identifier: NCT01542970. |

**Laitinen 2013**

| | |
|---|---|
| Trial name or title | Nutrition and pregnancy intervention study |
| Methods | Randomised controlled trial. |
| Participants | Pregnant obese women. |
| Interventions | Dietary supplement: comparison of probiotics, fish oil and their combination to placebo |
| Outcomes | Primary outcome measures: prevalence of GDM [time frame: assessed at gestational weeks 24-28].<br>Fasting glucose levels [time frame: assessed at the third trimester of pregnancy].<br>Prevalence of allergy in child [time frame: assessed at 12 and 24 months of age].<br>Secondary outcome measures: need for medication for management of gestational diabetes mellitus GDM (insulin or metformin) [time frame: during pregnancy]<br>Body composition of mother [time frame: during and after pregnancy].<br>Immunologic and metabolic markers [time frame: during and after pregnancy].<br>Fecal microbiota [time frame: before, during and after intervention].<br>Other outcome measures: body composition, growth, development and metabolic markers of the child [time frame: 0 to 24 months of age] |
| Starting date | September 2013. |
| Contact information | Kirsi Laitinen, Adjunct professor, Turku University Hospital, Turku, Finland, 20521, + 358 02 333 6063, kirsi.laitinen@utu.fi |

**Laitinen 2013** (*Continued*)

| | |
|---|---|
| Notes | ClinicalTrials.gov identifier: NCT01922791. |

**Liu 2013**

| | |
|---|---|
| Trial name or title | The effects of polyunsaturated fatty acids (PUFA) on allergic/atopic dermatitis |
| Methods | November 2013. |
| Participants | Pregnant women between 16 and 20 weeks. |
| Interventions | Dietary supplement: DHA + EPA.<br>Dietary supplement: high olive oil.<br>Dietary supplement: DHA. |
| Outcomes | Primary outcome measures: lipid analysis [time frame: baseline, delivery, within 1 week after delivery, 6 weeks postpartum, 4 months postpartum].<br>Metabolomics study of PUFA [time frame: baseline, delivery, within 1 week after delivery, 6 weeks postpartum, 4 months postpartum, 12 months postpartum].<br>Skin prick test to common allergens [time frame: 4 months postpartum, 12 months postpartum].<br>Clinical assessment of IgE-mediated allergic eczema [time frame: 4 months postpartum, 12 months postpartum].<br>Secondary outcome measures: fatty acid desaturase (FADS) phenotypes [time frame: baseline].<br>Immunoglobulin E (IgE) antibodies [time frame: baseline].<br>Immunological biomarkers [time frame: 4 months postpartum, 12 months postpartum].<br>Medically-confirmed adverse events collected throughout the study period [time frame: 12 months postpartum] |
| Starting date | November 2013. |
| Contact information | Huimin Liu, International Peace Maternity and Child Health Hospital of China welfare Institute, Shanghai, Shanghai, China, 200030, 86-20-82156129, huimin.liu@mjn.com |
| Notes | ClinicalTrials.gov Identifier:NCT01936194. |

GDM: gestational diabetes mellitus
DHA: docosahexaenoic acid
EPA: eicosapentaenoic acid
PUFA: polyunsaturated fatty acids
SPT: skin prick test

## DATA AND ANALYSES

### Comparison 1. n-3 LCPUFA supplementation versus placebo or no supplementation - any allergy

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Any allergies (with sensitisation): medically diagnosed IgE mediated | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 1.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 1.2 12 to 36 months | 2 | 823 | Risk Ratio (M-H, Fixed, 95% CI) | 0.66 [0.44, 0.98] |
| 1.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.86 [0.61, 1.20] |
| 2 Any allergies (+/- sensitisation): medically diagnosed/parental reported | 4 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 2.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 2.2 12 to 36 months | 2 | 823 | Risk Ratio (M-H, Fixed, 95% CI) | 0.89 [0.71, 1.11] |
| 2.3 Beyond 36 months | 3 | 1765 | Risk Ratio (M-H, Fixed, 95% CI) | 0.96 [0.84, 1.09] |

### Comparison 2. n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Food allergies (with sensitisation): medically diagnosed IgE mediated | 2 | | Risk Ratio (M-H, Random, 95% CI) | Subtotals only |
| 1.1 Up to 12 months | 1 | 117 | Risk Ratio (M-H, Random, 95% CI) | 0.13 [0.02, 0.95] |
| 1.2 12 to 36 months | 2 | 825 | Risk Ratio (M-H, Random, 95% CI) | 0.58 [0.18, 1.88] |
| 1.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Random, 95% CI) | 1.43 [0.63, 3.26] |
| 2 Food allergies (+/- sensitisation): medically diagnosed/parental reported | 4 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 2.1 Up to 12 months | 1 | 117 | Risk Ratio (M-H, Fixed, 95% CI) | 0.13 [0.02, 0.95] |
| 2.2 12 to 36 months | 4 | 973 | Risk Ratio (M-H, Fixed, 95% CI) | 0.72 [0.40, 1.30] |
| 2.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 1.43 [0.63, 3.26] |
| 3 Atopic dermatitis/Eczema (with sensitisation): medically diagnosed IgE mediated | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 3.1 Up to 12 months | 1 | 117 | Risk Ratio (M-H, Fixed, 95% CI) | 0.38 [0.13, 1.11] |
| 3.2 12 to 36 months | 2 | 823 | Risk Ratio (M-H, Fixed, 95% CI) | 0.61 [0.39, 0.95] |
| 3.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.84 [0.57, 1.23] |
| 4 Atopic dermatitis/Eczema (+/- sensitisation): medically diagnosed/parental reported | 6 | | Risk Ratio (M-H, Random, 95% CI) | Subtotals only |
| 4.1 Up to 12 months | 2 | 203 | Risk Ratio (M-H, Random, 95% CI) | 0.76 [0.22, 2.62] |

| | | | | |
|---|---|---|---|---|
| 4.2 12 to 36 months | 4 | 973 | Risk Ratio (M-H, Random, 95% CI) | 0.96 [0.69, 1.33] |
| 4.3 Beyond 36 months | 2 | 1237 | Risk Ratio (M-H, Random, 95% CI) | 0.88 [0.68, 1.13] |
| 5 Allergic rhinitis/Hay fever (with sensitisation): medically diagnosed IgE mediated | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 5.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 5.2 12 to 36 months | 2 | 825 | Risk Ratio (M-H, Fixed, 95% CI) | 0.47 [0.07, 3.06] |
| 5.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.83 [0.44, 1.54] |
| 6 Allergic rhinitis/Hay fever (+/- sensitisation): medically diagnosed/parental reported | 3 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 6.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 6.2 12 to 36 months | 2 | 805 | Risk Ratio (M-H, Fixed, 95% CI) | 0.53 [0.25, 1.12] |
| 6.3 Beyond 36 months | 2 | 1169 | Risk Ratio (M-H, Fixed, 95% CI) | 1.03 [0.81, 1.30] |
| 7 Asthma/Wheeze (with sensitisation): medically diagnosed IgE mediated | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 7.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 7.2 12 to 36 months | 2 | 824 | Risk Ratio (M-H, Fixed, 95% CI) | 0.86 [0.21, 3.49] |
| 7.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 1.10 [0.34, 3.58] |
| 8 Asthma/Wheeze (+/- sensitisation): medically diagnosed/parental reported | 7 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 8.1 Up to 12 months | 1 | 83 | Risk Ratio (M-H, Fixed, 95% CI) | 1.26 [0.54, 2.94] |
| 8.2 12 to 36 months | 4 | 955 | Risk Ratio (M-H, Fixed, 95% CI) | 0.93 [0.73, 1.18] |
| 8.3 Beyond 36 months | 3 | 1697 | Risk Ratio (M-H, Fixed, 95% CI) | 0.94 [0.78, 1.13] |

**Comparison 3. n-3 LCPUFA supplementation versus placebo or no supplementation - maternal and infant safety**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Maternal safety | 2 | | Risk Ratio (M-H, Random, 95% CI) | Subtotals only |
| 1.1 Postpartum bleeding | 2 | 2932 | Risk Ratio (M-H, Random, 95% CI) | 0.73 [0.49, 1.10] |
| 2 Infants safety | 4 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 2.1 Early childhood infections | 4 | 2280 | Risk Ratio (M-H, Fixed, 95% CI) | 0.99 [0.87, 1.12] |
| 2.2 Fever | 1 | 834 | Risk Ratio (M-H, Fixed, 95% CI) | 0.99 [0.74, 1.31] |

**Comparison 4. n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Skin prick test results - egg | 4 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 1.1 Up to 12 months | 2 | 203 | Risk Ratio (M-H, Fixed, 95% CI) | 0.44 [0.19, 1.04] |
| 1.2 12 to 36 months | 3 | 893 | Risk Ratio (M-H, Fixed, 95% CI) | 0.55 [0.39, 0.77] |
| 1.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.63 [0.30, 1.34] |
| 2 Skin prick test results - cows' milk | 4 | | Risk Ratio (M-H, Random, 95% CI) | Subtotals only |
| 2.1 Up to 12 months | 2 | 205 | Risk Ratio (M-H, Random, 95% CI) | 0.75 [0.24, 2.29] |
| 2.2 12 to 36 months | 3 | 897 | Risk Ratio (M-H, Random, 95% CI) | 0.68 [0.20, 2.34] |
| 2.3 Beyond 36 months | 0 | 0 | Risk Ratio (M-H, Random, 95% CI) | 0.0 [0.0, 0.0] |
| 3 Skin Prick Test results - peanut | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 3.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 3.2 12 to 36 months | 2 | 778 | Risk Ratio (M-H, Fixed, 95% CI) | 0.61 [0.34, 1.08] |
| 3.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.60 [0.30, 1.18] |
| 4 Skin prick test results - wheat | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 4.1 Up to 12 months | 1 | 117 | Risk Ratio (M-H, Fixed, 95% CI) | 3.74 [0.16, 89.85] |
| 4.2 12 to 36 months | 2 | 783 | Risk Ratio (M-H, Fixed, 95% CI) | 1.40 [0.29, 6.84] |
| 4.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 2.76 [0.56, 13.56] |
| 5 Skin prick test results - fish | 1 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 5.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 5.2 12 to 36 months | 1 | 666 | Risk Ratio (M-H, Fixed, 95% CI) | 6.36 [0.33, 122.65] |
| 5.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 1.84 [0.17, 20.17] |
| 6 Skin prick test results - inhalant allergen (pollens) | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 6.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 6.2 12 to 36 months | 2 | 779 | Risk Ratio (M-H, Fixed, 95% CI) | 0.44 [0.08, 2.30] |
| 6.3 Beyond 36 months | 1 | 580 | Risk Ratio (M-H, Fixed, 95% CI) | 0.77 [0.44, 1.34] |
| 7 Skin prick test results - house dust mite | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 7.1 Up to 12 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 7.2 12 to 36 months | 2 | 738 | Risk Ratio (M-H, Fixed, 95% CI) | 0.76 [0.18, 3.28] |
| 7.3 Beyond 36 months | 1 | 580 | Risk Ratio (M-H, Fixed, 95% CI) | 0.84 [0.48, 1.46] |
| 8 Skin prick test results - cat | 3 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 8.1 Up to 12 months | 1 | 86 | Risk Ratio (M-H, Fixed, 95% CI) | 0.79 [0.05, 12.25] |
| 8.2 12 to 36 months | 2 | 738 | Risk Ratio (M-H, Fixed, 95% CI) | 1.07 [0.39, 2.94] |
| 8.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 1.91 [0.98, 3.75] |
| 9 Skin prick test results - any allergen | 4 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 9.1 Up to 12 months | 2 | 201 | Risk Ratio (M-H, Fixed, 95% CI) | 0.59 [0.32, 1.09] |
| 9.2 12 to 36 months | 3 | 892 | Risk Ratio (M-H, Fixed, 95% CI) | 0.70 [0.53, 0.94] |
| 9.3 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.95 [0.74, 1.22] |

**Comparison 5. n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire)**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Food allergies: parental reported (non-validated questionnaires) | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 1.1 Up to 12 months | 1 | 695 | Risk Ratio (M-H, Fixed, 95% CI) | 6.44 [0.33, 124.23] |
| 1.2 12 to 36 months | 1 | 565 | Risk Ratio (M-H, Fixed, 95% CI) | 0.82 [0.36, 1.83] |
| 1.3 Beyond 36 months | 0 | 0 | Risk Ratio (M-H, Fixed, 95% CI) | 0.0 [0.0, 0.0] |
| 2 Eczema: parental reported (non-validated questionnaires) | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 2.1 Up to 12 months | 1 | 695 | Risk Ratio (M-H, Fixed, 95% CI) | 0.90 [0.65, 1.26] |
| 2.2 12 to 36 months | 2 | 1263 | Risk Ratio (M-H, Fixed, 95% CI) | 0.91 [0.74, 1.13] |
| 2.3 Beyond 36 months | 2 | 746 | Risk Ratio (M-H, Fixed, 95% CI) | 0.95 [0.77, 1.18] |
| 3 Allergic rhinitis/Hay fever: parental reported (non-validated questionnaires) | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 3.1 Up to 12 months | 1 | 695 | Risk Ratio (M-H, Fixed, 95% CI) | 4.60 [0.22, 95.48] |
| 3.2 12 to 36 months | 1 | 565 | Risk Ratio (M-H, Fixed, 95% CI) | 0.71 [0.26, 1.96] |
| 3.3 Beyond 36 months | 1 | 109 | Risk Ratio (M-H, Fixed, 95% CI) | 1.56 [0.78, 3.12] |
| 4 Asthma/Wheeze: parental reported (non-validated questionnaires) | 3 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 4.1 Up to 12 months | 2 | 1529 | Risk Ratio (M-H, Fixed, 95% CI) | 1.07 [0.73, 1.56] |
| 4.2 12 to 36 months | 3 | 2134 | Risk Ratio (M-H, Fixed, 95% CI) | 0.94 [0.79, 1.11] |
| 4.3 Beyond 36 months | 2 | 745 | Risk Ratio (M-H, Fixed, 95% CI) | 1.02 [0.72, 1.45] |
| 5 Any allergies: parental reported (non-validated questionnaires) | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 5.1 Up to 12 months | 1 | 695 | Risk Ratio (M-H, Fixed, 95% CI) | 0.90 [0.65, 1.26] |
| 5.2 12 to 36 months | 1 | 565 | Risk Ratio (M-H, Fixed, 95% CI) | 0.95 [0.74, 1.21] |
| 5.3 Beyond 36 months | 1 | 110 | Risk Ratio (M-H, Fixed, 95% CI) | 0.92 [0.64, 1.30] |

**Comparison 6. Timing of supplementation - prenatal versus postnatal versus pre and postnatal subgroup**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Any allergies: last available time (with sensitisation): medically diagnosed IgE mediated | 2 | 823 | Risk Ratio (M-H, Fixed, 95% CI) | 0.82 [0.59, 1.13] |
| 1.1 Prenatal | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.86 [0.61, 1.20] |
| 1.2 Prenatal and postnatal | 1 | 117 | Risk Ratio (M-H, Fixed, 95% CI) | 0.52 [0.17, 1.59] |
| 2 Any allergies: last available time (+/- sensitisation): medically diagnosed/parental reported | 4 | 1882 | Risk Ratio (M-H, Fixed, 95% CI) | 0.95 [0.83, 1.07] |
| 2.1 Prenatal | 2 | 1234 | Risk Ratio (M-H, Fixed, 95% CI) | 0.90 [0.75, 1.07] |

| | | | | |
|---|---|---|---|---|
| 2.2 Postnatal | 1 | 531 | Risk Ratio (M-H, Fixed, 95% CI) | 1.05 [0.86, 1.28] |
| 2.3 Prenatal and postnatal | 1 | 117 | Risk Ratio (M-H, Fixed, 95% CI) | 0.78 [0.44, 1.38] |

**Comparison 7. Allergy risk - high risk versus unselected risk subgroup**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Any allergy: last available time | 4 | 1882 | Risk Ratio (M-H, Fixed, 95% CI) | 0.95 [0.83, 1.07] |
| 1.1 High risk | 2 | 823 | Risk Ratio (M-H, Fixed, 95% CI) | 0.91 [0.77, 1.07] |
| 1.2 Unselected risk | 2 | 1059 | Risk Ratio (M-H, Fixed, 95% CI) | 1.00 [0.82, 1.21] |

**Comparison 8. n-3 LCPUFA supplementation versus placebo or no supplementation - sensitivity analysis**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Any allergies (with sensitisation): medically diagnosed IgE mediated | 1 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 1.1 12 to 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.68 [0.44, 1.05] |
| 1.2 Beyond 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.86 [0.61, 1.20] |
| 2 Any allergies (+/- sensitisation): medically diagnosed/parental reported | 2 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 2.1 12 to 36 months | 1 | 706 | Risk Ratio (M-H, Fixed, 95% CI) | 0.91 [0.71, 1.16] |
| 2.2 Beyond 36 months | 2 | 1237 | Risk Ratio (M-H, Fixed, 95% CI) | 0.98 [0.86, 1.12] |

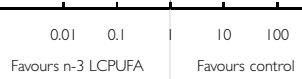
## Analysis 1.1. Comparison 1 n-3 LCPUFA supplementation versus placebo or no supplementation - any allergy, Outcome 1 Any allergies (with sensitisation): medically diagnosed IgE mediated.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 1 n-3 LCPUFA supplementation versus placebo or no supplementation - any allergy

Outcome: 1 Any allergies (with sensitisation): medically diagnosed IgE mediated

| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Up to 12 months | | | | | |
| **Subtotal (95% CI)** | **0** | **0** | | | **Not estimable** |
| Total events: 0 (n-3 LCPUFA), 0 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: not applicable | | | | | |
| 2 12 to 36 months | | | | | |
| Furuhjelm 2009 | 4/54 | 9/63 | | 15.6 % | 0.52 [ 0.17, 1.59 ] |
| Makrides 2010 | 32/368 | 43/338 | | 84.4 % | 0.68 [ 0.44, 1.05 ] |
| **Subtotal (95% CI)** | **422** | **401** | | **100.0 %** | **0.66 [ 0.44, 0.98 ]** |
| Total events: 36 (n-3 LCPUFA), 52 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.20, df = 1 (P = 0.65); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 2.03 (P = 0.042) | | | | | |
| 3 Beyond 36 months | | | | | |
| Makrides 2010 | 55/368 | 59/338 | | 100.0 % | 0.86 [ 0.61, 1.20 ] |
| **Subtotal (95% CI)** | **368** | **338** | | **100.0 %** | **0.86 [ 0.61, 1.20 ]** |
| Total events: 55 (n-3 LCPUFA), 59 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.90 (P = 0.37) | | | | | |



## Analysis 1.2. Comparison 1 n-3 LCPUFA supplementation versus placebo or no supplementation - any allergy, Outcome 2 Any allergies (+/- sensitisation): medically diagnosed/parental reported.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 1 n-3 LCPUFA supplementation versus placebo or no supplementation - any allergy

Outcome: 2 Any allergies (+/- sensitisation): medically diagnosed/parental reported

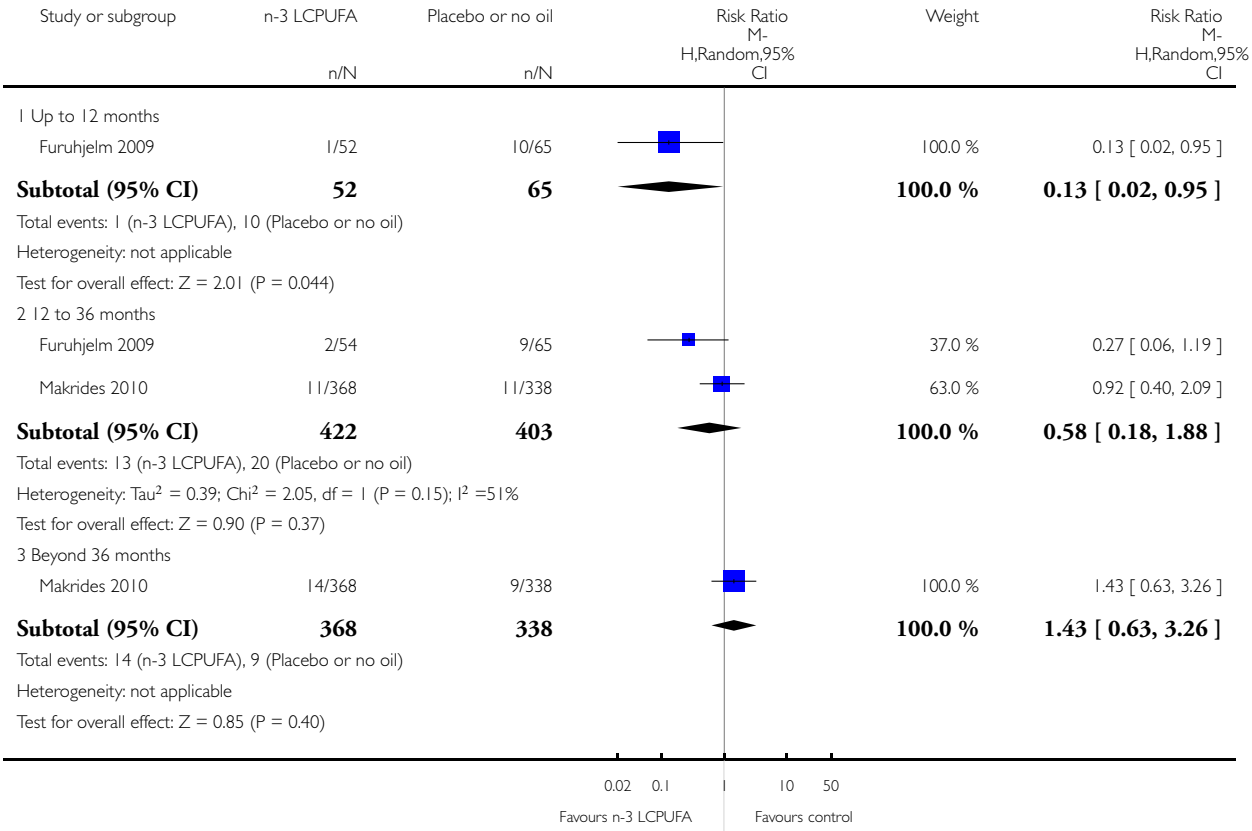
| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Up to 12 months | | | | | |
| **Subtotal (95% CI)** | **0** | **0** | | | **Not estimable** |
| Total events: 0 (n-3 LCPUFA), 0 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: not applicable | | | | | |
| 2 12 to 36 months | | | | | |
| Furuhjelm 2009 | 14/54 | 21/63 | | 16.2 % | 0.78 [ 0.44, 1.38 ] |
| Makrides 2010 | 95/368 | 96/338 | | 83.8 % | 0.91 [ 0.71, 1.16 ] |
| **Subtotal (95% CI)** | **422** | **401** | | **100.0 %** | **0.89 [ 0.71, 1.11 ]** |
| Total events: 109 (n-3 LCPUFA), 117 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.24, df = 1 (P = 0.62); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 1.05 (P = 0.29) | | | | | |
| 3 Beyond 36 months | | | | | |
| Makrides 2009 | 113/260 | 112/271 | | 39.5 % | 1.05 [ 0.86, 1.28 ] |
| Makrides 2010 | 146/368 | 145/338 | | 54.4 % | 0.92 [ 0.78, 1.10 ] |
| Olsen 1992 | 11/263 | 17/265 | | 6.1 % | 0.65 [ 0.31, 1.37 ] |
| **Subtotal (95% CI)** | **891** | **874** | | **100.0 %** | **0.96 [ 0.84, 1.09 ]** |
| Total events: 270 (n-3 LCPUFA), 274 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 2.04, df = 2 (P = 0.36); $I^2$ =2% | | | | | |
| Test for overall effect: Z = 0.64 (P = 0.52) | | | | | |

## Analysis 2.1. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 1 Food allergies (with sensitisation): medically diagnosed IgE mediated.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 1 Food allergies (with sensitisation): medically diagnosed IgE mediated

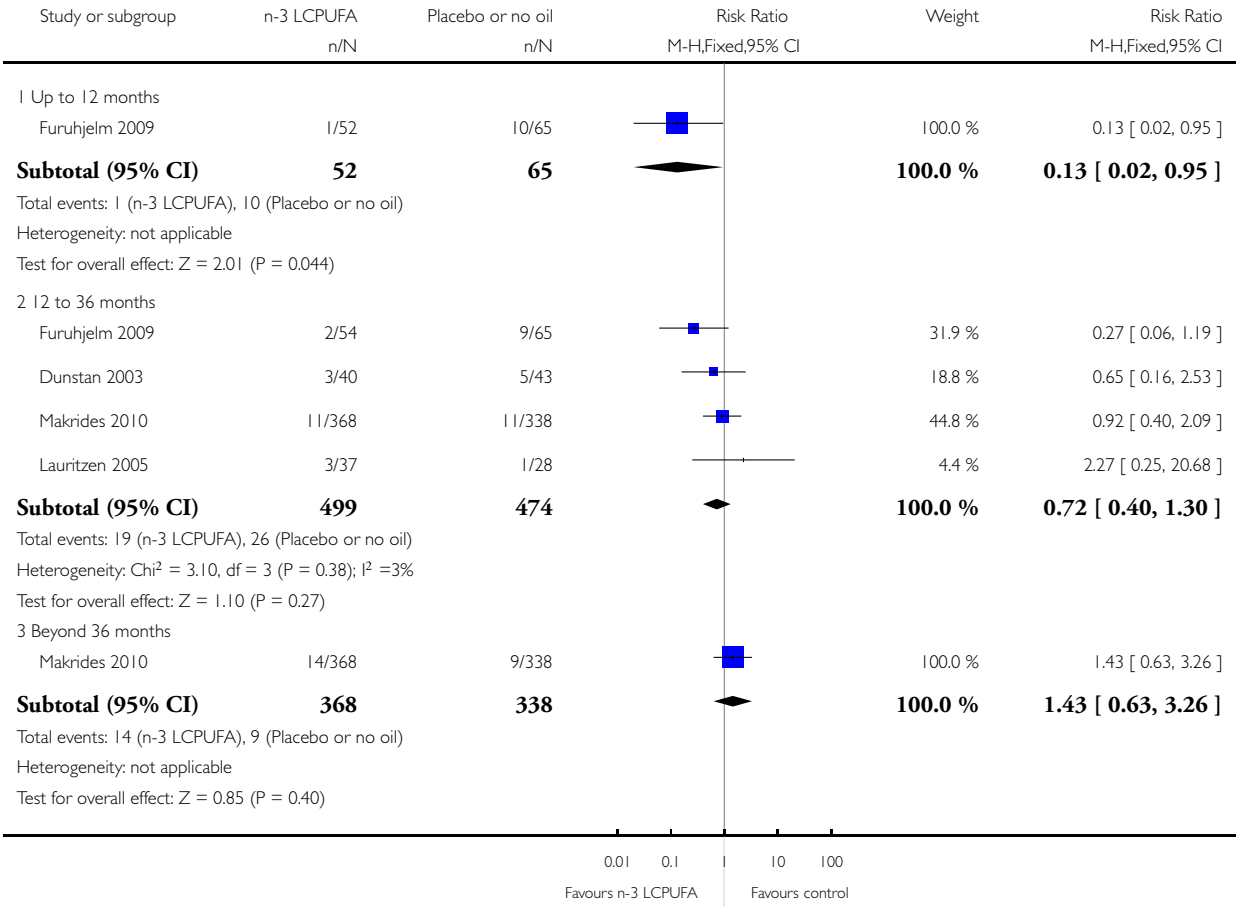


## Analysis 2.2. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 2 Food allergies (+/- sensitisation): medically diagnosed/parental reported.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 2 Food allergies (+/- sensitisation): medically diagnosed/parental reported

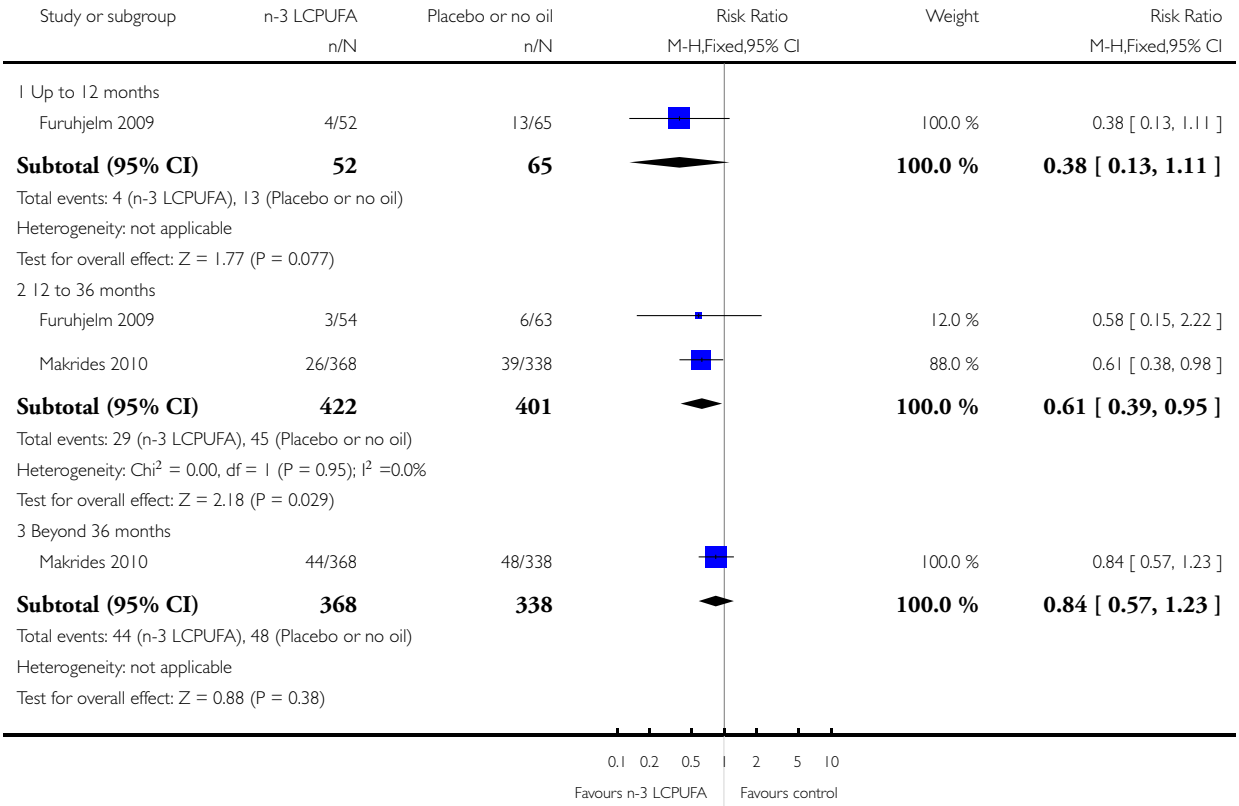


**Analysis 2.3. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 3 Atopic dermatitis/Eczema (with sensitisation): medically diagnosed IgE mediated.**

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 3 Atopic dermatitis/Eczema (with sensitisation): medically diagnosed IgE mediated

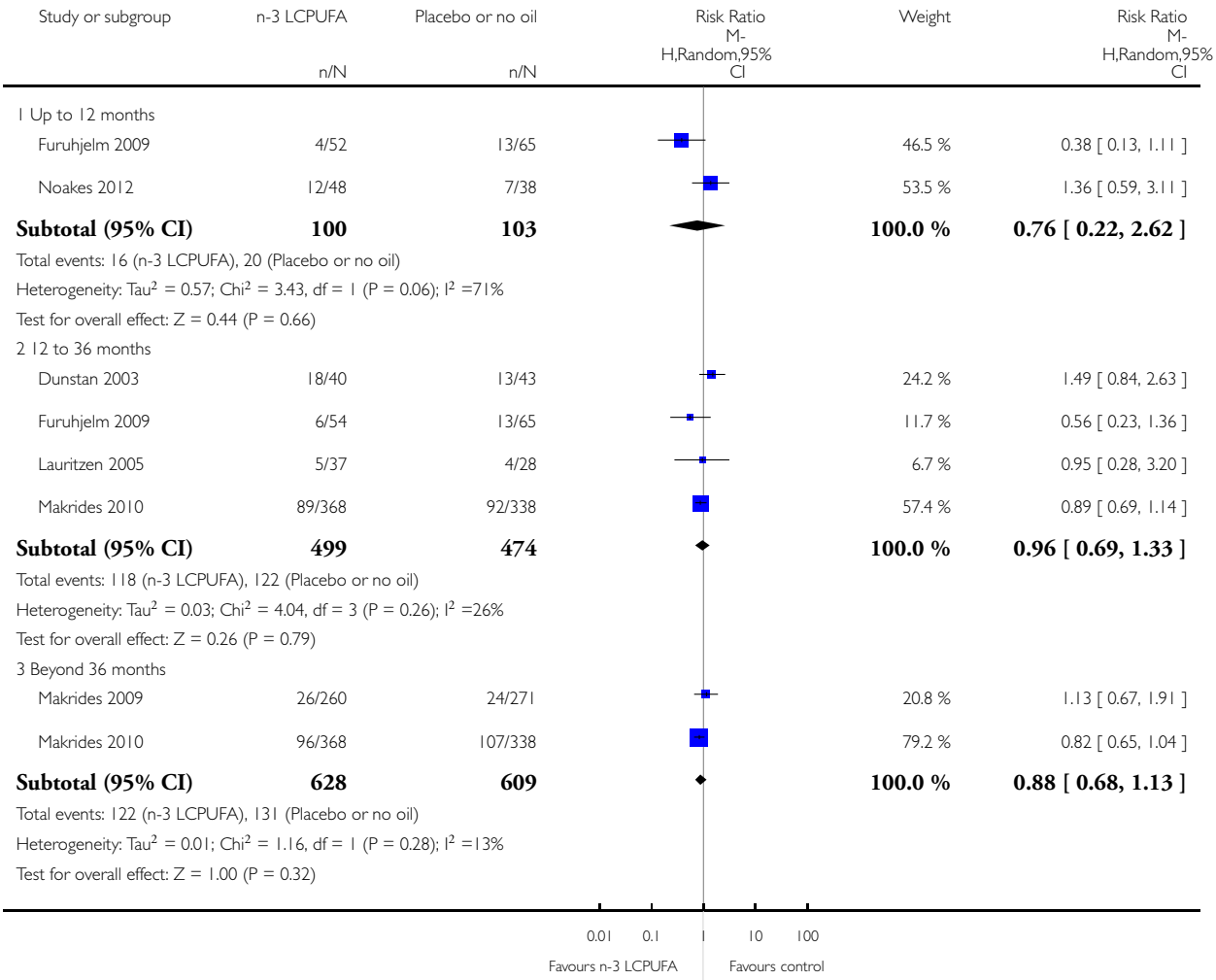


## Analysis 2.4. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 4 Atopic dermatitis/Eczema (+/- sensitisation): medically diagnosed/parental reported.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 4 Atopic dermatitis/Eczema (+/- sensitisation): medically diagnosed/parental reported

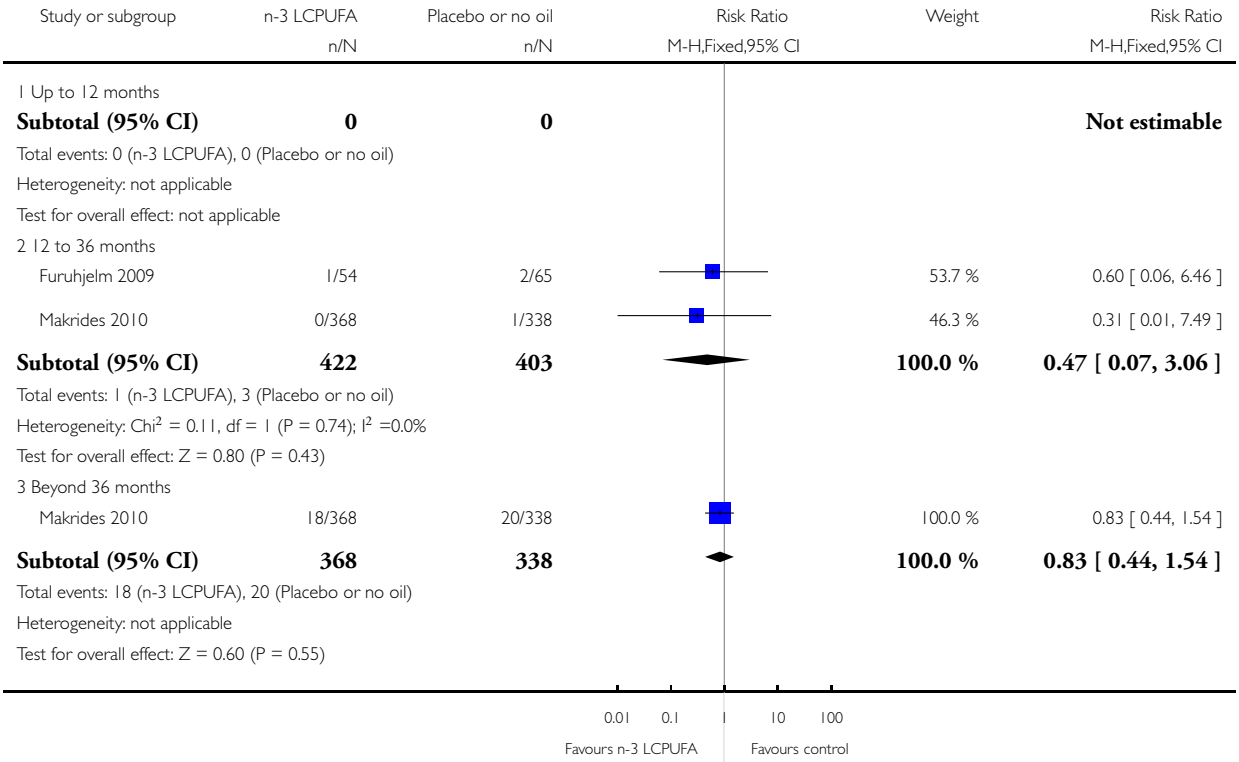


**Analysis 2.5. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 5 Allergic rhinitis/Hay fever (with sensitisation): medically diagnosed IgE mediated.**

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 5 Allergic rhinitis/Hay fever (with sensitisation): medically diagnosed IgE mediated



## Analysis 2.6. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 6 Allergic rhinitis/Hay fever (+/- sensitisation): medically diagnosed/parental reported.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 6 Allergic rhinitis/Hay fever (+/- sensitisation): medically diagnosed/parental reported

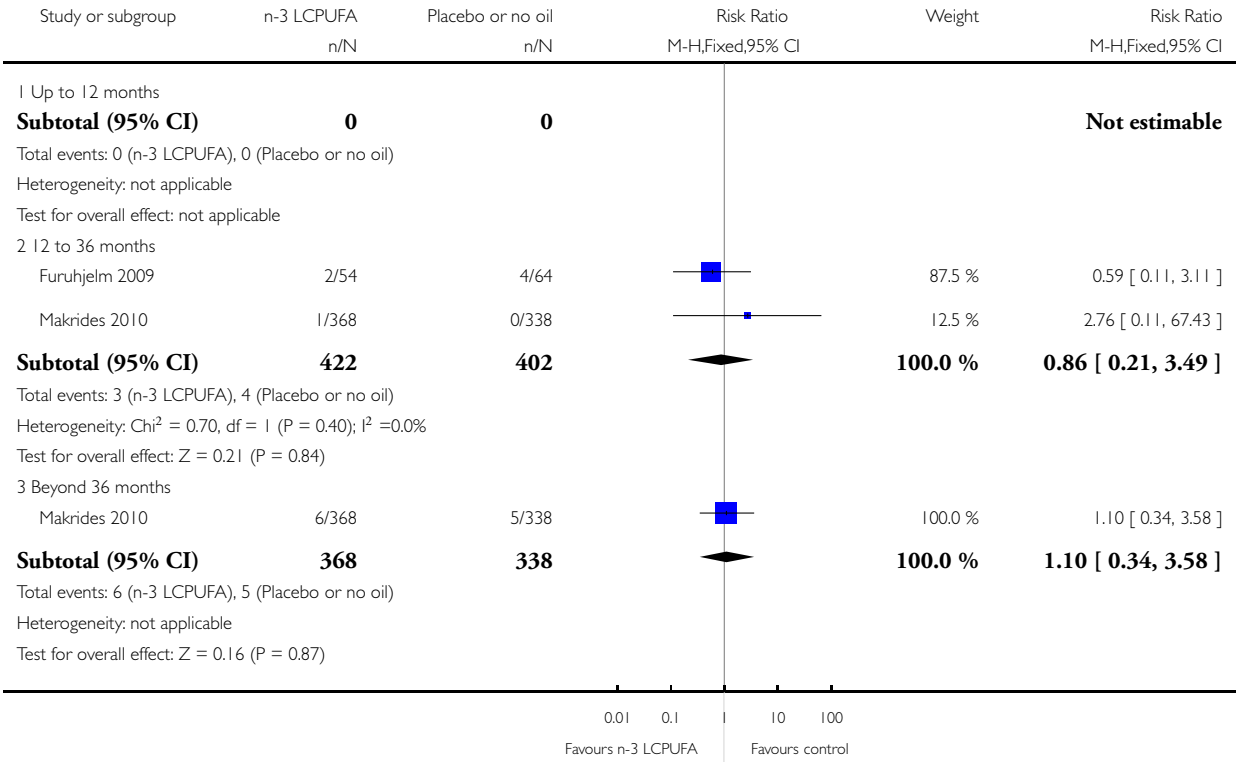
| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Up to 12 months | | | | | |
| **Subtotal (95% CI)** | **0** | **0** | | | **Not estimable** |
| Total events: 0 (n-3 LCPUFA), 0 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: not applicable | | | | | |
| 2 12 to 36 months | | | | | |
| Furuhjelm 2009 | 2/54 | 2/65 | | 9.8 % | 1.20 [ 0.18, 8.26 ] |
| Makrides 2010 | 8/360 | 16/326 | | 90.2 % | 0.45 [ 0.20, 1.04 ] |
| **Subtotal (95% CI)** | **414** | **391** | | **100.0 %** | **0.53 [ 0.25, 1.12 ]** |
| Total events: 10 (n-3 LCPUFA), 18 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.83, df = 1 (P = 0.36); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 1.67 (P = 0.095) | | | | | |
| 3 Beyond 36 months | | | | | |
| Makrides 2009 | 61/260 | 65/271 | | 58.1 % | 0.98 [ 0.72, 1.33 ] |
| Makrides 2010 | 53/333 | 44/305 | | 41.9 % | 1.10 [ 0.76, 1.59 ] |
| **Subtotal (95% CI)** | **593** | **576** | | **100.0 %** | **1.03 [ 0.81, 1.30 ]** |
| Total events: 114 (n-3 LCPUFA), 109 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.24, df = 1 (P = 0.62); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.25 (P = 0.80) | | | | | |

## Analysis 2.7. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 7 Asthma/Wheeze (with sensitisation): medically diagnosed IgE mediated.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 7 Asthma/Wheeze (with sensitisation): medically diagnosed IgE mediated



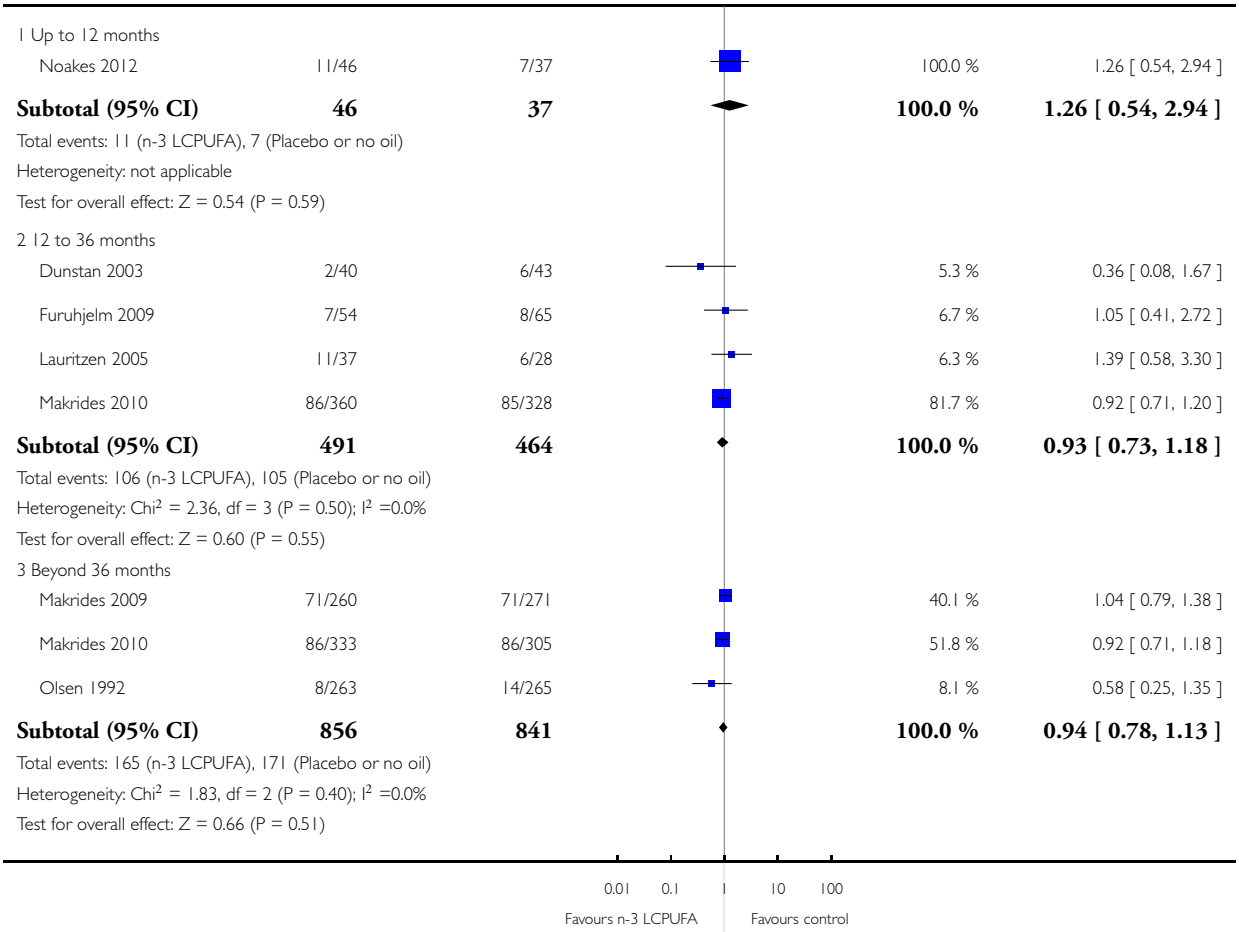
## Analysis 2.8. Comparison 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy, Outcome 8 Asthma/Wheeze (+/- sensitisation): medically diagnosed/parental reported.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 2 n-3 LCPUFA supplementation versus placebo or no supplementation - specific forms of allergy

Outcome: 8 Asthma/Wheeze (+/- sensitisation): medically diagnosed/parental reported

| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Up to 12 months | | | | | |
| Noakes 2012 | 11/46 | 7/37 | | 100.0 % | 1.26 [ 0.54, 2.94 ] |
| **Subtotal (95% CI)** | **46** | **37** | | **100.0 %** | **1.26 [ 0.54, 2.94 ]** |
| Total events: 11 (n-3 LCPUFA), 7 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.54 (P = 0.59) | | | | | |
| 2 12 to 36 months | | | | | |
| Dunstan 2003 | 2/40 | 6/43 | | 5.3 % | 0.36 [ 0.08, 1.67 ] |
| Furuhjelm 2009 | 7/54 | 8/65 | | 6.7 % | 1.05 [ 0.41, 2.72 ] |
| Lauritzen 2005 | 11/37 | 6/28 | | 6.3 % | 1.39 [ 0.58, 3.30 ] |
| Makrides 2010 | 86/360 | 85/328 | | 81.7 % | 0.92 [ 0.71, 1.20 ] |
| **Subtotal (95% CI)** | **491** | **464** | | **100.0 %** | **0.93 [ 0.73, 1.18 ]** |
| Total events: 106 (n-3 LCPUFA), 105 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 2.36, df = 3 (P = 0.50); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.60 (P = 0.55) | | | | | |
| 3 Beyond 36 months | | | | | |
| Makrides 2009 | 71/260 | 71/271 | | 40.1 % | 1.04 [ 0.79, 1.38 ] |
| Makrides 2010 | 86/333 | 86/305 | | 51.8 % | 0.92 [ 0.71, 1.18 ] |
| Olsen 1992 | 8/263 | 14/265 | | 8.1 % | 0.58 [ 0.25, 1.35 ] |
| **Subtotal (95% CI)** | **856** | **841** | | **100.0 %** | **0.94 [ 0.78, 1.13 ]** |
| Total events: 165 (n-3 LCPUFA), 171 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 1.83, df = 2 (P = 0.40); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.66 (P = 0.51) | | | | | |

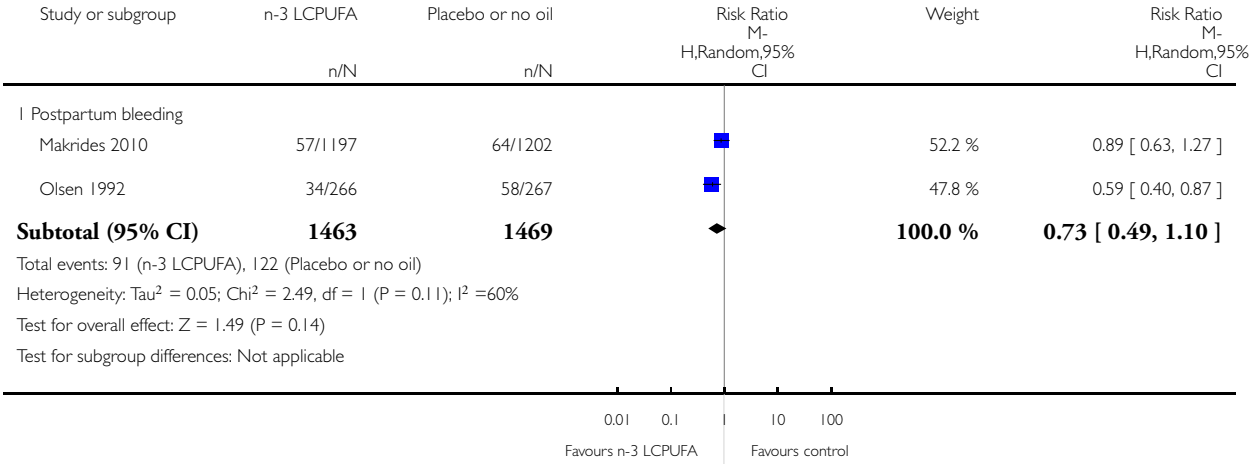


## Analysis 3.1. Comparison 3 n-3 LCPUFA supplementation versus placebo or no supplementation - maternal and infant safety, Outcome 1 Maternal safety.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 3 n-3 LCPUFA supplementation versus placebo or no supplementation - maternal and infant safety

Outcome: 1 Maternal safety



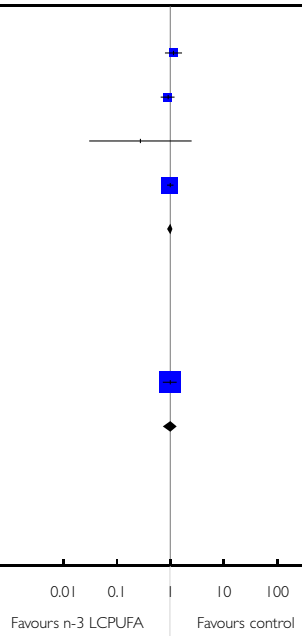
## Analysis 3.2. Comparison 3 n-3 LCPUFA supplementation versus placebo or no supplementation - maternal and infant safety, Outcome 2 Infants safety.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 3 n-3 LCPUFA supplementation versus placebo or no supplementation - maternal and infant safety

Outcome: 2 Infants safety

| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Early childhood infections | | | | | |
| Makrides 2009 | 53/322 | 48/335 | | 15.0 % | 1.15 [ 0.80, 1.65 ] |
| Makrides 2010 | 65/368 | 66/338 | | 21.9 % | 0.90 [ 0.66, 1.23 ] |
| Noakes 2012 | 1/46 | 3/37 | | 1.1 % | 0.27 [ 0.03, 2.47 ] |
| Ramakrishnan 2010 | 194/420 | 193/414 | | 62.0 % | 0.99 [ 0.86, 1.15 ] |
| **Subtotal (95% CI)** | **1156** | **1124** | | **100.0 %** | **0.99 [ 0.87, 1.12 ]** |
| Total events: 313 (n-3 LCPUFA), 310 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 2.32, df = 3 (P = 0.51); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.19 (P = 0.85) | | | | | |
| 2 Fever | | | | | |
| Ramakrishnan 2010 | 77/420 | 77/414 | | 100.0 % | 0.99 [ 0.74, 1.31 ] |
| **Subtotal (95% CI)** | **420** | **414** | | **100.0 %** | **0.99 [ 0.74, 1.31 ]** |
| Total events: 77 (n-3 LCPUFA), 77 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.10 (P = 0.92) | | | | | |

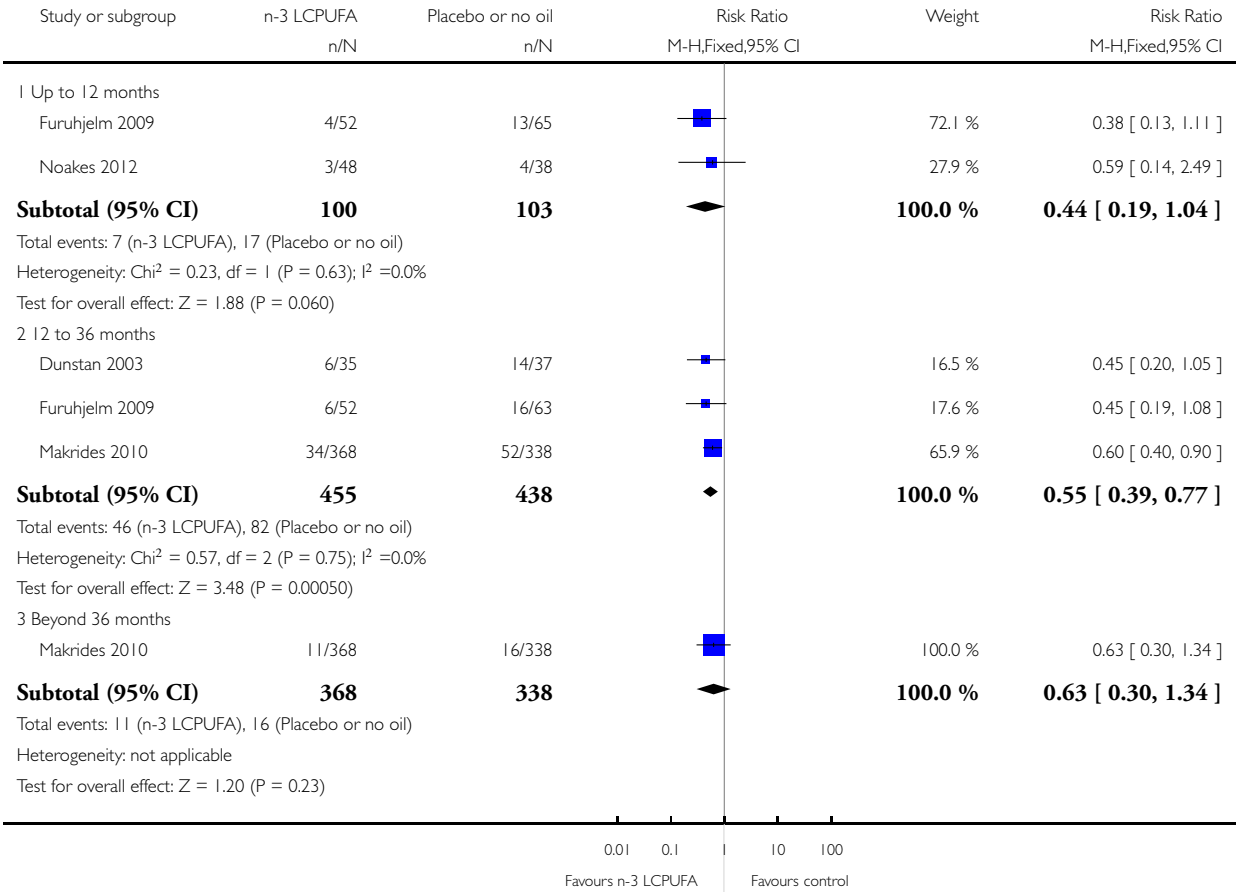


## Analysis 4.1. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 1 Skin prick test results - egg.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 1 Skin prick test results - egg

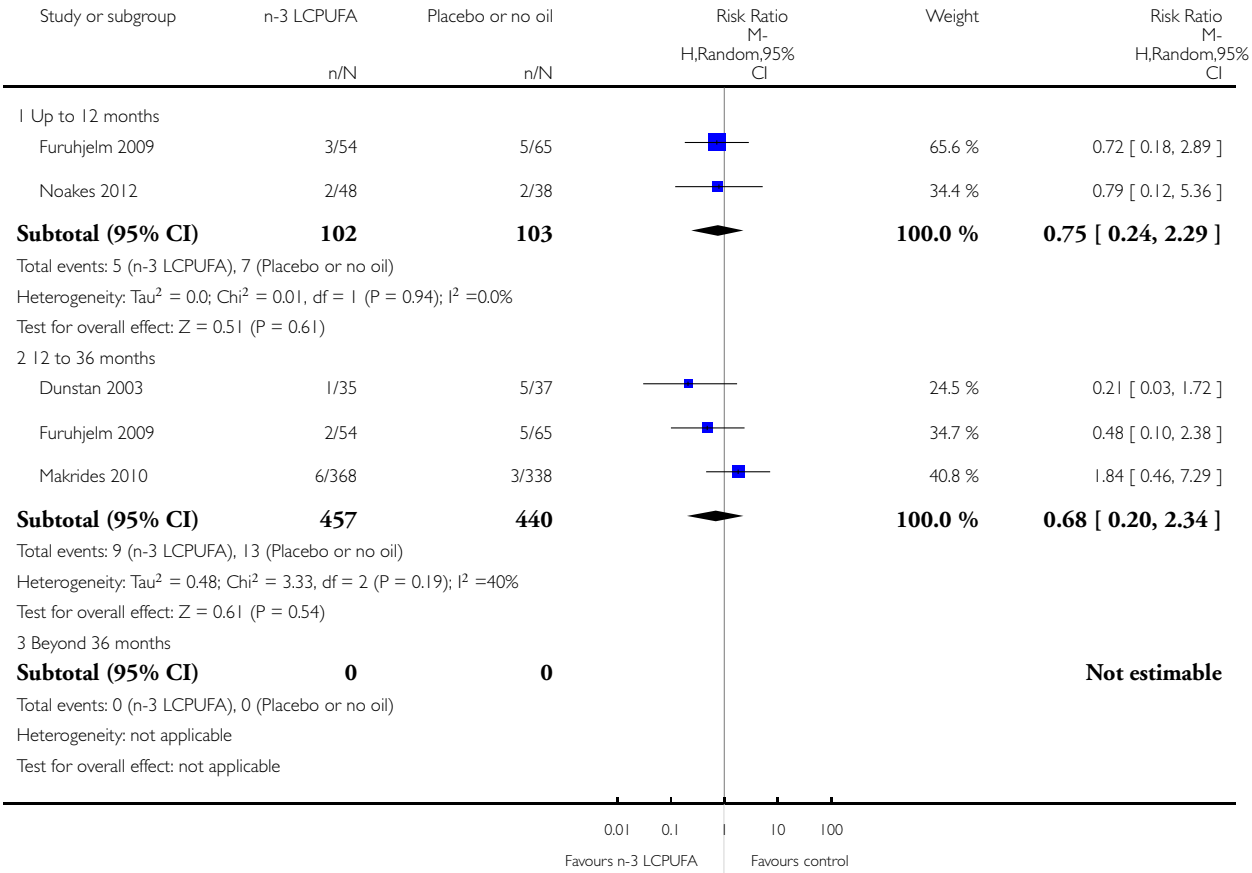


## Analysis 4.2. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 2 Skin prick test results - cows' milk.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 2 Skin prick test results - cows' milk



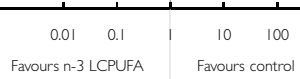
## Analysis 4.3. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 3 Skin Prick Test results - peanut.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 3 Skin Prick Test results - peanut

| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Up to 12 months | | | | | |
| **Subtotal (95% CI)** | **0** | **0** | | | **Not estimable** |
| Total events: 0 (n-3 LCPUFA), 0 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: not applicable | | | | | |
| 2 12 to 36 months | | | | | |
| Dunstan 2003 | 3/35 | 6/37 | | 20.3 % | 0.53 [ 0.14, 1.95 ] |
| Makrides 2010 | 15/368 | 22/338 | | 79.7 % | 0.63 [ 0.33, 1.19 ] |
| **Subtotal (95% CI)** | **403** | **375** | | **100.0 %** | **0.61 [ 0.34, 1.08 ]** |
| Total events: 18 (n-3 LCPUFA), 28 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.05, df = 1 (P = 0.82); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 1.71 (P = 0.088) | | | | | |
| 3 Beyond 36 months | | | | | |
| Makrides 2010 | 13/368 | 20/338 | | 100.0 % | 0.60 [ 0.30, 1.18 ] |
| **Subtotal (95% CI)** | **368** | **338** | | **100.0 %** | **0.60 [ 0.30, 1.18 ]** |
| Total events: 13 (n-3 LCPUFA), 20 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 1.48 (P = 0.14) | | | | | |



## Analysis 4.4. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 4 Skin prick test results - wheat.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 4 Skin prick test results - wheat

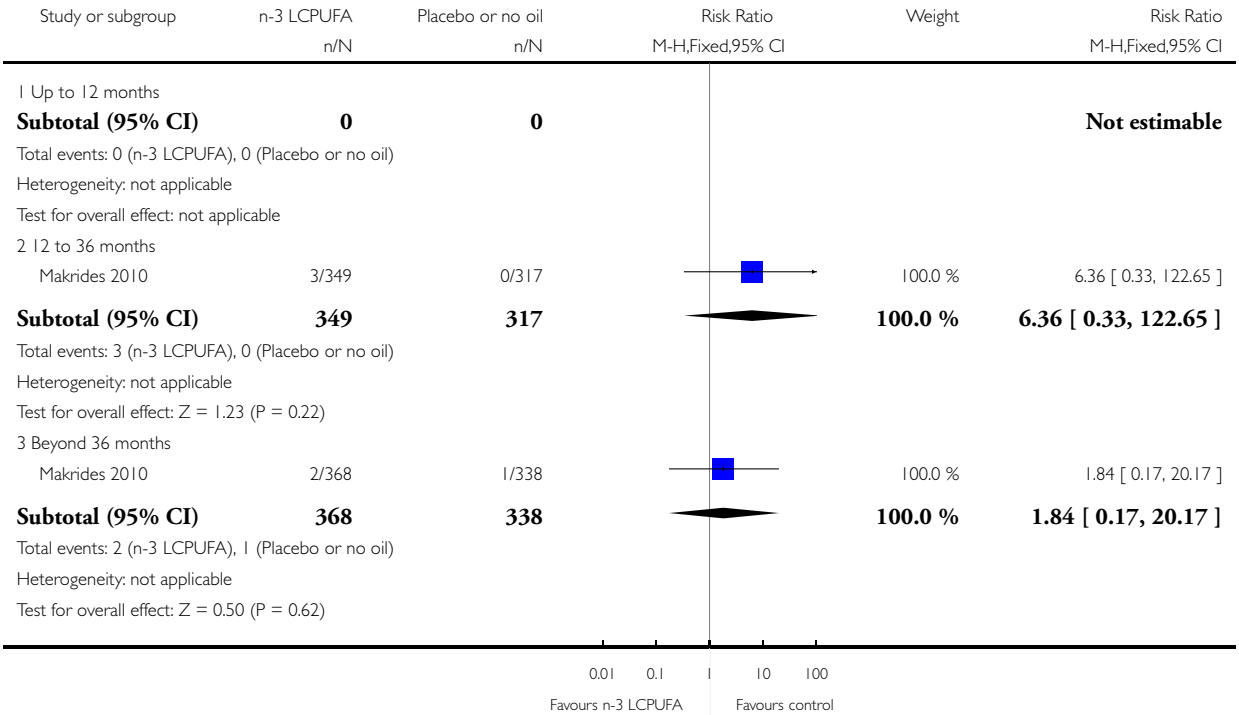
| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Up to 12 months | | | | | |
| Furuhjelm 2009 | 1/52 | 0/65 | | 100.0 % | 3.74 [ 0.16, 89.85 ] |
| **Subtotal (95% CI)** | **52** | **65** | | **100.0 %** | **3.74 [ 0.16, 89.85 ]** |
| Total events: 1 (n-3 LCPUFA), 0 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.81 (P = 0.42) | | | | | |
| 2 12 to 36 months | | | | | |
| Furuhjelm 2009 | 1/52 | 0/65 | | 17.5 % | 3.74 [ 0.16, 89.85 ] |
| Makrides 2010 | 2/349 | 2/317 | | 82.5 % | 0.91 [ 0.13, 6.41 ] |
| **Subtotal (95% CI)** | **401** | **382** | | **100.0 %** | **1.40 [ 0.29, 6.84 ]** |
| Total events: 3 (n-3 LCPUFA), 2 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.55, df = 1 (P = 0.46); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.42 (P = 0.67) | | | | | |
| 3 Beyond 36 months | | | | | |
| Makrides 2010 | 6/368 | 2/338 | | 100.0 % | 2.76 [ 0.56, 13.56 ] |
| **Subtotal (95% CI)** | **368** | **338** | | **100.0 %** | **2.76 [ 0.56, 13.56 ]** |
| Total events: 6 (n-3 LCPUFA), 2 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 1.25 (P = 0.21) | | | | | |

## Analysis 4.5. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 5 Skin prick test results - fish.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 5 Skin prick test results - fish

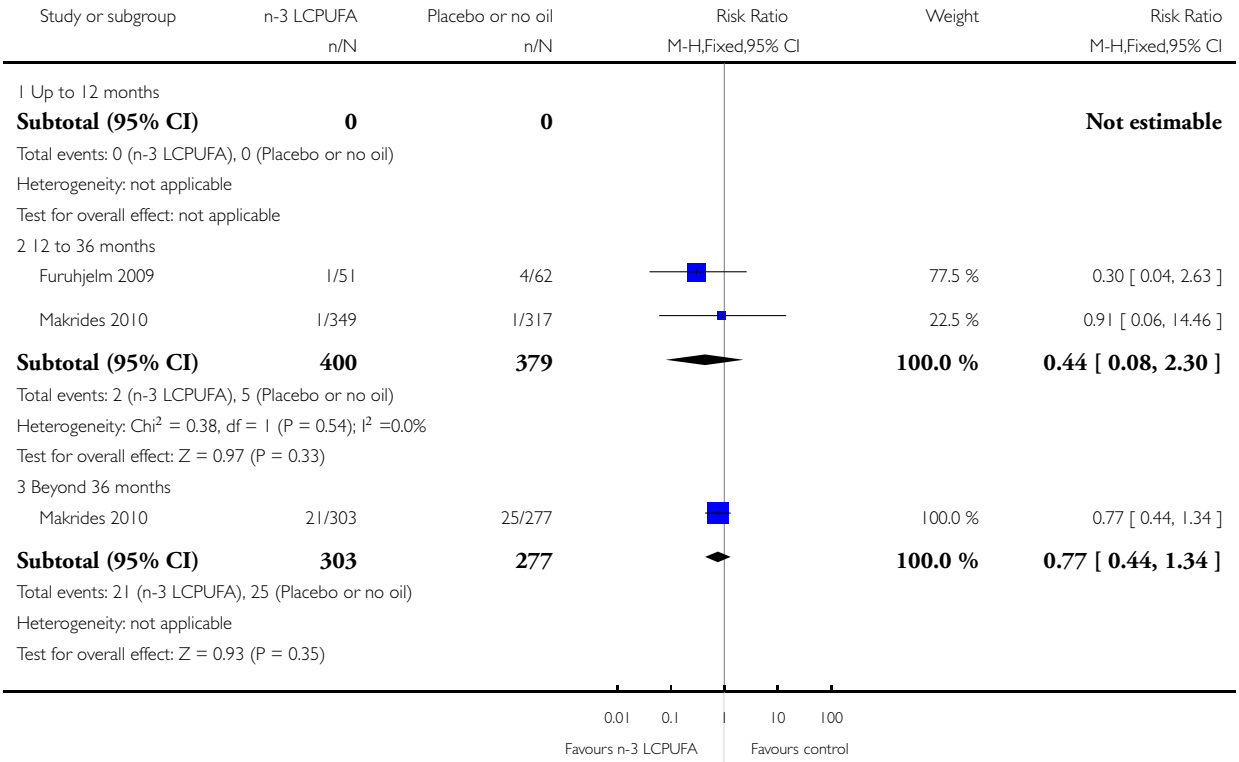


## Analysis 4.6. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 6 Skin prick test results - inhalant allergen (pollens).

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 6 Skin prick test results - inhalant allergen (pollens)

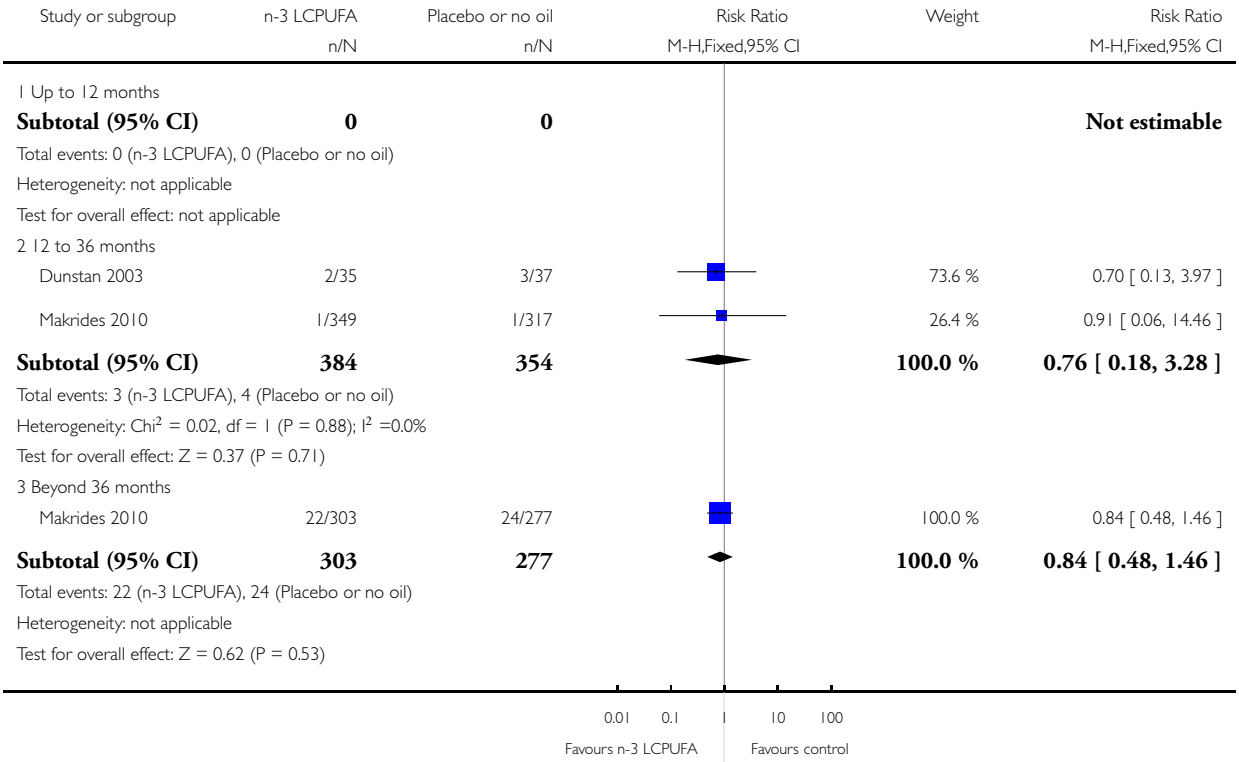


## Analysis 4.7. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 7 Skin prick test results - house dust mite.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 7 Skin prick test results - house dust mite

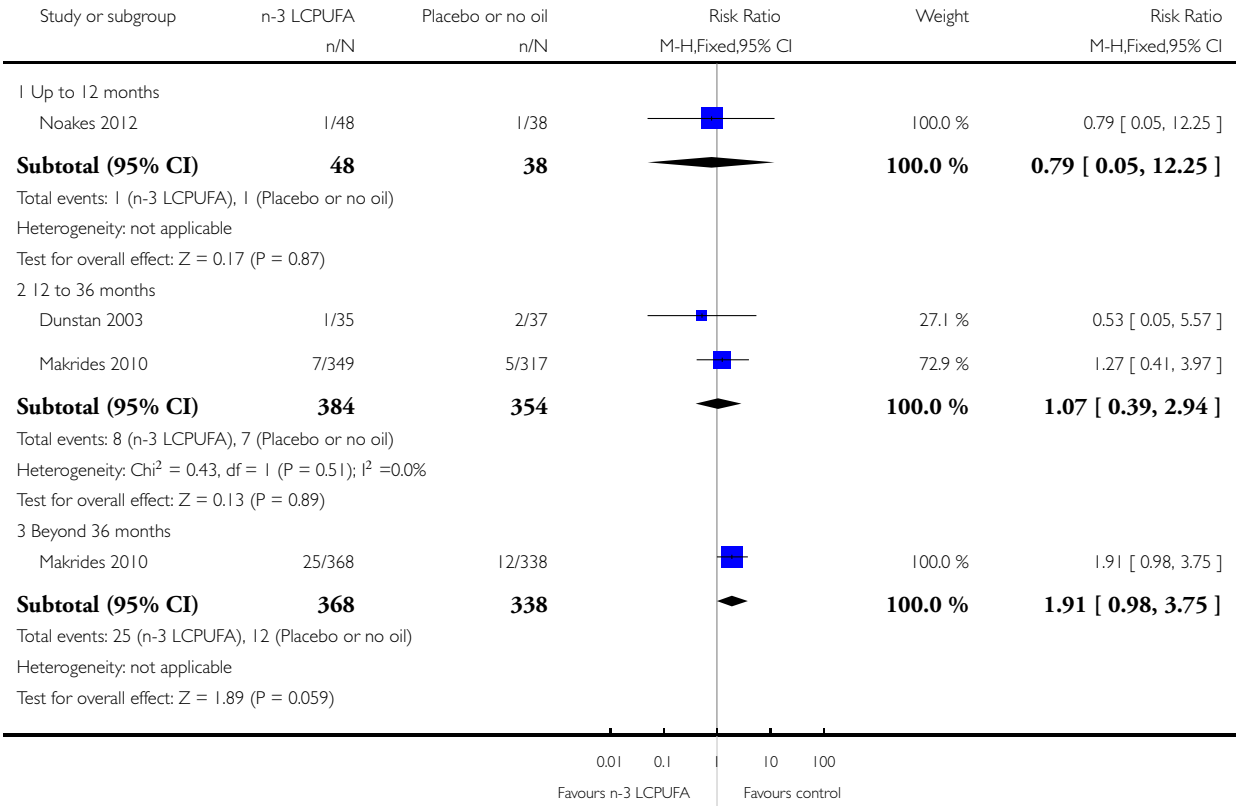


## Analysis 4.8. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 8 Skin prick test results - cat.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 8 Skin prick test results - cat

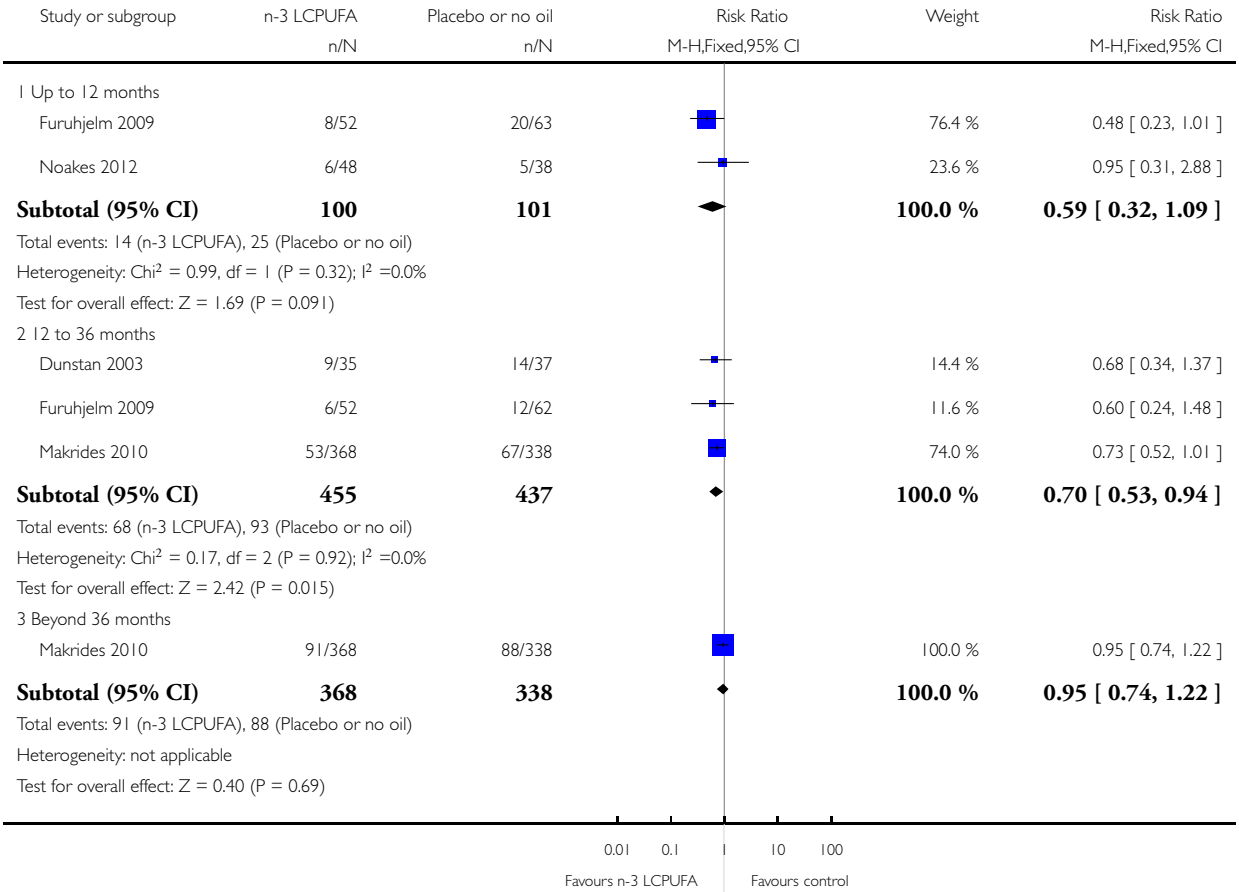


## Analysis 4.9. Comparison 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation, Outcome 9 Skin prick test results - any allergen.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 4 n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation

Outcome: 9 Skin prick test results - any allergen

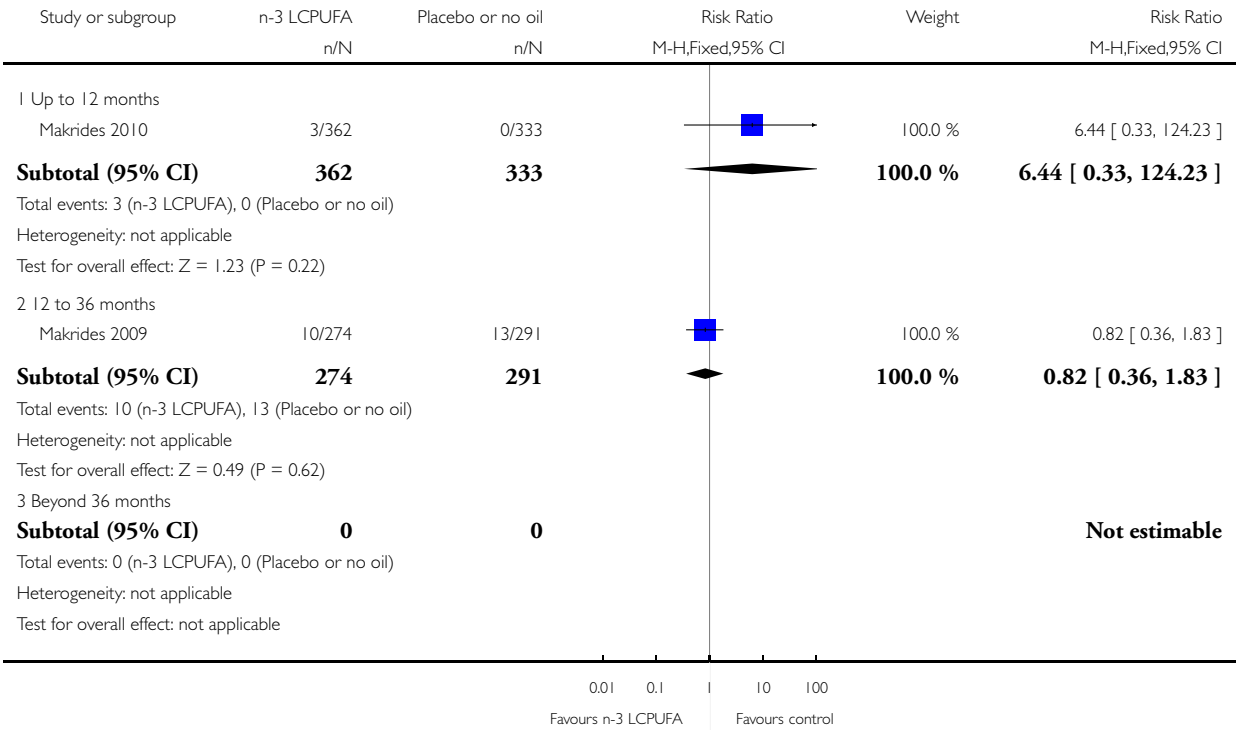


**Analysis 5.1. Comparison 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire), Outcome 1 Food allergies: parental reported (non-validated questionnaires).**

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire)

Outcome: 1 Food allergies: parental reported (non-validated questionnaires)



## Analysis 5.2. Comparison 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire), Outcome 2 Eczema: parental reported (non-validated questionnaires).

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire)

Outcome: 2 Eczema: parental reported (non-validated questionnaires)

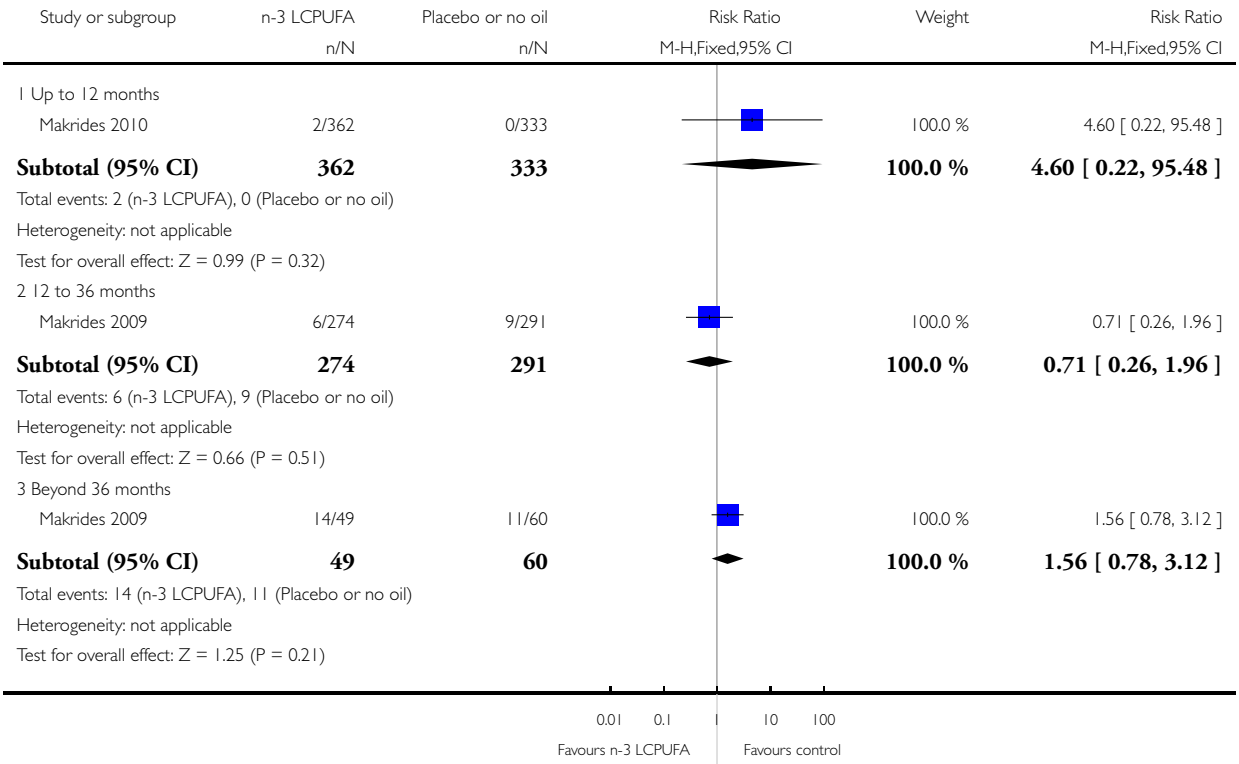
| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Up to 12 months | | | | | |
| Makrides 2010 | 57/362 | 58/333 | | 100.0 % | 0.90 [ 0.65, 1.26 ] |
| **Subtotal (95% CI)** | **362** | **333** | | **100.0 %** | **0.90 [ 0.65, 1.26 ]** |
| Total events: 57 (n-3 LCPUFA), 58 (Placebo or no oil) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.59 (P = 0.55) | | | | | |
| 2 12 to 36 months | | | | | |
| Makrides 2009 | 44/274 | 50/291 | | 35.6 % | 0.93 [ 0.65, 1.35 ] |
| Makrides 2010 | 83/365 | 84/333 | | 64.4 % | 0.90 [ 0.69, 1.17 ] |
| **Subtotal (95% CI)** | **639** | **624** | | **100.0 %** | **0.91 [ 0.74, 1.13 ]** |
| Total events: 127 (n-3 LCPUFA), 134 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.02, df = 1 (P = 0.88); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.83 (P = 0.41) | | | | | |
| 3 Beyond 36 months | | | | | |
| Makrides 2009 | 17/49 | 19/59 | | 14.3 % | 1.08 [ 0.63, 1.84 ] |
| Makrides 2010 | 101/333 | 99/305 | | 85.7 % | 0.93 [ 0.74, 1.18 ] |
| **Subtotal (95% CI)** | **382** | **364** | | **100.0 %** | **0.95 [ 0.77, 1.18 ]** |
| Total events: 118 (n-3 LCPUFA), 118 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.23, df = 1 (P = 0.63); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.43 (P = 0.67) | | | | | |

**Analysis 5.3. Comparison 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire), Outcome 3 Allergic rhinitis/Hay fever: parental reported (non-validated questionnaires).**

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire)

Outcome: 3 Allergic rhinitis/Hay fever: parental reported (non-validated questionnaires)

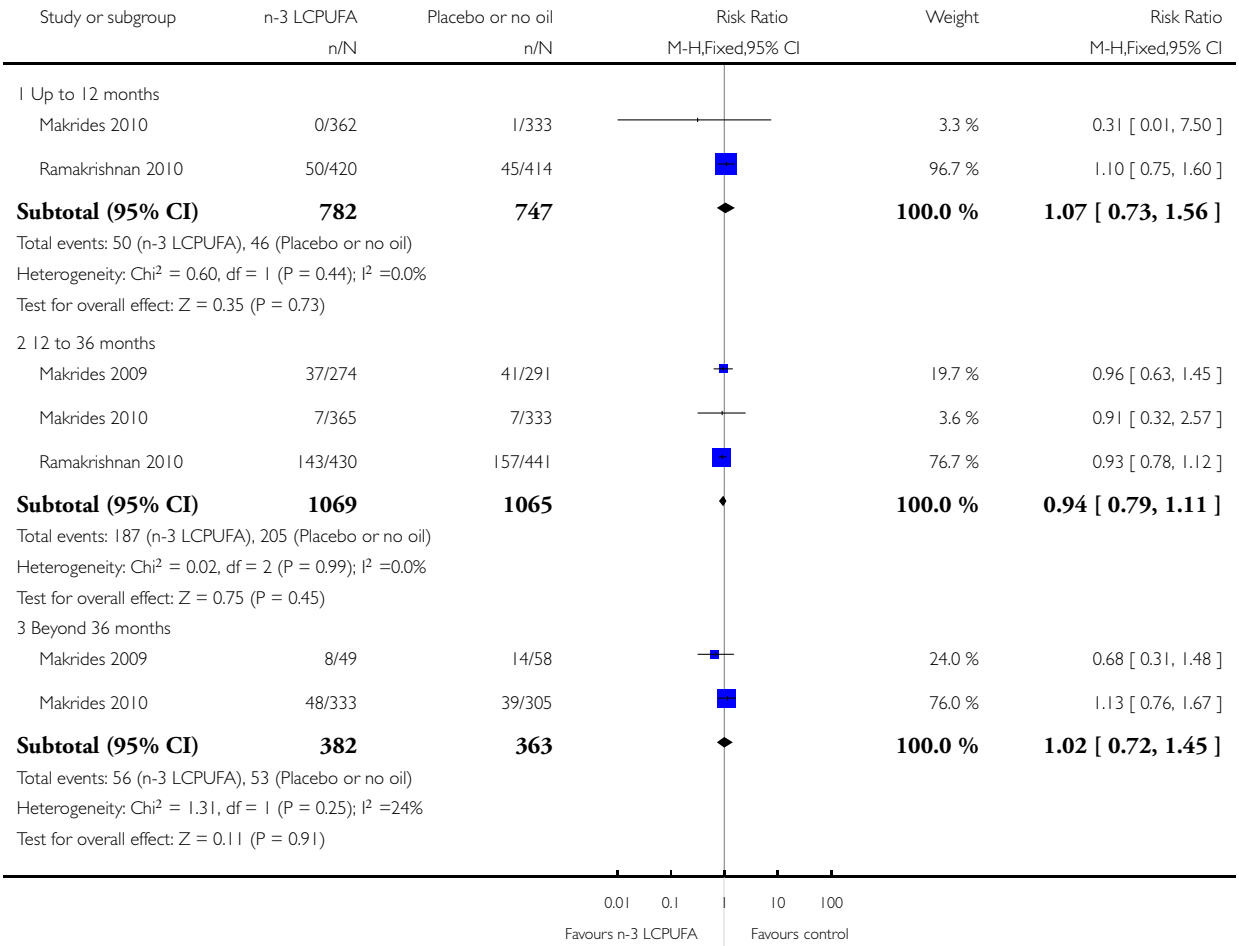


## Analysis 5.4. Comparison 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire), Outcome 4 Asthma/Wheeze: parental reported (non-validated questionnaires).

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire)

Outcome: 4 Asthma/Wheeze: parental reported (non-validated questionnaires)

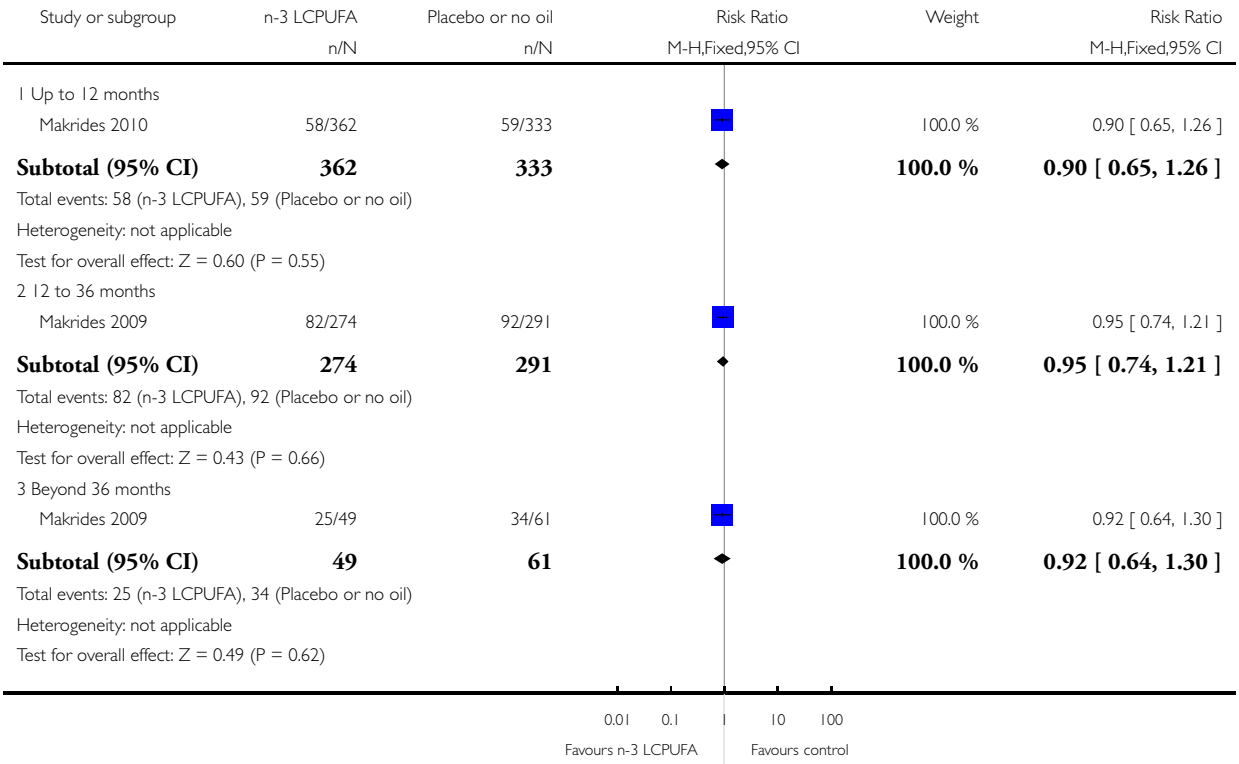


**Analysis 5.5. Comparison 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire), Outcome 5 Any allergies: parental reported (non-validated questionnaires).**

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 5 n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy (non-validated questionnaire)

Outcome: 5 Any allergies: parental reported (non-validated questionnaires)

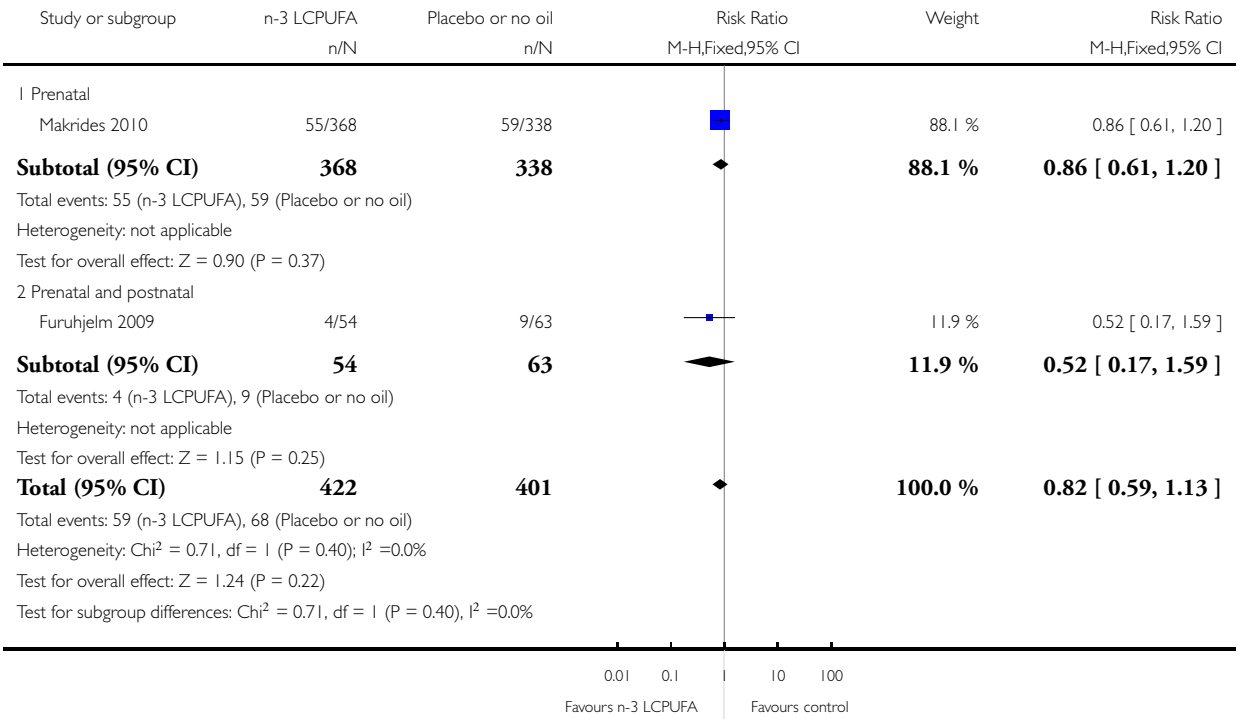


**Analysis 6.1. Comparison 6 Timing of supplementation - prenatal versus postnatal versus pre and postnatal subgroup, Outcome 1 Any allergies: last available time (with sensitisation): medically diagnosed IgE mediated.**

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 6 Timing of supplementation - prenatal versus postnatal versus pre and postnatal subgroup

Outcome: 1 Any allergies: last available time (with sensitisation): medically diagnosed IgE mediated

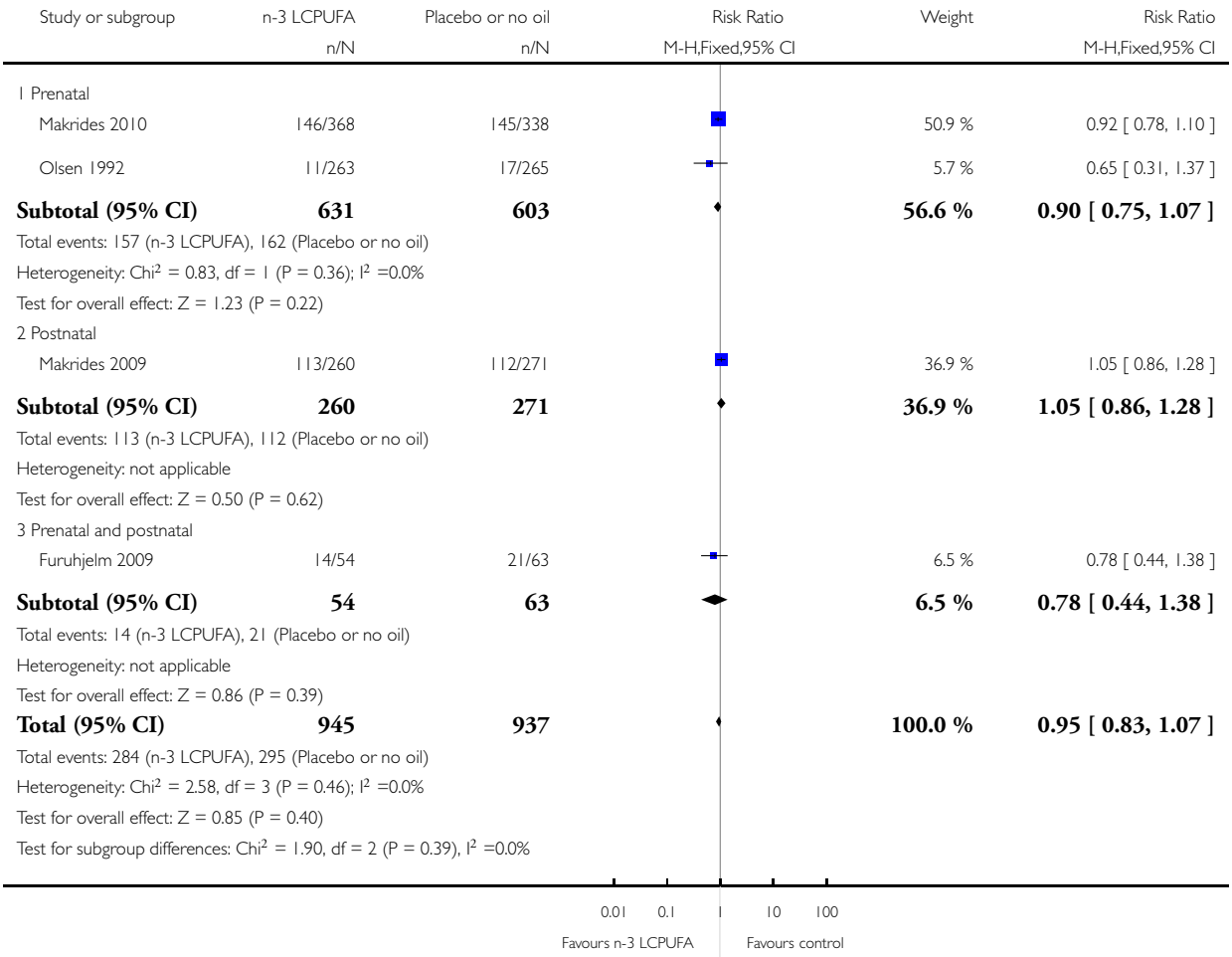


## Analysis 6.2. Comparison 6 Timing of supplementation - prenatal versus postnatal versus pre and postnatal subgroup, Outcome 2 Any allergies: last available time (+/- sensitisation): medically diagnosed/parental reported.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 6 Timing of supplementation - prenatal versus postnatal versus pre and postnatal subgroup

Outcome: 2 Any allergies: last available time (+/- sensitisation): medically diagnosed/parental reported



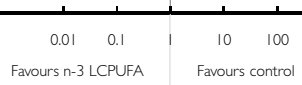
## Analysis 7.1. Comparison 7 Allergy risk - high risk versus unselected risk subgroup, Outcome 1 Any allergy: last available time.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 7 Allergy risk - high risk versus unselected risk subgroup

Outcome: 1 Any allergy: last available time

| Study or subgroup | n-3 LCPUFA n/N | Placebo or no oil n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 High risk | | | | | |
| Furuhjelm 2009 | 14/54 | 21/63 | | 6.5 % | 0.78 [ 0.44, 1.38 ] |
| Makrides 2010 | 146/368 | 145/338 | | 50.9 % | 0.92 [ 0.78, 1.10 ] |
| **Subtotal (95% CI)** | **422** | **401** | | **57.4 %** | **0.91 [ 0.77, 1.07 ]** |
| Total events: 160 (n-3 LCPUFA), 166 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 0.32, df = 1 (P = 0.57); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 1.12 (P = 0.26) | | | | | |
| 2 Unselected risk | | | | | |
| Makrides 2009 | 113/260 | 112/271 | | 36.9 % | 1.05 [ 0.86, 1.28 ] |
| Olsen 1992 | 11/263 | 17/265 | | 5.7 % | 0.65 [ 0.31, 1.37 ] |
| **Subtotal (95% CI)** | **523** | **536** | | **42.6 %** | **1.00 [ 0.82, 1.21 ]** |
| Total events: 124 (n-3 LCPUFA), 129 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 1.54, df = 1 (P = 0.21); $I^2$ =35% | | | | | |
| Test for overall effect: Z = 0.02 (P = 0.99) | | | | | |
| **Total (95% CI)** | **945** | **937** | | **100.0 %** | **0.95 [ 0.83, 1.07 ]** |
| Total events: 284 (n-3 LCPUFA), 295 (Placebo or no oil) | | | | | |
| Heterogeneity: $Chi^2$ = 2.58, df = 3 (P = 0.46); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.85 (P = 0.40) | | | | | |
| Test for subgroup differences: $Chi^2$ = 0.52, df = 1 (P = 0.47), $I^2$ =0.0% | | | | | |

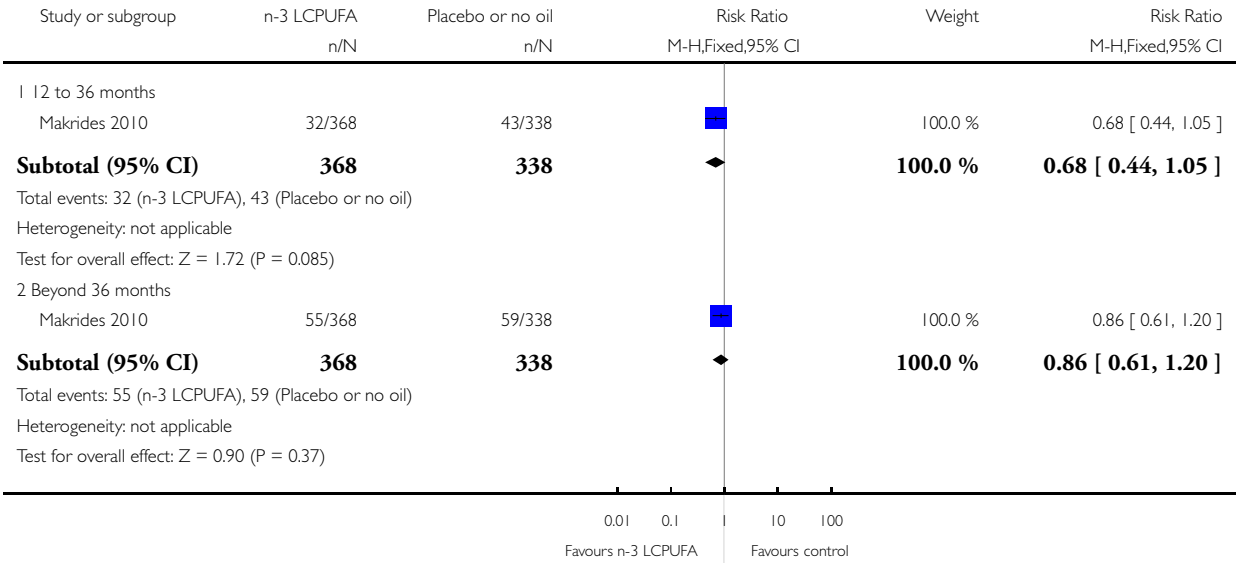


## Analysis 8.1. Comparison 8 n-3 LCPUFA supplementation versus placebo or no supplementation - sensitivity analysis, Outcome 1 Any allergies (with sensitisation): medically diagnosed IgE mediated.

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 8 n-3 LCPUFA supplementation versus placebo or no supplementation - sensitivity analysis

Outcome: 1 Any allergies (with sensitisation): medically diagnosed IgE mediated

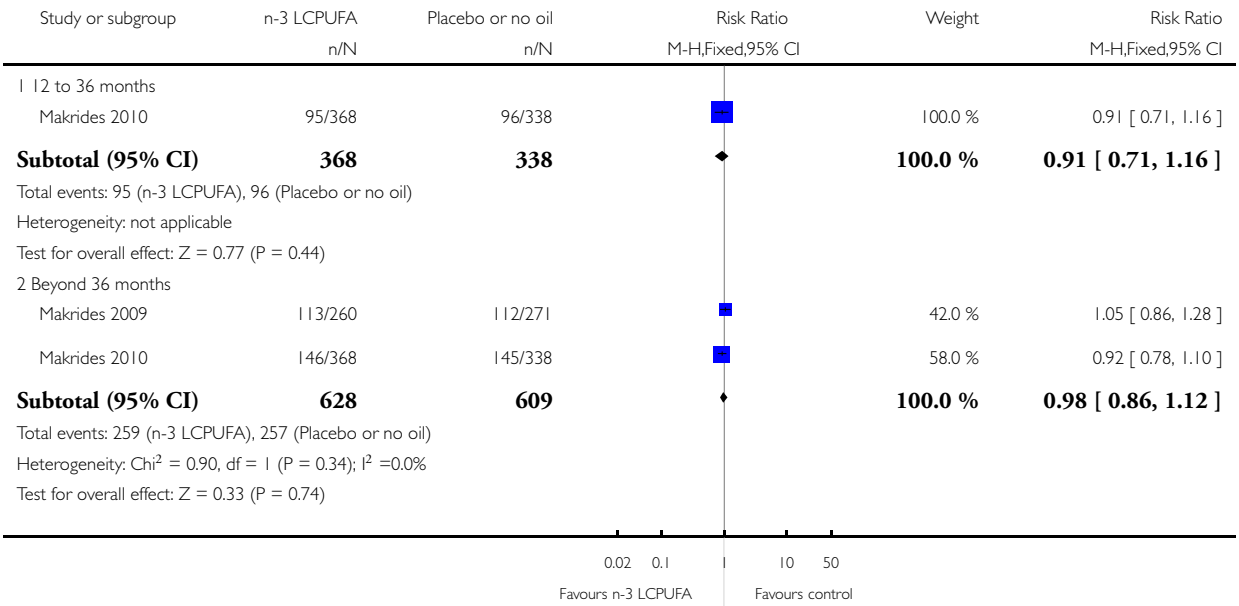


**Analysis 8.2. Comparison 8 n-3 LCPUFA supplementation versus placebo or no supplementation - sensitivity analysis, Outcome 2 Any allergies (+/- sensitisation): medically diagnosed/parental reported.**

Review: Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood

Comparison: 8 n-3 LCPUFA supplementation versus placebo or no supplementation - sensitivity analysis

Outcome: 2 Any allergies (+/- sensitisation): medically diagnosed/parental reported




## ADDITIONAL TABLES

**Table 1. The effects of n-3 LCPUFA supplementation on IgE-mediated allergies using pooled analysis RR (M-H, Fixed/Random, 95% CI)**

| *Allergy (IgE mediated)* | *Assessed Age* | *No of studies* | *No of participants* | *n-3 LCPUFA* | | *Placebo* | | *Effect Estimate RR [95% CI]* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Events* | *Total* | *Events* | *Total* | |
| *Food allergy* | < 12 months | 1 | 117 | 1 | 52 | 10 | 65 | **0.13 [0.02 to 0.95]** * |
| | 12-36 months | 2 | 825 | 13 | 422 | 20 | 403 | 0.58 [0.18 to 1.88] R |
| | ≥ 36 months | 1 | 706 | 14 | 368 | 9 | 338 | 1.43 [0.63 to 3.26] |
| *Eczema* | < 12 months | 1 | 117 | 4 | 52 | 13 | 65 | 0.38 [0.13 to 1.11] |

**Table 1. The effects of n-3 LCPUFA supplementation on IgE-mediated allergies using pooled analysis RR (M-H, Fixed/Random, 95% CI)** (*Continued*)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12-36 months | 2 | 823 | 29 | 422 | 45 | 401 | **0.61 [0.39 to 0.95]** * |
| | ≥ 36 months | 1 | 706 | 44 | 368 | 48 | 338 | 0.84 [0.57 to 1.23] |
| *Allergic rhinitis* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 825 | 1 | 422 | 3 | 403 | 0.47 [0.07 to 3.06] |
| | ≥ 36 months | 1 | 706 | 18 | 368 | 20 | 338 | 0.83 [0.44 to 1.54] |
| *Asthma* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 824 | 3 | 422 | 4 | 402 | 0.86 [0.21 to 3.49] |
| | ≥ 36 months | 1 | 706 | 6 | 368 | 5 | 338 | 1.10 [0.34 to 3.58] |
| *Any allergies* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 823 | 36 | 422 | 52 | 401 | **0.66 [0.44 to 0.98]** * |
| | ≥ 36 months | 1 | 706 | 55 | 368 | 59 | 338 | 0.86 [0.61 to 1.20] |

*significant P < 0.05, * * significant P < 0.005, NE = Not estimable , $^{R=}$ Random-effects estimate

**Table 2. The effects of n-3 LCPUFA supplementation on all allergies using pooled analysis RR (M-H, Fixed, 95% CI)**

| ***Allergy*** (IgE mediated or not) | ***Assessed Age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Placebo*** | | ***Effect Estimate RR [95% CI]*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Food allergy* | < 12 months | 1 | 117 | 1 | 52 | 10 | 65 | **0.13 [0.02, 0.95]** * |
| | 12-36 months | 4 | 973 | 19 | 499 | 26 | 474 | 0.72 [0.40 to 1.30] |
| | ≥ 36 months | 1 | 706 | 14 | 368 | 9 | 338 | 1.43 [0.63 to 3.26] |
| *Eczema* | < 12 months | 2 | 203 | 16 | 100 | 20 | 103 | 0.76 [0.22 to 2.62]$^{R}$ |

**Table 2. The effects of n-3 LCPUFA supplementation on all allergies using pooled analysis RR (M-H, Fixed, 95% CI)** (*Continued*)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12-36 months | 4 | 973 | 118 | 499 | 122 | 474 | 0.96 [0.69 to 1.33]$^{R}$ |
| | ≥ 36 months | 2 | 1237 | 122 | 628 | 131 | 609 | 0.88 [0.68 to 1.13]$^{R}$ |
| *Allergic rhinitis* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 805 | 10 | 414 | 18 | 391 | 0.53 [0.25 to 1.12] |
| | ≥ 36 months | 2 | 1169 | 114 | 593 | 109 | 576 | 1.03 [0.81 to 1.30] |
| *Asthma* | < 12 months | 1 | 83 | 11 | 46 | 7 | 37 | 1.26 [0.54 to 2.94] |
| | 12-36 months | 4 | 955 | 106 | 491 | 105 | 464 | 0.93 [0.73 to 1.18] |
| | ≥ 36 months | 3 | 1697 | 165 | 856 | 171 | 841 | 0.94 [0.78 to 1.13] |
| *Any allergies* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 823 | 109 | 422 | 117 | 401 | 0.89 [0.71 to 1.11] |
| | ≥ 36 months | 3 | 1765 | 270 | 891 | 274 | 874 | 0.96 [0.84 to 1.09] |

*significant P < 0.05, * * significant P < 0.005, NE= Not estimable , $^{R=}$ Random-effects estimate

**Table 3. The effects of n-3 LCPUFA supplementation on skin prick results for allergens using pooled analysis RR (M-H, Fixed, 95% CI)**

| ***Skin prick results*** | ***Assessed Age*** | ***No of studies*** | ***No of participants*** | ***n-3 LCPUFA*** | | ***Placebo*** | | ***Fixed Effect estimate RR [95% CI]*** |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Events*** | ***Total*** | ***Events*** | ***Total*** | |
| *Egg* | < 12 months | 2 | 203 | 7 | 100 | 17 | 103 | 0.44 [0.19 to 1.04] |
| | 12-36 months | **3** | **893** | **46** | **455** | **82** | **438** | **0.55 [0.39 to 0.77]** * * |
| | ≥ 36 months | 1 | 706 | 11 | 368 | 16 | 338 | 0.63 [0.30 to 1.34] |
| *Cow's milk* | < 12 months | 2 | 205 | 5 | 102 | 7 | 103 | 0.75 [0.24 to 2.29]$^{R}$ |

**Table 3. The effects of n-3 LCPUFA supplementation on skin prick results for allergens using pooled analysis RR (M-H, Fixed, 95% CI)** (*Continued*)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12-36 months | 3 | 897 | 9 | 457 | 13 | 440 | 0.68 [0.20 to 2.34][R] |
| | ≥ 36 months | 0 | 0 | | | | | NE |
| *Peanut* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 778 | 18 | 403 | 28 | 375 | 0.61 [0.34 to 1.08] |
| | ≥ 36 months | 1 | 706 | 13 | 368 | 20 | 338 | 0.60 [0.30 to 1.18] |
| *Wheat* | < 12 months | 1 | 117 | 1 | 52 | 0 | 65 | 3.74 [0.16 to 89.85] |
| | 12-36 months | 2 | 783 | 3 | 401 | 2 | 382 | 1.40 [0.29 to 6.84] |
| | ≥ 36 months | 1 | 706 | 6 | 368 | 2 | 338 | 2.76 [0.56 to 13.56] |
| *Fish* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 1 | 666 | 3 | 349 | 0 | 317 | 6.36 [0.33 to 122.65] |
| | ≥ 36 months | 1 | 706 | 2 | 368 | 1 | 338 | 1.84 [0.17 to 20.17] |
| *Inhalant allergens (pollens)* | < 12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 779 | 2 | 400 | 5 | 379 | 0.44 [0.08 to 2.30] |
| | ≥ 36 months | 1 | 580 | 21 | 303 | 25 | 277 | 0.77 [0.44 to 1.34] |
| *House dust mite* | <12 months | 0 | 0 | | | | | NE |
| | 12-36 months | 2 | 738 | 3 | 384 | 4 | 354 | 0.76 [0.18 to 3.28] |
| | ≥36 months | 1 | 580 | 22 | 303 | 24 | 277 | 0.84 [0.48 to 1.46] |
| *Cat* | < 12 months | 1 | 86 | 1 | 48 | 1 | 38 | 0.79 [0.05 to 12.25] |
| | 12-36 months | 2 | 738 | 8 | 384 | 7 | 354 | 1.07 [0.39 to 2.94] |

**Table 3. The effects of n-3 LCPUFA supplementation on skin prick results for allergens using pooled analysis RR (M-H, Fixed, 95% CI)** (*Continued)*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ≥ 36 months | 1 | 706 | 25 | 368 | 12 | 338 | 1.91 [0.98 to 3.75] |
| *Any allergen (one or more allergen)* | < 12 months | 2 | 201 | 14 | 100 | 25 | 101 | 0.59 [0.32 to 1.09] |
| | 12-36 months | **3** | **892** | **68** | **455** | **93** | **437** | **0.70 [0.53 to 0.94]** * |
| | ≥ 36 months | 1 | 706 | 91 | 368 | 88 | 338 | 0.95 [0.74 to 1.22] |

*significant P < 0.05, * * significant P < 0.005, NE = Not estimable

# APPENDICES

## Appendix 1. PubMed search strategy

1. pregnancy[mh]
2. pregnan*[tiab]
3. maternal exchange*[tiab]
4. transplacental exposure*[tiab]
5. gestat*[tiab]
6. fetal Development [mesh]
7. Fetal Development [tiab]
8. Fetal Programming* [tiab]
9. fetal growth[tiab]
10. Foetal Development [tiab]
11. Foetal Programming* [tiab]
12. foetal growth[tiab]
13. Gestational Age*[tiab]
14. Fetal Age*[tiab]
15. foetal age*[tiab]
16. Breast Feeding[mh]
17. breast feeding[tiab]
18. breast fed[tiab]
19. lactating mother*[tiab]
20. breastfeeding[tiab]
21. Postpartum Period[mh]
22. Postpartum[tiab])
23. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16OR 17 OR 18 OR 19 OR 20 OR 21 OR 22
24. fish oils[mh]
25. fish oil*[tiab]
26. docosahexaenoic acid*[tiab]

27. cod liver oil[tiab]
28. omega 3 fatty acid*[tiab]
29. n 3 fatty acid*[tiab]
30. eicosapentaenoic acid[tiab]
31. n 3 pufa)
32. 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31
33. hypersensitivity[mh]
34. hypersensitivit*[tiab]
35. allerg*[tiab]
36. environmental illness*[tiab]
37. atopic dermatitis[tiab]
38. anaphyla*[tiab]
39. atopic dermatitis[tiab]
40. eczema[tiab]
41. urticaria*[tiab]
42. hives[tiab])
43. 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39 OR 40 OR 41 OR 42
44. 23 AND 32 AND 43
45. randomized controlled trial[pt]
46. controlled clinical trial[pt]
47. randomized controlled trials[mh]
48. random allocation[mh]
49. double-blind method[mh]
50. single-blind method[mh]
51. clinical trial[pt]
52. clinical trials[mh]
53. clinical trial[tw]
54. ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw]))
55. (placebos[mh] OR placebo*[tw] OR random*[tw] OR research design [mh:noexp] OR comparative study[pt] OR evaluation studies as topic[mh] OR follow-up studies[mh] OR prospective studies[mh] OR control[tw] OR controlled[tw] OR prospectiv*[tw] OR volunteer*[tw])
56. 45 OR 46 OR 47 OR 48 OR 49 OR 50 OR 51 OR 52 OR 53 OR 54 OR 55
57. NOT animals[mh] NOT (human[mh] and animals[mh])
58. 44 AND 56
59. 58 NOT 57

## Appendix 2. CINAHL (via EBSCOhost) search strategy

1. (((singl* OR doubl* OR trebl* OR tripl*) AND (mask* OR blind*)) OR (placebo* OR random* OR "research design*" OR "comparative stud*" OR "evaluation stud*" OR "follow-up stud*" OR control OR controlled OR prospectiv* OR volunteer*)) NOT (animals NOT human))
2. (pregnan* OR "maternal exchange*" OR "transplacental exposure*" OR gestat* OR "fetal development" OR "fetal programming*" OR "fetal growth" OR "foetal development" OR "foetal programming*" OR "foetal growth" OR "fetal age*" OR "foetal age*" OR "breast feeding" OR "breast fed" OR "lactating mother*" OR breastfeeding OR postpartum)
3. ("fish oil*" OR "docosahexaenoic acid*" OR "cod liver oil" OR "omega 3 fatty acid*" OR "n 3 fatty acid*" OR "eicosapentaenoic acid" OR "n 3 pufa")
4. (hypersensitivit* OR allerg* OR "environmental illness*" OR "atopic dermatitis" OR anaphyla* OR eczema OR urticaria* OR hives)
5. 1 AND 2 AND 3 AND 4

## Appendix 3. Scopus search strategy

1. (pregnan* OR "maternal exchange" OR "transplacental exposure" OR gestat* OR "fetal development" OR "fetal programming" OR "fetal growth" OR "foetal development" OR "foetal programming" OR "foetal growth" OR "Gestational Age" OR "fetal age" OR "foetal age" OR "breast feeding" OR "breast fed" OR "lactating mother" OR breastfeeding OR postpartum)
2. ("fish oil" OR "docosahexaenoic acid" OR "cod liver oil" OR "omega 3 fatty acid" OR "n 3 fatty acid" OR "eicosapentaenoic acid" OR "n 3 pufa")
3. (hypersensitivit* OR allerg* OR "environmental illness" OR "atopic dermatitis" OR anaphyla* OR "atopic dermatitis" OR eczema OR urticaria* OR hives)
4. ("randomized controlled trial" OR "randomised controlled trial" OR "random allocation" OR "double blind" OR "single blind" OR "clinical trial*" OR ((singl* OR doubl* OR trebl* OR tripl*) AND (mask* OR blind*)) OR placebo* OR "comparative stud" OR "follow up stud" OR "prospective stud" OR rct OR "systematic review" OR "meta analys" OR metaanalys*)
5. 1 AND 2 AND 3 AND 4

## Appendix 4. Web of Knowledge search strategy

1. pregnan*
2. "maternal exchange*"
3. "transplacental exposure*"
4. gestat*
5. "Fetal Development*"
6. "Fetal Programming*"
7. "fetal growth"
8. "Foetal Development*"
9. "Foetal Programming*"
10. "foetal growth"
11. "Gestational Age*"
12. "Fetal Age*"
13. "foetal age*"
14. "breast feeding*"
15. "breast fed"
16. "lactating mother*"
17. breastfeeding
18. postpartum
19. 1 OR 2 OR 3 OR4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18
20. "fish oil*"
21. "docosahexaenoic acid*"
22. "cod liver oil*"
23. "omega 3 fatty acid*"
24. "n 3 fatty acid*"
25. "eicosapentaenoic acid*"
26. "n 3 pufa*"
27. 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26
28. hypersensitivit*
29. allerg*
30. "environmental illness*"
31. "atopic dermatitis"
32. anaphyla*
33. "atopic dermatitis"
34. eczema
35. urticaria*
36. hives

37. 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36
38. 19 AND 27 AND 372124
39. ("randomized controlled trial*" OR "controlled clinical trial*" OR "randomised controlled trial*" OR "random allocation*" OR "double blind" OR "single blind" OR "clinical trial*" OR ((singl* OR doubl* OR trebl* OR tripl*) AND (mask* OR blind*)) OR placebo* OR "comparative stud*" OR"follow up stud*" OR "prospective stud*" OR rct OR "systematic review*" OR "meta analys*" OR metaanalys*)
40. 38 AND 39

## Appendix 5. ClinicalTrials.gov search strategy

**http://clinicaltrials.gov**

(hypersensitivity OR hypersensitive OR allergy) AND ("fish oil" OR docosahexaenoic OR "omega 3 fatty acid") AND (pregnancy OR pregnant OR postpartum OR fetal OR foetal OR "breast feeding" OR breastfeeding)

## Appendix 6. Data analysis

Data and analyses
Comparison 1: n-3 LCPUFA supplementation versus placebo or no supplementation - any allergy
Analysis 1.1; Analysis 1.2
Comparison 2: Secondary Outcomes: n-3 LCPUFA supplementation versus placebo or no supplementation - specific type of allergy
Analysis 2.1; Analysis 2.2; Analysis 2.3; Analysis 2.4; Analysis 2.5; Analysis 2.6; Analysis 2.7; Analysis 2.8
Comparison 3: Secondary Outcomes: n-3 LCPUFA supplementation versus placebo or no supplementation - maternal and infant safety
Analysis 3.1; Analysis 3.2
Comparison 4: Secondary Outcomes: n-3 LCPUFA supplementation versus placebo or no supplementation - allergen sensitisation
Analysis 4.1; Analysis 4.2; Analysis 4.3; Analysis 4.4; Analysis 4.5; Analysis 4.6; Analysis 4.7; Analysis 4.8; Analysis 4.9
Comparison 5: Secondary Outcomes: n-3 LCPUFA supplementation versus placebo or no supplementation - parent's reports of allergy
Analysis 5.1; Analysis 5.2; Analysis 5.3; Analysis 5.4; Analysis 5.5
Comparison 6: Subgroup: Timing of supplementation
Analysis 6.1; Analysis 6.2
Comparison 7: Subgroup: Allergy risk
Analysis 7.1
Comparison 8: Sensitivity analysis
Analysis 8.1; Analysis 8.2

## CONTRIBUTIONS OF AUTHORS

AWG and MM were responsible for conceiving the review. AWG, MM and CTC designed, developed and wrote the review. MM and CTC provided a methodological perspective and a clinical perspective and provided general advice on the review. AWG and CTC were responsible for co-ordinating the review.

## DECLARATIONS OF INTEREST

Maria Makrides (MM) serves on scientific advisory boards for Nestle, Fonterra and Nutricia. Associated honoraria are paid to the Women's and Children's Health Research Institute to support conference travel and continuing education for post graduate students and early career researchers. MM is an investigator on two trials included in this review (Makrides 2009; Makrides 2010).

Carmel T Collins is an investigator on two trials included in this review (Makrides 2009; Makrides 2010).

Anoja W Gunaratne (AWG) was involved in the follow-up at 7 years corrected age of Makrides 2009 and at 3 years of age of Makrides 2010.

The Makrides 2009 and Makrides 2010 trials were independently assessed for inclusion, risk of bias and data extracted by AWG and a third party (Karen Best).

## SOURCES OF SUPPORT


### Internal sources

- Women's & Children's Health Research Institute, The University of Adelaide, Adelaide, Australia.

### External sources

- NHMRC Center for Research Excellence in Food for Future Austalians (ID App1035530), Australia.
- Maria Makrides was supported by a Fellowship from National Health Medical Research Council (NHMRC) (ID App1061704), Australia.
- Carmel T Collins was supported by a Postdoctoral Research Fellowship from the MS McLeod Research Fund of the Women's and Children's Hospital Foundation, Australia.


## DIFFERENCES BETWEEN PROTOCOL AND REVIEW

1. Due to different methods of reporting across trials, we deemed it inappropriate to conduct analyses of cumulative allergies across time points.

2. In the protocol we specified the primary outcome only as allergy. We have refined this for the review, with any allergy being our primary outcome (medically diagnosed IgE mediated; and medically diagnosed IgE mediated and/or parental report). Specific forms of allergy (food allergy, eczema, allergic rhinitis and asthma/wheeze) are now secondary outcomes.

3. Trials that reported 'wheeze' were included in the allergy outcomes for asthma, with the outcome being changed to asthma/wheeze.

4. The data were reported at last time point in the subgroup analysis comparisons.

5. Maternal safety was added as a secondary safety outcome (e.g. postpartum haemorrhage or infection) due to the theoretical risk of harm associated with higher doses of n-3 LCPUFA.

6. In the Objectives, we made it clearer that the aim of this review was to assess the effect of maternal n-3 LCPUFA supplementation in mothers during pregnancy and/or lactation on the allergy outcomes of in their children.

**Published peer reviewed papers and abstracts from this thesis**

**Gunaratne AW, Makrides M, Collins CT. Maternal prenatal and /or postnatal n-3 fish oil supplementation for preventing allergies in early childhood (Protocol) (Appendix 2.1)** Published in the Cochrane Collaboration and published in the Cochrane Library 2012, Issue 9.
**The body of the work that appears in this protocol is mainly relevant to the systematic review presented in the Chapter 2 and 6.**

**Gunaratne AW, Makrides M, Collins CT. Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood (Review) (Appendix 6).** Cochrane Database of Systematic Reviews 2015, Issue 7. Art. No.: CD010085. DOI: 10.1002/14651858.CD010085.pub2
**The body of the work that appears in this review is mainly relevant to the systematic review presented in the Chapter 2 and 6.**

**Gunaratne AW, Makrides M, Collins CT.**

***Maternal supplementation with long chain polyunsaturated fatty acids (LCPUFA) to prevent childhood allergies: a systematic review.***

**This was presented as an oral presentation in the 17th Congress of the Federation of Asian and Oceania Perinatal Societies (FAOPS) and the 16th Annual Congress of the Perinatal Society of Australia and New Zealand (PSANZ) – FAOPS & PSANZ Sydney in 2012.**

**Background:** Allergies have become more prevalent in Western societies over the last 20 years. Dietary consumption of n-3 LCPUFA has declined over the same period of time. This, together with the known role of n-3 LCPUFA in inhibiting inflammation, has resulted in speculation that n-3 LCPUFA may prevent allergy development.
**Method:** Standard Cochrane review methods were used. CENTRAL (The Cochrane Library), PubMed (1966 to August 2011), EMBASE (1974 to August 2011) and CINAHL (1984 to August2011) databases were searched to identify randomised controlled trials evaluating the effect of maternal n-3 LCPUFA supplementation compared with placebo or no treatment on allergic outcomes.
**Results:** Six trials involving 2261 children were included in the review. Five trials reported allergic disease for children below 3 years of age and two trials reported over 3 years of age. N-3 LCPUFA supplements significantly reduced the risk of IgE mediated eczema (Risk Ratio [RR] 0.61, 95% CI 0.39, 0.95, P=0.03) and any IgE mediated allergies (RR 0.66, 95% CI 0.44, 0.98, P=0.04) in children between one to three years of age. IgE mediated food allergy was significantly reduced in the first year of life (RR 0.13, 95% CI 0.02, 0.95, P=0.04). When all allergies were grouped together, regardless of whether they were IgE mediated, the groups did not differ.
**Conclusions:** Maternal n-3 LCPUFA supplementation reduces IgE mediated allergies including food allergy and eczema in children less than 3 years of age. More studies are needed to observe longer term allergy outcomes.

**The body of the work that appears in this conference abstract is drawn from the Chapter 2 and has been published in the Journal of Paediatrics and Child Health.**

**Gunaratne AW, Makrides M, Collins CT. Maternal prenatal and /or postnatal n-3 fish oil supplementation for preventing allergies in early childhood-Systematic review**

As an invited speaker, I did an oral presentation, Nutrition Update Day in Adelaide on the 6th, July 2012.

**The body of the work that I presented in this topic is mainly relevant to the systematic review presented in the Chapter 2.**

**Gunaratne AW, Palmer DJ, Sullivan T, Gold MS, Collins CT, Makrides M.**

***Validity of parental reported eczema vs. medically diagnosed eczema 12 month old infants***

**Abstract presented as a poster presentation in the 18th Annual Congress of the Perinatal Society of Australia and New Zealand (PSANZ) Perth in 2014.**

**Background:** Medical diagnosis is considered the gold standard for detecting childhood allergies; however this approach can be costly. We aimed to determine the validity of parental reports of eczema.

**Method:** 706 children at higher risk of allergic disease participating in a randomised controlled trial were medically assessed for allergic disease at 12 months of age. Parental reports of eczema symptoms and local doctor diagnosed eczema were collected prior to the study standardized medical assessment. Agreement between parental reports and medical diagnosis was assessed using Cohen's Kappa, while diagnostic accuracy was summarised using the positive predictive value (PPV), negative predictive value (NPV), sensitivity (SN) and specificity (SP).

**Results:** 97% (n=684) children were assessed for eczema by the study doctor with 176 (26%) diagnosed with eczema. Parents reported 95 (14%) to have eczema symptoms and parental reports of local doctor diagnosis of eczema was 167 (24%).

Agreement with study doctor diagnosed eczema was moderate for parental report of eczema symptoms (kappa=0.49, 95%CI 0.41-0.57) and good for parental reports of local doctor diagnosed eczema (kappa=0.76, 95%CI 0.70-0.82). Compared to the gold standard of study doctor diagnosed eczema, parental reports of eczema symptoms had poorer diagnostic accuracy (PPV 84%, NPV 83%, SN 44%, SP 97%) than parental reports of local doctor diagnosed eczema (PPV 84%, NPV 93%, SN 80%, SP 95%).

**Conclusions:** Parental reports of doctor diagnosed eczema were more reliable and accurate than parental reports of eczema symptoms. This is an important study design consideration when investigating the incidence of eczema in infancy.

**The body of the work that appears in this conference abstract is drawn from the results in the Chapter 4 and has been published in the Journal of Paediatrics and Child Health.**