The Effect of Prenatal Supplementation
with Omega 3 Long Chain Poly-
unsaturated Fatty Acids (n-3 LCPUFA) on
Childhood Allergic Disease
at Six Years of Age

Karen P Best RGN, RM

This thesis is submitted for the degree of Doctor of Philosophy

Discipline of Paediatrics
Faculty of Heath Sciences
University of Adelaide
June 2015
# Table of Contents

Table of Contents ........................................................................................................... ii

List of Tables ................................................................................................................... ix

List of Figures .................................................................................................................. xi

Abstract ............................................................................................................................ xii

Declaration ......................................................................................................................... xiv

Publications & presentations in support of this thesis ................................................... xvi

Acknowledgements ......................................................................................................... xviii

Abbreviations ................................................................................................................... xx

1 Introduction & Background .......................................................................................... 1

   Introduction .................................................................................................................... 2

   Allergic Disease .......................................................................................................... 5

   Sensitisation ................................................................................................................. 6

   The Atopic March ....................................................................................................... 11

   Atopic Eczema ............................................................................................................ 13

   Allergic Rhinitis/Rhino-conjunctivitis ..................................................................... 15

   IgE-mediated Allergic Asthma ................................................................................... 16

   Long Chain Polyunsaturated Fatty Acids (LCPUFA) .............................................. 19

   Current dietary intake of PUFA ............................................................................... 21

   LCPUFA and Pregnancy ......................................................................................... 23

   LCPUFA and Allergy ............................................................................................. 24

   Supporting evidence of prenatal n-3 LCPUFA ...................................................... 25
A Systematic Review of the Literature Omega-3 LCPUFA intake during pregnancy and allergic disease outcomes in the offspring: A systematic review and meta-analysis of observational studies and randomised controlled trials

Introduction .................................................................................................................. 27

Methods ......................................................................................................................... 29
  Search Strategy .............................................................................................................. 30

Results ............................................................................................................................. 33
  Participants ..................................................................................................................... 34
  Exposure/Intervention .................................................................................................. 50
  Clinical Outcomes ....................................................................................................... 51

Quality of Observational Studies ................................................................................. 52

Quality of RCTS ............................................................................................................. 54

Results of Observational Studies .................................................................................. 56
  Eczema .......................................................................................................................... 56
  Rhino-conjunctivitis/Hayfever ..................................................................................... 57
  Asthma/wheeze .............................................................................................................. 57
  Sensitisation .................................................................................................................. 58

Results of RCTs .............................................................................................................. 58
  Eczema .......................................................................................................................... 58
  Rhino-conjunctivitis ..................................................................................................... 60
  Asthma .......................................................................................................................... 60
  Sensitisation .................................................................................................................. 61

Discussion ....................................................................................................................... 64
Methods and study design of the six year allergy follow up of children at high hereditary risk of atopy born to mothers supplemented with n-3 LCPUFA during pregnancy

Introduction

Aim

Hypothesis

Participants & Methods

The Parent Trial

DOMInO Trial design and dietary treatments

DOMInO Trial randomisation & Blinding

Enrolment to 1 & 3 year allergy follow-up

6 year allergy follow participation

Primary Outcome

Secondary Outcomes

Trial Quality Outcomes

The Six Year Appointment

Study Invitation

Locating families

During the 6 year Appointment

After the 6 year appointment

Off-site Assessments

Assessment by Correspondence

Skin Prick Test

SPT Procedure

Safety

Allergen extracts

Assessment of Allergic Disease

Eczema

Eczema
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinitis &amp; Rhino-conjunctivitis</td>
<td>99</td>
</tr>
<tr>
<td>Wheeze</td>
<td>100</td>
</tr>
<tr>
<td>Sensitisation</td>
<td>102</td>
</tr>
<tr>
<td>Case Report Form (CRF)</td>
<td>103</td>
</tr>
<tr>
<td>Anthropometrics</td>
<td>106</td>
</tr>
<tr>
<td>Child Health Questionnaire (CHQ)</td>
<td>107</td>
</tr>
<tr>
<td>Study Management</td>
<td>109</td>
</tr>
<tr>
<td>Steering Committee</td>
<td>110</td>
</tr>
<tr>
<td>Web Based Management Information System</td>
<td>111</td>
</tr>
<tr>
<td>Research Staff Assistance</td>
<td>113</td>
</tr>
<tr>
<td>Data Management</td>
<td>114</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>115</td>
</tr>
<tr>
<td>Secondary outcomes</td>
<td>116</td>
</tr>
<tr>
<td>Missing Data</td>
<td>117</td>
</tr>
<tr>
<td>Results of the six year allergy follow up of children at high hereditary risk of atopy born to mothers supplemented with n-3 LCP UFAs during pregnancy</td>
<td>118</td>
</tr>
<tr>
<td>Introduction</td>
<td>119</td>
</tr>
<tr>
<td>Sample and participant flow</td>
<td>120</td>
</tr>
<tr>
<td>Non completers</td>
<td>122</td>
</tr>
<tr>
<td>Baseline Characteristics</td>
<td>123</td>
</tr>
<tr>
<td>Compliance with the intervention</td>
<td>125</td>
</tr>
<tr>
<td>SPT Completion</td>
<td>126</td>
</tr>
<tr>
<td>Assessment Location</td>
<td>127</td>
</tr>
<tr>
<td>Primary Outcome Analysis</td>
<td>128</td>
</tr>
<tr>
<td>Sensitivity analysis</td>
<td>129</td>
</tr>
<tr>
<td>Secondary Outcomes</td>
<td>131</td>
</tr>
<tr>
<td>Incidence of Eczema Symptoms</td>
<td>131</td>
</tr>
<tr>
<td>Severity of Eczema Symptoms</td>
<td>132</td>
</tr>
</tbody>
</table>
Longitudinal analysis of childhood allergy outcomes of children at high hereditary risk of atopy born to mothers supplemented with n-3 LCPUFA during pregnancy

Introduction

Aim

Hypothesis

Methods
Bibliography .................................................................................................................. 204

Appendix 1: Participant Information Sheet ................................................................. 217

Appendix 2: Participant Consent Form ......................................................................... 220

Appendix 3: Updated Contact Details Form ............................................................... 222

Appendix 4: Appointment Confirmation Letter ......................................................... 223

Appendix 5: HREC Amendment Request to use Facebook ....................................... 224

Appendix 6: Skin Prick Test SOP ............................................................................. 232

Appendix 7: Skin prick test results for parents ......................................................... 237

Appendix 8: Certificate for child ............................................................................. 238

Appendix 9: GP letter with skin prick test results ................................................... 239

Appendix 10: HREC Amendment Request – In home skin prick testing ............... 240

Appendix 11: Case Report Form (CRF) .................................................................... 247

Appendix 12: Child Health Questionnaire (CHQ) .................................................... 269

Appendix 13: CRF completion Instructions ................................................................ 274

Appendix 14: HREC Amendment Request – Verbal Consent ............................... 288

Appendix 15: Statistical Analysis Plan .................................................................... 294

Appendix 16: Guidelines for storage and monitoring of allergens .................... 313

Appendix 17: ASCIA Abstract ................................................................................. 316

Appendix 18: PSANZ Abstract ................................................................................. 317
List of Tables

Table 1-1  Alternative diagnostic techniques to determine sensitisation .......... 8
Table 1-2  Global median n-3 LCPUFA intakes (mg/day) ................................. 21
Table 2-1  Prospective Observational Studies of maternal fish or n-3 LCPUFA intake during pregnancy and allergic disease in the offspring .......... 35
Table 2-2  Randomised controlled trials (RCTs) of maternal n-3 LCPUFA supplementation during pregnancy and allergic disease in the offspring .................................................................................................................. 46
Table 2-3  Summary of risk of bias assessment for included RCTs ............. 55
Table 3-1  Secondary outcome descriptions .................................................. 74
Table 3-2  Additional secondary outcome descriptions ................................ 75
Table 3-3  List of allergen extracts used ......................................................... 95
Table 3-4  Case report form (CRF) sections .................................................. 104
Table 3-5  CHQ-PF50 Health Concepts (Domains) ........................................ 108
Table 4-1  Baseline demographic and clinical characteristics ....................... 124
Table 4-2  Locations where 6 year assessments were completed ............... 127
Table 4-3  Severity of eczema symptoms at 6 years .................................... 133
Table 4-4  Severity of wheeze and parent reported asthma at 6 years ............ 136
Table 4-5  Categories of wheezing phenotypes with and without sensitisation 138
Table 4-6  Incidence & Severity of Rhinitis/Rhino-conjunctivitis .................... 141
Table 4-7  Sensitisation to individual allergen extracts at 6 years of age .... 143
Table 4-8  Six year follow up study quality outcomes – SPT Completion .... 145
Table 4-9  Child Health Questionnaire ........................................................... 147
Table 4-10 Six year follow up study quality outcomes - Blinding .............. 150
Table 4-11 Socio-economic status, carer education and occupation ............. 152
Table 4-12 Environment Characteristics at 6 years ....................................... 154
Table 4-13  Child’s Current DHA Intake at 6 years ................................................................. 155
Table 4-14  Child Dietary Characteristics at 6 years.............................................................. 157
Table 4-15  Anthropometrics and Physical Activity .............................................................. 159
Table 4-16  Paracetamol and Ibuprofen use at 6 years.......................................................... 160
Table 5-1    Allergen extracts tested at 1, 3 & 6 years............................................................ 173
Table 5-2    Allergic disease symptom questions at 1, 3 & 6 years................................. 174
Table 5-3    Effect of n-3 LCPUFA supplementation on individual allergic disease symptoms at 1, 3 & 6 years ........................................................................ 179
Table 5-4    Longitudinal analysis of treatment effect (n-3 LCPUFA vs control) on individual allergic disease symptoms across all years ......................... 180
Table 5-5    Risk of allergic disease with sensitisation between n-3 LCPUFA and control groups across 1-6 years.......................................................... 181
Table 5-6    Effect of n-3 LCPUFA supplementation on sensitisation at 1, 3 & 6 years ................................................................................................................. 185
Table 5-7    Overall association of egg sensitisation at 1 year to D. farinae sensitisation at 6 years........................................................................................................ 187
Table 5-8    Association of egg sensitisation at 1 year to D. farinae sensitisation at 6 years in the n-3 LCPUFA group ................................................................. 188
Table 5-9    Association of egg sensitisation at 1 year to D. farinae sensitisation at 6 years in the control group ................................................................. 189
Table 5-10   Longitudinal analysis of treatment effect (n-3 LCPUFA vs control) on any sensitisation and sensitisation to individual allergen extracts across all years ................................................................................. 190
Table 5-11   Risk of sensitisation between n-3 LCPUFA and control groups across 1-6 years ............................................................................................................. 191
List of Figures

Figure 1-1 Immunoglobulin-E (IgE) and allergic reactions (12) ................................................................................................. 5
Figure 1-2 Sensitisation rates to food (egg and milk) and inhalant allergens (29) ............................................................... 12
Figure 1-3 Prevalence of sensitisation to inhalant allergens and the incidence of poly-sensitisation vs mono-sensitisation (29) ........................................................................................................... 12
Figure 1-4 Classes of essential fatty acids and conversion pathways (59) ................................................................. 20
Figure 2-1 Flow chart of literature search and eligibility of included studies .......................................................... 33
Figure 2-2 Incidence of confirmed ‘atopic’ eczema (with sensitisation) at 12 months ..................................................... 59
Figure 2-3 Incidence of ‘any’ eczema (with or without sensitisation) 0-12months ............................................................... 59
Figure 2-4 Cumulative incidence of IgE mediated rhino-conjunctivitis 0-3 years ......................................................... 60
Figure 2-5 Incidence of ‘any positive skin prick test (SPT) 0-12 months ................................................................. 61
Figure 2-6 Sensitisation to egg between 0-12 months ........................................................................................................ 63
Figure 2-7 Sensitisation to ‘any food’ at 12 months ........................................................................................................ 63
Figure 3-1 Skin Prick Test – Prick and lift action (148) ........................................................................................................ 88
Figure 3-2 Child (‘Cleo’) undergoing SPT procedure whilst watching a movie .............................................................. 89
Figure 3-3 Diagram of SPT wheal measurement ........................................................................................................ 90
Figure 3-4 Labelled Allergen Extracts for the 6 year assessment ................................................................................ 92
Figure 4-1 Flow diagram of the nested allergy follow-up cohort of the DOMInO trial .................................................. 121
Figure 4-2 Reasons for refusal to participate in the 6 year allergy follow-up ........................................................... 122
Figure 4-3 Reasons for non-completion of skin prick test at the 6 year assessment .................................................. 126
Figure 4-4 Incidence of IgE-mediated allergic disease symptoms with sensitisation defined as positive skin prick test ≥3mm at 6 years ................................................................................... 128
Figure 4-5 Primary outcome sensitivity analysis ........................................................................................................ 130
Figure 4-6 Symptoms of eczema with and without sensitisation at 6 years and parent reported eczema ever ........................................................................................................................................ 131
Figure 4-7 Symptoms of wheeze with and without sensitisation at 6 years and parent reported asthma ever ........................................................................................................................................ 134
Figure 4-8 Symptoms of rhinitis and rhino-conjunctivitis with and without sensitisation at 6 years and parent reported hayfever ever ........................................................................................... 139
Figure 4-9 Sensitisation to individual allergen extracts at 6 years of age ............................................................................... 142
Figure 5-1 Sensitisation pattern 1-6 years ................................................................................................................ 183
Abstract

There is general consensus that the remarkable increase in allergic disease over the last 30-40 years is due to environmental influences including lifestyle and diet. Due to a number of factors associated with an industrialised world, the gross imbalance of n-6 (omega 6) and n-3 (omega 3) polyunsaturated fatty acids (PUFA) in our diet is no longer concordant with our genetically determined biology. Data from clinical and animal studies suggest that dietary n-3 LCPUFA in early life may influence immune system development and immune cell function reducing inflammatory responses, however clinically beneficial effects are more conflicting.

I conducted a systematic review of the literature including observational studies of increased maternal dietary intake of n-3 PUFA and RCT evidence of prenatal n-3 LCPUFA supplementation on outcomes of allergic disease in the offspring. Whilst limitations of cohort studies are well recognised, the concordance between outcomes from both study designs is noteworthy and suggestive of benefits. The paucity of RCT evidence beyond early childhood however, makes it difficult to draw any strong conclusions regarding the effect prenatal n-3 LCPUFA supplementation.

The six year allergy follow up study was a double blind randomised controlled trial designed to investigate the effect of supplementation of women with a fetus at high risk of atopy with 900mg of n-3 LCPUFA or a blended vegetable oil (with no n-3 LCPUFA) on outcomes of allergic disease in the offspring. 668 families were invited to take part in an allergy assessment to determine the incidence of allergic disease symptoms (eczema, wheeze or allergic rhinitis) and sensitisation to determine food and aeroallergen sensitisation.
603 children (90.2% of eligible cohort) completed an allergy assessment at six years of age. Results show that n-3 LCPUFA supplementation in pregnancy does not reduce the overall incidence of IgE-mediated allergic disease at six years of age, 116/367 (31.48%) vs 106/336 (31.46%) control, aRR 1.04 (0.82, 1.33), p=0.73. However, secondary outcomes suggest that the intervention reduces the incidence of ‘sensitisation to house dust mite’ and parent reported ‘hayfever ever’, 49/367 (13.42%) vs 68/336 (20.30%), aRR 0.67 (0.44, 1.00), p=0.0495; 81/367 (22.05%) vs 98/336 (29.05%), aRR: 0.77 (0.59, 1.01), p=0.055 respectively.

This cohort of children with high hereditary risk of allergy also completed assessment of allergic disease and sensitisation at 1 and 3 years of age. A longitudinal analysis was performed on 1, 3 and 6 year data indicating that there was not enough evidence to conclude that the relative risk of sensitisation (n-3 LCPUFA vs control) changed over time or was associated with any outcomes of allergic disease or sensitisation across all years.

There are plausible mechanisms by which increasing maternal dietary n-3 LCPUFA intake may modulate the fetal immune system and subsequent development of allergic disease in infants at risk of atopic disease. Although my results did not show a reduction in overall IgE associated disease at 6 years or impact on longitudinal outcomes (1, 3 and 6 years), they are consistent with previous studies and suggestive of benefits of prenatal n-3 LCPUFA supplementation on certain aspects of allergic disease, namely sensitisation. My results support the necessity to further investigate these outcomes and their relationship to the clinical expression of disease.
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. This work, 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 for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

The systematic review and meta-analysis in this thesis (Chapter 2) is currently with the editors of the American Journal of Clinical Nutrition. I am first author and main contributor to the paper, written under the guidance of my supervisors Professor Maria Makrides, A/Professor Mike Gold and Professor Declan Kennedy.

I confirm that I personally completed the majority of the six year allergy assessments. When it became necessary to enlist the help of research staff of the Child Nutrition Research Centre to complete some of the 6 year allergy assessments (due to the number of assessments required and multiple clinic locations) I coordinated all aspects of study management including training, delegation and quality assurance.
I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

____________________  _____/____/____
Karen P Best
Publications & presentations in support of this thesis

Publications


Published Abstracts

Conference Presentations


Best K, Sullivan T, Gold M, Kennedy D, Martin J, Palmer D, Makrides M. Six Year Follow Up of Children at High Hereditary Risk of Allergy, Born To Mothers Supplemented With Docosahexaenoic Acid (DHA) in the DOMInO Trial. Perinatal Society of Australia & New Zealand, Melbourne Victoria, April 2015, (Oral presentation and abstract)
Acknowledgements

First and foremost I would like to thank the children who took part in this research project and their families. Without their unselfish commitment, this study would not have been possible.

Thank you to the staff of the Child Nutrition Research Centre for assisting with scheduling and re-scheduling of appointments, completion of assessments and general research support.

To the many South Australian metropolitan clinics and country Hospitals that accommodated me to complete the six year assessments, your support was invaluable in achieving follow-up of as many children as possible.

Thank you to my supervisors A/Professor Mike Gold and Professor Declan Kennedy for your guidance at steering committee meetings. A special thank you to Dr James Martin, not technically my supervisor but still attended every meeting with advice and clinical knowledge and thanks also to Tom Sullivan, the most amazing statistician and really nice person.
Thank you to the MS McLeod Foundation for provision of my Paediatric/Child Health Nursing PhD Scholarship.

A special thank you to my partner Jason and my children, Chloe, Liam and Lucy for your love, support and faith in my ability.

The final acknowledgement is for my principal supervisor, Professor Maria Makrides, thank you for your expert advice and guidance throughout this project. I have learned so much from you over the years and continue to do so. I am extremely grateful for the opportunities that you have provided me.

This research was supported by the National Health and Medical Research Council.
Abbreviations

AA: Arachidonic acid
ACTRN: Australian clinical trials registry number
ALA: Alpha-linolenic acid
ANZCO: Australian & New Zealand Coding of Occupations
aRR: adjusted relative risk
CNRC: Child Nutrition Research Centre
CI: Confidence interval
CHQ: Child Health Questionnaire
CNRC: Child Nutrition Research Centre
CRF: Case report form
D. farinae: Dermatophagoides farinae
D. pteronyssinus: Dermatophagoides pteronyssinus
DHQ: Diet history questionnaire
DHA: Docosahexaenoic acid
DMAC: Data Management & Analysis Centre
DOMInO: Docosahexaenoic Acid to optimise maternal and infant outcomes
EFA: Essential fatty acid
EMBASE: Excerpta Medica Database
EPA: Eicosapentaenoic Acid
FFQ: Food Frequency Questionnaire
FMC: Flinders Medical Centre
GCP: Good Clinical Practice
HDM: House dust mite
HLA: Human-leucocyte antigen
HREC: Human Research Ethics Committee
IgE: Immunoglobulin-E
ITT: Intention to treat
LA: Linoleic Acid
n: number of participants
NHMRC: National Health and Medical Research Council
PUFA: Poly unsaturated fatty acid
RCT: Randomised controlled trial
RR: Relative risk
SEIFA: Socio-Economic Indexes for Areas
SCORAD: standardised scoring system for atopic dermatitis
SMS: Short message service
SOP: Standard operating procedure
SPT: Skin prick test
WCH: Women’s & Children’s Hospital
WCHN: Women’s & Children’s Health Network
Introduction & Background
Introduction

Epidemiologic evidence on allergy and asthma indicate a relentless increase in prevalence over the past several decades (1). Australia has the one of the highest rates of allergy in the western world (2) with nearly 20% of the population (4.1 million) suffering at least one allergic disease in 2007 (3). Allergies are currently associated with 4 of the top 10 most common long-term (chronic) self-reported illnesses in youth aged 12-24 years in Australia (3). As children are the majority of those affected, this prevalence is expected to rise as they reach adulthood and allergies persist. Whilst the prevalence of asthma has plateaued in recent years food allergy prevalence continues to rise affecting an estimated 10% of the population (4) Fivefold increases in hospitalisation rates for food allergy-related anaphylaxis in 0–4-year-olds have been reported in the United Kingdom, United States and Australia in recent decades (5).

The worldwide epidemic of allergic disease has escalated too rapidly to be attributed to genetic changes alone and the cause is considered to be a consequence of a changing environment. There is growing awareness that profound environmental change to everyday lifestyle in industrialised countries is discordant with our ancient genetically determined biology. The increase in allergic disease over the last 30 – 40 years suggest that this environmental variation may be responsible for unmasking a genetic predisposition (6) and there is an ongoing search for causal associations that will help to identify strategies to reverse the trend. A number of features associated with a ‘western’ lifestyle have been suggested to be in part, contributory to the increased prevalence of asthma and allergy. There is strong epidemiological evidence that increasing socio-economic conditions and the resulting improvement in hygiene standards may be an
influencing factor. Declining family size, fewer respiratory infections, greater use of antibiotics early in life, less contact with farm animals and a general decrease in the amount of microbial exposure in childhood collectively referred to as the ‘hygiene hypothesis’ are all factors that have been linked to the current allergy epidemic (1). There is growing evidence that diet may play a major role in the increase in allergic disease, in particular the dramatic changes to the type of fats consumed in the western diet (8). Since the advent of the vegetable oil industry and replacement of saturated fats with plant oils the balance of fat in the western diet now consists of an overwhelming predominance of inflammatory omega 6 polyunsaturated fatty acids (n-6 PUFA) at the expense of anti-inflammatory omega-3 PUFA (n-3).

Cohort studies have observed associations of maternal dietary intake of n-3 long chain PUFA (LCPUFA) during pregnancy and allergic disease in the offspring and randomised controlled trials (RCTs) have investigated the effects of prenatal supplementation with n-3 LCPUFA or a placebo on outcomes of allergic disease. I conducted a comprehensive systematic review and meta-analysis of the available evidence (including observational studies and RCTs) and concluded that although suggestive of benefits, the current evidence neither supports or refutes the hypothesis that n-3 LCPUFA supplementation during pregnancy will reduce the incidence of allergic disease in the offspring. Observational studies with follow up of the child at school age are suggestive of benefits of increased maternal dietary intake of n-3 LCPUFA during pregnancy on the symptoms of allergic respiratory disease and sensitisation to aeroallergens however no RCTs have assessed outcomes in this age group. As a result, a new follow up study of a nested allergy cohort of a high-quality RCT that is detailed in the thesis, was implemented to
assess the effect of n-3 LCPUFA supplementation during pregnancy on symptoms of allergic disease in the school age child - “The Six year Allergy follow-up of children at high hereditary risk of atopy born to mothers supplemented with n-3 LCPUFA during pregnancy”.

The following chapter details the aetiology of IgE mediated allergic disease including the atopic march. The basic function of n-3 LCPUFA is discussed as well as current intakes in the general population and pregnant women.
Allergic Disease

Allergic disease is defined as hypersensitivity or an exaggerated reaction of the immune system to external substances (usually a protein) that are harmless to most people (allergens) \(^9, 10\). This immune response which results in “sensitisation” is classed as a Type I or immediate hypersensitivity reaction \(^11\). When a susceptible individual is exposed to an allergen, immunoglobulin E (IgE) antibodies, specific to the antigen, are created and bind to IgE receptors on mast cell surfaces. When re-exposed, the allergen, via the IgE antibodies, binds to mast cells, cross-linking the IgE leading to degranulation and the release of histamine and other inflammatory mediators, resulting in ‘sensitisation’, Figure 1-1.

![Figure 1-1 Immunoglobulin-E (IgE) and allergic reactions](image)

Figure 1-1 Immunoglobulin-E (IgE) and allergic reactions \(^12\)
Release of inflammatory mediators causes contraction of smooth muscle cells, vasodilation, increased vascular permeability and platelet aggregation (13). These reactions can affect a single tissue or organ or multiple organs depending on local or general re-exposure to the allergen. This IgE response presents as an allergic reaction, manifesting clinically as atopic eczema (atopic dermatitis, eczema), atopic asthma IgE mediated asthma), allergic rhinitis (hay fever, rhinitis, rhino-conjunctivitis) and food allergy or anaphylaxis.

The genetic predisposition to this over-production of IgE in response to allergen exposure is termed “atopy” and is the strongest identifiable predisposing factor for development of atopic asthma (14) and other allergies. Pre-disposition to atopy is multifactorial, the strongest predictor being family history. Children born into atopic families are 50-80% more likely to develop allergic diseases compared to those with no family history (20%) (15). This risk appears to be higher if both parents are atopic as opposed to only one parent and also if the mother (as compared to the father) is atopic (15). Up to 40% of Australian children have evidence of allergic sensitisation (16) but not all will develop symptoms of allergic disease, the onset appears to be due a complex interplay between the genes associated with atopy and environmental influences.

Sensitisation

Assessment of sensitisation by skin testing has been used for investigation into allergy as early as 1865 when Blackley discovered that IgE mediated allergic diseases are caused by exposure to allergens (17). Lewis and Grant first described the prick puncture test in 1924 and this method was widely used from the 1970’s onward following modification by Pepys (18). The primary objective of skin prick
testing is to ascertain whether an individual has an IgE initiated response to an allergen or allergens when introduced to the skin, therefore confirming that the individual has been ‘sensitised’. A number of alternative diagnostic techniques have been used over the years to confirm sensitisation in IgE-mediated allergic disease, Table 1-1 (19).
### Table 1-1 Alternative diagnostic techniques to determine sensitisation

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Indications for use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgE</td>
<td>Levels of total IgE antibody can be estimated from a blood sample</td>
<td>Elevated IgE does not prove that symptoms are due to allergy, and a normal IgE level does not exclude allergy, also there is NO relationship between the level of IgE and symptom severity. Not routinely recommended in allergy testing. Total IgE is often (but not always) raised in people with allergies and in those with internal parasites.</td>
</tr>
<tr>
<td>Specific IgE</td>
<td>The amount of IgE directed against specific allergens can be measured from a blood sample.</td>
<td>Testing is often performed when severe eczema or dermographism prevent accurate testing by SPT or when the patient is taking medications (such as antihistamines or tricyclic antidepressants) that interfere with accurate testing. May give misleadingly falsely positive or false negative results.</td>
</tr>
<tr>
<td>Intra-dermal testing</td>
<td>A small amount of very dilute allergen is injected into the upper layers of the skin</td>
<td>More sensitive test than SPT but more uncomfortable and more likely to lead to false positive and clinically irrelevant results. Not recommended for routine use for aeroallergens and foods, but may have a place in venom and drug allergy diagnosis. It carries a greater risk of anaphylaxis and should be limited to those with specialist training.</td>
</tr>
<tr>
<td>Scratch Testing</td>
<td>Allergens are applied to the skin and the skin is scratched with a lancet</td>
<td>Poor reliability and greater patient discomfort than SPT. Not recommended.</td>
</tr>
<tr>
<td>Test</td>
<td>Method</td>
<td>Indications for use</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Patch Testing</td>
<td>Allergens applied to area of skin and left in place for 48 hours.</td>
<td>Relevant to contact hypersensitivity and some other forms of delayed-type hypersensitivity. It is conducted mainly by dermatologists and some immunologists, and is not relevant to immediate or IgE-mediated allergy</td>
</tr>
</tbody>
</table>

The skin prick test (SPT) is recognised as the gold standard core diagnostic test to determine sensitisation and is recommended as the primary method for the diagnosis of IgE mediated allergies in most allergic diseases (21). Skin prick testing is considered to be a low risk procedure however there are reports of adverse reactions in the literature. These have occurred mainly in adults with pre-existing conditions and generally occur with multiple extract testing and predominantly to fresh foods (22). Adverse reactions in children are less common and reports are conflicting as to how often they occur (23). A population-based study of paediatric food allergy conducted in 2010 reported no adverse reactions or anaphylaxis from 2464 twelve month old infants who underwent skin prick testing to common foods (24). A Swedish study designed to assess prevalence and possible risk factors of adverse reactions to skin prick testing in children reported 14 adverse reactions out of 5,908 children undergoing SPT. Seven of these were generalised adverse reactions and required medication, yielding a 0.12% risk. The other seven were vaso-vagal reactions giving the same risk, 0.12%. Identified risk factors for generalised reactions were low age (<1 year) and active eczema (25).
The advantages of SPT over other methods of testing are the relative sensitivity and specificity, rapid results, flexibility, low cost, good tolerability, and clear demonstration of IgE response. However the test is subject to some operator, observer and interpretation variability and must be performed in accordance with strict guidelines to ensure consistency of results.
Allergic disease is characterised by a typical sequence of sensitisation and symptoms which can last many years or even the whole life of the individual. This developmental pattern of allergic disease is termed the ‘atopic or allergic march’ expressing the common course of IgE antibody response and expression of clinical symptoms. The time it takes to become sensitised to an allergen varies from person to person and characteristically evolves in the order of exposure; food, indoor allergens, outdoor allergens. Typically, the first IgE responses (directed to food proteins) appear in infants during the first months of life, most commonly directed to hen’s egg and cow’s milk \(^{(26)}\). Symptoms of eczema and food allergy are usually the first indication of allergic disease at this time, reaching their highest prevalence during the first 2 years of life \(^{(27)}\). As the atopic march progresses a number of children will outgrow their eczema and food allergy, however, sensitisation to indoor aeroallergens (cat, dog, house dust mite (HDM) and mould) occurs with increasing prevalence, Figure 1-2. This is generally followed by sensitisation to outdoor allergens (grasses and pollens). Aeroallergen sensitisation (environmental allergens) is generally associated with symptoms of allergic respiratory disease including atopic asthma and allergic rhinitis/rhinoconjunctivitis which steadily increase in prevalence into the school years often persisting throughout adulthood. Frequently these conditions co-exist with varying severity from minor discomfort to life threatening reactions \(^{(28)}\).
Chapter One

Figure 1-2 Sensitisation rates to food (egg and milk) and inhalant allergens (29)

Early sensitisation to food protein (hen’s egg and cow’s milk) predicts later sensitisation to aeroallergens and allergic disease, especially in children with atopic heredity or early atopic symptoms (30). The major risk factor for atopic asthma is early sensitisation to either foods in the first year of life (odds ratio, 12.3) or aeroallergens (odds ratio, 4.6) in the first 2 years of life (31). With increasing age, the incidence of sensitisation to a number of allergens (poly-sensitisation) is higher than that of mono-sensitisation, Figure 1-3 (29).

Figure 1-3 Prevalence of sensitisation to inhalant allergens and the incidence of poly-sensitisation vs mono-sensitisation (29)
Atopic Eczema

Eczema is an inflammatory, chronically relapsing, non-contagious, pruritic skin disease (32) and is often the first symptom of the atopic march. Eczema is the most common inflammatory skin disease of childhood, (33) however not all eczema is atopic. A systematic review has shown that the prevalence of allergic sensitisation among eczema sufferers ranges widely, between 7% and 75% (34). Eczema has also been known as dermatitis, atopic dermatitis and atopic eczema/dermatitis syndrome. In 2003 the World Allergy Organisation Nomenclature Task force recommended that ‘eczema’ would be the agreed term and that ‘atopic eczema’ would be the term used if there was demonstrable IgE association (32). Among the 56 countries that took part in the International Study of Asthma and Allergies in Childhood (ISAAC), the prevalence of eczema varied significantly from 0.3% to 20.5% but showed consistent trends in increasing disease prevalence over time (35). As with other allergic diseases, propensity is linked to both genetic and environmental factors, 70-80% of eczema sufferers have co-existing allergic disease (asthma, allergic rhinitis or food allergy) and/or IgE mediated sensitisation (36). Atopic eczema with sensitisation to environmental allergens is a major risk factor for progression along the atopic march to allergic rhinitis (75%) and asthma (50%) during the first 6 years of life (31, 37). Early onset sensitisation and severity of eczema is also predictive of the persistence and progression of asthma (27). 70% of individuals with severe atopic eczema develop asthma compared with 20-30% with mild atopic eczema and approximately 8% in the general population (28).

Symptoms of eczema often present in the first few months of life with the majority of cases diagnosed before the age of five years (38). The cause of eczema is unknown although there is evidence that it is linked to a disrupted epidermal
barrier function as well as an abnormal immune response \(^{(39)}\). The current widely accepted diagnostic measure for eczema is based on the Hanifin and Rajka criteria \(^{(40)}\). This method is most often used by trained specialists and involves the presence or absence of 33 minor features. This was somewhat impractical for epidemiological surveys and therefore a working group of 16 leading dermatologists identified a minimum list of reliable discriminators for diagnosing a typical case of eczema for the International Study of Asthma and Allergy in Childhood (ISAAC) \(^{(41)}\). The combination of a chronic itchy rash with flexural involvement had both high sensitivity (0.80) and specificity (0.97) when compared with eczema diagnosis by standardised dermatologist assessment \(^{(42)}\).

Moderate-to-severe eczema can have a profound effect on the quality of life for both sufferers and their families. In addition to the effects of the stigma of a visible skin disease, the child also suffers sleep loss from intractable itching and soreness. Additional factors such as frequent doctor visits and the need for special clothing and messy topical applications all add to the burden of disease \(^{(43)}\). A study evaluating the impact of eczema on the child and family have reported that 63% of children with eczema had current sleep problems and most had had sleep disturbance at some time \(^{(44)}\).
Allergic Rhinitis/Rhino-conjunctivitis

Allergic rhinitis (IgE mediated rhinitis, hayfever) is an inflammatory condition affecting nasal mucosal membranes with or without ocular involvement (rhino-conjunctivitis). In sensitised individuals, aeroallergens including pollens, moulds and animal dander provoke an IgE mediated allergic response. Symptoms commonly present as nasal discomfort with sneezing, discharge and/or congestion but may also include itching of the eyes (rhino-conjunctivitis), ears and throat, headaches, fatigue and sleeping difficulties. Allergic rhinitis is a common disease affecting from 5 to 50% of the world population and its prevalence is increasing. The ISAAC showed that ‘seasonal’ allergic rhinitis (hay fever) affects up to 50% of adolescents in developed countries.

Although not usually a severe disease (and often trivialised), allergic rhinitis has a significant impact on quality of life and substantial socioeconomic consequences, and it is associated with multiple comorbidities, including asthma. Studies on the prevalence of asthma in patients with rhinitis varies considerably, but has been reported to be as high as 80%.
Chapter One

16

IgE-mediated Allergic Asthma

Asthma is a chronic lung disease that inflames and narrows the airways and represents a spectrum of conditions with different pathophysiological mechanisms. By international standards the prevalence of asthma in Australia is relatively high with over 2 million Australians suffering the disease in 2007-2008 (48). Asthma represents the leading cause of burden of disease among children aged 0–14 years, contributing 17.4% of lost years of healthy life in that age group (49). Asthma is also one of the most common causes of hospital admission and visits to the doctor in young children (49). The most common type of asthma is IgE mediated or atopic asthma, with 80% of childhood asthma and >50% of adult asthma reported to be atopic in origin (50). Currently there is no single reliable test or standardised diagnostic criteria for asthma and there is a lack of consensus in defining asthma in the medical literature (51). A recent systematic review showed that of 122 publications investigating risk factors associated with childhood asthma, 60 varying definitions were used (52). Clinical diagnosis in children involves a history of recurrent or persistent wheeze, a consistent clinical response to an inhaled bronchodilator or preventer and the absence of physical findings that suggest an alternative diagnosis (53). Diagnosis of asthma before the age of five is extremely difficult due to the many and varied respiratory wheezing conditions that are common in this age group. Population studies have shown that approximately one in three children will have at least one episode of wheezing prior to age three (54). However this wheezing is transient in more than half of these children and does not increase their risk of asthma in later life (55). Regardless of when symptoms occur, presence of atopy (IgE sensitisation) is associated with an increased risk for persistent wheeze and reduced lung function at six years of age, and is more likely to be an indicator of asthma (56). There is poor agreement between physician
diagnosis of asthma as well as definitions of different phenotypes of childhood wheezing disorders (54). The European Respiratory Society assembled a task force with the purpose of producing guidelines for the treatment of wheezing in children aged <six years based on all of the available evidence (54). Whilst not useful for clinical diagnosis, the following retrospective description of duration of wheeze, as used in epidemiological studies, are considered most appropriate to report asthma related symptoms (53, 57).
- Early Transient wheeze
  Wheezing in first 3 years of life but no wheezing at 6 years

- Late onset persistent wheeze
  No wheezing in first 3 years of life but wheezing at 6 years

- Early onset persistent wheeze
  Wheezing in first 3 years and continue to wheeze at 6 years

Assessment of asthma by questionnaire for the purpose of outcome assessments in clinical research is challenging. In the absence of a physical examination, questions thought to be most reliable in capturing incidence of asthma are those that include symptom-based components such as 'wheeze'. These types of questions do not rely on lay understanding of asthma and therefore are more sensitive than items only inquiring about 'asthma'. This was the approach adopted by the ISAAC study and included in their asthma module. Validation studies of the ISSAC questionnaire against respiratory physician assessment in the diagnosis of asthma have found questionnaire responses to be both sensitive 0.85 (0.73–0.93) and specific 0.81 (0.76–0.86). \(^{55, 58}\)
Long Chain Polyunsaturated Fatty Acids (LCPUFA)

Dietary fat is an important source of energy for all biological activities in human beings. Types of fats include monounsaturated, polyunsaturated and saturated fats. Whilst saturated fatty acids and some monounsaturated fatty acids can be synthesised from carbohydrates and proteins, we are unable to synthesise linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (18:3n-3) which are consequently labelled ‘essential fatty acids' (EFA) as they must be obtained in the diet. When consumed in their plant based form, ALA and LA undergo a series of biological processes to convert them to their longer chain derivatives, arachidonic acid (AA; 20:4n-6) from LA, and long-chain n-3 PUFA (LCPUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) from ALA, Figure 1-4.
The same enzymes (de-saturases and elongases) are required by both classes of fatty acids (LA and ALA) to enable conversion to their long chain derivatives. High levels of n-6 LA in the diet inhibit incorporation of DHA and EPA into tissues, resulting in generally low n-3 LCPUFA status (60). The rate of conversion of ALA to LCPUFA varies in individuals but it is generally low (∼5% EPA & <1% DHA). Fatty acid conversion can affected by several factors including background n-6 PUFA intake, dietary cholesterol, and gender-related hormones (61). There are distinct differences in structure and function of n-3 compared to n-6 LCPUFA’s. AA (from n-6) is present in high proportions in human inflammatory cells. AA is the precursor of 2-series prostaglandins and 4-series leukotriene’s, which are highly-active mediators of inflammation with a major role in allergic inflammation (62).
Current dietary intake of PUFA

There is considerable inter and intra-population variability in dietary n-3 LCPUFA intake, Table 1-2. The amount of n-3 LCPUFA consumed in Western cultures is up to five fold lower than high fish and seafood eating countries like Japan (63).

Table 1-2 Global median n-3 LCPUFA intakes (mg/day)

<table>
<thead>
<tr>
<th>Country</th>
<th>Population</th>
<th>Year of data collection</th>
<th>Median intakes (mg/day)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenland</td>
<td>Eskimos</td>
<td>1976</td>
<td>13000</td>
<td>[14]</td>
</tr>
<tr>
<td>Canada</td>
<td>Inuit of Nunavik</td>
<td>1992</td>
<td>2115</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>James Bay Cree</td>
<td>1992</td>
<td>800</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Quebec</td>
<td>1992</td>
<td>170</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Quebec</td>
<td>~2008</td>
<td>207*</td>
<td>[20]</td>
</tr>
<tr>
<td>Japan</td>
<td>Kyushu, SW island of Japan</td>
<td>1999</td>
<td>905</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>INTERLIPID Study</td>
<td>2003</td>
<td>810</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>Aito Town</td>
<td>2003</td>
<td>310</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>INTERLIPID study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Japanese living in Hawaii</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>All regions of France</td>
<td>1995</td>
<td>364</td>
<td>[19]</td>
</tr>
<tr>
<td>Nth Sth Europe</td>
<td>7 centres in Europe</td>
<td>2003</td>
<td>239</td>
<td>[21]</td>
</tr>
<tr>
<td>Belgium women</td>
<td>Women living in Flanders</td>
<td>2009</td>
<td>199</td>
<td>[22]</td>
</tr>
<tr>
<td>Germany</td>
<td>German Nutrition Survey</td>
<td>1998</td>
<td>160</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>6 multi-ethnic communities</td>
<td>2002</td>
<td>100</td>
<td>[25]</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Rotterdam coronary calcification study</td>
<td>1993</td>
<td>97*</td>
<td>[26]</td>
</tr>
</tbody>
</table>
Common food sources of LA include vegetable oils, such as soybean, safflower, and corn oil, nuts and seeds. The longer chain member of the n-6 series, AA is found mostly in animal sources such as meat, poultry, egg yolk and breast milk. ALA predominantly comes from flaxseeds, walnuts canola and their oils. The most common food source of the n-3 series LCPUFA (DHA & EPA) is dark-meat fin-fish or “fatty” fish, like tuna, salmon, mackerel, herring, and sardines. A relatively balanced amount of n-6 to n-3 fatty acids is considered ideal for optimal health, however, over the last 30-40 years the composition of fat in western diets has changed dramatically. Consumption of LA has increased exponentially since the advent of the vegetable oil industry and replacement of saturated fats with plant oils. Changes to agriculture and aquaculture farming methods have further increased dietary n-6 overload as domestic livestock and fish are fed on n-6 rich grains altering the fatty acid profile of the meat. Compounding this effect, the increase in dietary n-6 PUFA has coincided with a decrease in the amount of whole food, fish consumption and the increased consumption of processed foods. This predominance of LA in most western diets (5 to 20-fold greater amounts than ALA) result in inflammatory AA being incorporated into cellular phospholipids, and in the process, displacing anti-inflammatory DHA and EPA.

Cumulatively, these changes to the diet in industrialised countries, has fundamentally altered the nutritional characteristics of ancestral traditional diets. These profound changes in the type of fat consumed parallel the increasing prevalence of allergic disease, (atopic eczema, allergic rhinitis and atopic asthma) posing the hypothesis that there may be a causal relationship.
LCPUFA and Pregnancy

Pregnancy and infancy are periods of development during which the fatty acid supply is particularly significant \(^{(60)}\). Several studies have shown that maternal intake of fatty acids during pregnancy is a major determinant of the fatty acid status of infants at birth \(^{(66-69)}\). As the synthesis of LCPUFA in the fetus and placenta is low, both the maternal LCPUFA status and placental function are critical for their supply to the fetus \(^{(70)}\). The metabolic demand for n-3 LCPUFA, in particular DHA is increased during pregnancy, especially the last trimester when large amounts are deposited in the fetal retina and brain. Irrespective of diet, maternal plasma concentrations of DHA increase by \(\approx52\%\), between 10 and 40 weeks of pregnancy \(^{(71)}\). The requirement for n-3 LCPUFA during pregnancy for optimal fetal neurological growth and development is well documented \(^{(72, 73)}\). The World Health Organization (WHO) recommends pregnant women consume at least 200 mg DHA daily \(^{(74)}\) in order to provide the fetus with the estimated requirement of 70 mg/day \(^{(75)}\). This recommended intake of DHA can be achieved by consuming one to two portions of sea fish per week, including fatty fish. However, as demonstrated by the Australian Longitudinal Study on Women's Health, mean fish intake of pregnant women and for women who had given birth within the last 12 months, were well below the suggested intakes by Food Standards Australia and New Zealand \(^{(76)}\). The mean intake of n-3 LCPUFA, DHA in Australian women of child bearing age is 106 mg/day, although consumption is disproportionately distributed and median intake is only 15mg/day \(^{(77)}\).
LCPUFA and Allergy

The extensive immuno-modulatory properties of n-3 LCPUFA are well recognised \(^{78-80}\) however the evidence involving n-3 LCPUFA supplementation in established disease has been unconvincing \(^{81}\). A Cochrane review & meta-analysis conducted in 2002 concluded that there was little evidence to recommend that people with asthma supplement or modify their diet to increase intake of omega-3 fatty acids in order to improve their symptoms \(^{82}\). However, the period of fetal immune system development is more susceptible to influence and there are plausible mechanisms by which diets high in n-3 LCPUFA may modulate the development of IgE mediated allergic disease. Diets high in n-3 LCPUFA, particularly DHA increase cell membrane n-3 LCPUFA and inhibit synthesis of inflammatory AA, reduce prostaglandin-E synthesis and pro-inflammatory cytokine responses known to be associated with allergies. There is increasing evidence demonstrating modification of immune function and influence on perinatal immune programming at a number of different stages in this complex process \(^{83-86}\).

Immune responses to antigens have been observed in the fetus as early as 25 weeks gestation \(^{87}\) with symptoms of allergic disease often occurring within months of birth. This indicates that the events that lead to immune dysregulation are initiated in early development, supporting the potential influence of prenatal interventions.
Supporting evidence of prenatal n-3 LCPUFA

A number of epidemiological studies and randomised controlled trials (RCTs) have been conducted over the last ten years to investigate whether an increased maternal intake of n-3 LCPUFA during pregnancy will lower the risk of developing childhood allergies. Although RCTs are the strongest study design for drawing causal inferences regarding relations between exposures, the complexities of nutrient actions and interactions cannot always be adequately addressed through any single research design. It has been suggested that due to limitations of RCTs assessing single nutrient effects, the totality of the available evidence should be utilised to determine nutrient disease associations (59). I therefore conducted a systematic review of the literature including observational studies and RCTs to identify, assess and synthesise the available evidence, see Chapter 2.
A Systematic Review of the Literature

Omega-3 LCPUFA intake during pregnancy and allergic disease outcomes in the offspring:
A systematic review and meta-analysis of observational studies and randomised controlled trials
Introduction

There is general consensus that the worldwide prevalence of allergic disease has escalated too rapidly to be attributed to genetic changes alone. Although the cause may have mixed aetiology, it is widely accepted that the current allergy epidemic is attributable to a changing environment, including lifestyle factors and diet. Dietary ratios of n-6 (omega-6) to n-3 (omega-3) polyunsaturated fatty acids (PUFAs) have changed from an equal balance of n-6:n-3 (1:1) to almost 30:1 (n-6:n-3) in some Western cultures (64). These profound changes in the type of fat consumed coincidentally parallel the increasing prevalence of atopy and allergic disease (atopic eczema, IgE mediated rhino-conjunctivitis, IgE mediated allergic asthma), posing the hypothesis that this imbalance may have a causal relationship. When consumed, n-3 and n-6 compete for the same enzymes to convert them into their long chain derivatives (LCPUFA). Diets high in n-6 PUFA via increased consumption of linoleic acid (LA) rich vegetable oils and arachidonic acid (AA) from meat, result in a predominance of pro-inflammatory AA in tissues at the expense of n-3 LCPUFA. This leads to biochemical and physiological changes consistent with a greater propensity to an inflammatory allergic response (88). Plausible mechanisms therefore exist whereby diets high in n-3 LCPUFA may modulate the development of immunoglobulin E (IgE) mediated allergic disease and regulate immune responses. Data from clinical and animal studies suggest that dietary n-3 LCPUFA in early life may influence immune system development and immune cell function reducing inflammatory responses, however clinically beneficial effects are more conflicting (87). Initiating events of allergic disease occur early in immune development with antigen specific reactivity detected in cord blood at birth and as early as 23 weeks gestation in the fetus (87). Hence, there
may be a window of opportunity to modulate the fetal immune system before it has been programmed to an allergic phenotype by increased maternal supply of anti-inflammatory n-3 LCPUFA. Published reviews on the effect of n-3 LCPUFA in the primary prevention of allergic disease have been conducted, however, not all are systematic and most don't include observational studies. Two reviews pooled results of n-3 LCPUFA exposure from fetal life, infancy and childhood, increasing variability of results and potentially diluting any causal effect. In the complex field of nutrition and health outcomes, multiple levels of evidence should be considered. I have therefore included epidemiological studies observing a n-3 PUFA/LCPUFA dietary exposure and RCTs with a n-3 LCPUFA intervention and subsequent associations or effects on IgE mediated allergic disease. By limiting this systematic review to exposures or interventions that commenced in the intra-partum period, we aim to develop a clearer understanding of the effect to the developing fetus, prior to commencement of the atopic march and establishment of allergic disease symptoms.
Methods

Prospective studies including longitudinal observational studies and randomised controlled trials (RCTs) were included. Observational studies were included if they examined an association between maternal fish or n-3 LCPUFA intake during pregnancy and clinical outcomes of allergic disease or sensitisation in the offspring. RCTs and quasi-randomised trials that evaluated an intervention modifying maternal n-3 LCPUFA intake during pregnancy with a parallel control group or placebo on the clinical outcomes of allergic disease (eczema, rhino-conjunctivitis, asthma) or sensitisation in the offspring were eligible for inclusion in the review. Animal studies, cross sectional studies, retrospective and case control studies were excluded.

All included participants were pregnant women, regardless of gestation, and their offspring. There was no restriction placed on the atopic pre-disposition of the women or the offspring or age of follow up of the child for allergic disease. Included studies were required to report maternal intake of n-3 LCPUFA via dietary intake or supplement during the pregnancy period. Studies of maternal n-3 LCPUFA consumption or supplementation in the postnatal period only (breast feeding or direct supplementation of the infant) were excluded. The primary outcome measure of this review is the incidence of atopic disease (i.e. IgE mediated allergic disease) or sensitisation in the offspring during infancy, childhood or adolescence. Presence of IgE mediated allergic disease is defined as a clinician diagnosis, parental report of symptoms of allergic disease or doctor diagnosis. Sensitisation is defined as a positive skin prick test (SPT) or IgE serology indicating sensitisation. Studies reporting immune biomarkers by
laboratory assessment, in the absence of evaluation of symptoms or clinical diagnosis of allergic disease in the offspring, were excluded.

Search Strategy

I supplemented my search by cross-checking the reference lists of relevant retrieved publications identified by the search and recent review articles. No date restrictions were imposed although results were limited to human studies in the English language. The titles and abstracts of all articles retrieved by the search were reviewed by one author (KB) to assess eligibility for inclusion in the review. If there was insufficient information in the abstract to warrant exclusion of an article, the full text of the article was retrieved to determine eligibility. Any uncertainty regarding inclusion of publications was resolved following discussion with a second reviewer (MM).

Data relating to dietary exposure or interventions, outcomes, potential effect confounders and study quality were extracted by use of a standardised data extraction form. Extracted information included characteristics of study participants, type of exposure measure (cohort), maternal exposure/supplementation to fish or n3-LCPUFA, timing and length of intervention, type and timing of outcome measure/s. Studies included in this review varied markedly in terms of the methods, timing and type of intervention and timing and type of reported outcome measures. Study results for all observational studies and a number of RCTs were reported descriptively including their applicability and limitations. Meta-analysis was only considered for RCTs with comparable timing of outcome assessment (age of offspring). Outcomes of allergic disease that met this criterion include; ‘atopic eczema’, ‘sensitisation to egg’, ‘sensitisation to any food’ and ‘any sensitisation’. Asthma symptoms and rhino-conjunctivitis were unable to be combined due to heterogeneity between definition of outcomes between studies and the young age of follow up (2 & 3 years). Pre-planned analysis of allergic disease outcomes were grouped according to age at assessment of
outcome. Sensitivity analysis was conducted to investigate the influence of a single study on the overall estimate, P<0.05 was considered statistically significant for all tests. All analyses were performed using Review Manager Software 5.3 and heterogeneity was assessed by comparing the confidence intervals (CIs) of the results of individual studies and with the I² statistic. When there was an absence of significant heterogeneity, the results were pooled by using a fixed-effect model. When substantial heterogeneity was detected (I² >50%), possible causes were explored, and a random-effects model was used. Continuous outcomes are reported as mean differences (MDs) with 95% CIs.
Results

The search returned a total of 1121 publications. After removing duplicates, 533 publications remained. Of these, 472 publications were excluded because they did not meet the predefined inclusion criteria. A total of 13 publications from 10 prospective cohort studies and 7 publications representing 5 unique RCTs were included in the review, Figure 2-1. Three trials with comparable timing of outcome assessments for; ‘any sensitisation’, ‘sensitisation to egg’ and ‘sensitisation to any food’ and two trials with atopic eczema outcomes at 12 months were combined in meta-analysis.

Figure 2-1 Flow chart of literature search and eligibility of included studies
Participants

All studies were conducted in industrialized countries. Seven of the observational studies enrolled healthy pregnant women presenting at antenatal or obstetric care clinics between 5 and 39 weeks gestation irrespective of atopy status (96-101). One study from the Netherlands selectively enrolled participants depending upon their nationality (102) and another restricted enrolment to Danish speaking participants. (103). Two remaining studies enrolled full term infants, collecting maternal diet information retrospectively (104-107). One of these studies (three publications included in this review) was a nested nutrition follow up of a diabetes prediction and prevention study in Finland. Mothers were enrolled following birth and confirmation that their child was at risk of developing diabetes by the presence of human leucocyte antigen (HLA) in cord blood (105, 106, 108). Characteristics and results of observational studies are presented in Table 2-1.
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Exposure Assessment</th>
<th>Maternal Exposure Categories</th>
<th>Outcome Assessment</th>
<th>Results</th>
<th>Adjusted Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maslova et al., Denmark 2013</td>
<td>n=28,936 Danish speaking women from Danish National Birth Cohort 1996-2002</td>
<td>360-item FFQ at 25 weeks gestation (previous 4w); Fish intake via telephone interview x2</td>
<td>High vs low fish exposure; Zero intake ≤ Monthly mean (21g/w) &gt; Monthly Weekly-Low frequency Weekly-High frequency (292g/w) Mean fish intake or LCPUFA=NR</td>
<td>Parent report doctor-diagnosed asthma, wheeze symptoms and number of wheezing episodes at 18m</td>
<td>Zero maternal fish intake vs high frequency intake (by phone interview) associated with an increased risk of child asthma diagnosis OR=1.30; (95% CI 1.05, 1.63, P=0.02). Assessment of fish intake by FFQ showed no association.</td>
<td>Parental education, occupation, maternal age, parity, pre-pregnancy BMI, smoking and exercise during pregnancy, gestational weight gain, BF duration, BW, GA, sex, parental history of asthma and allergies</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Miyake et al. Japan, 2013 (109)</td>
<td>n=1354 Kyushu Okinawa Maternal and child Health study 2007-2008</td>
<td>150-item DHQ at 5–39 weeks gestation (previous 4w)</td>
<td>Quartile medians for fish g/d Q1 (23.4); Q2 (37.5); Q3 (49.8); Q4 (71.3) Mean intake fish=46.9g/d; n-3 PUFA=2.3g/d</td>
<td>Parent report of symptoms of wheeze and eczema by ISAAC at 23-29m</td>
<td>No significant association between maternal fish intake and wheeze or eczema. Maternal intake of EPA and EPA plus DHA associated with reduced risk of wheeze OR 0.73; (95%CI, 0.50-1.04, P for trend = 0.02), 0.70; (95%CI, 0.49-1.00 P for trend =0.02)</td>
<td>Maternal age, gestation, region, parity, maternal and paternal education, asthma, atopic eczema, and rhino-conjunctivitis, maternal smoking in pregnancy, family income, BW, sex, household smoking during first year, BF duration</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Leermakers et al., Netherlands, 2013 (102)</td>
<td>n=2796 Dutch only women from Generation-R study recruited at antenatal visit 2002-2006</td>
<td>293-item semi-quantitative FFQ at 10-21 weeks gestation (previous 3m)</td>
<td>Total-, lean- and fatty fish consumption. Median Intake total fish=83g/w; fatty fish=32g/w</td>
<td>Parent report of wheezing and doctor-attended eczema by adapted ISAAC at 1, 2, 3 &amp; 4yrs</td>
<td>No associations of maternal total fish consumption with childhood wheezing at 1–4 years. Maternal consumption of 35–69 g fatty fish per week vs no fatty fish increased the overall risk of eczema OR=1.17; (95% CI, 1.00-1.38). Maternal consumption of ≥70gm fatty fish per week was not associated with eczema OR=1.06; (95% CI, 0.88-1.38). No association with maternal total or lean fish consumption and childhood eczema</td>
<td>Maternal age, parity, SES, asthma or atopy, vegetable intake, peri-conception folic acid, education, psychological distress, pre-pregnancy BMI, smoking and alcohol during pregnancy, pets, BW, GA, sex, BF, day care, lower respiratory tract infections, complementary feeding</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
<td>---------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Nwaru et al., Finland, 2012 (109)</td>
<td>n=2441 Infants from the Finnish Type 1 Diabetes Prediction &amp; Prevention (DIPP) Study 1997-2004</td>
<td>181-item FFQ given to participants at delivery, retrieved at 3 months post-partum (intake in 8th month of pregnancy)</td>
<td>Dietary fats and fatty acids per day Total n-3 PUFA intake (g/d) Q1 (&lt;5.55); Q2 &amp; Q3 (5.55-7.34); Q4 (7.35-11.28) Total n-3 PUFA from fish (g/d) Q1 (&lt;0.38); Q2 &amp; Q3 (0.38–0.98); Q4 (0.99–5.50) Mean Intake n-3 PUFA=3·2g/d</td>
<td>Parent report of rhinoconjunctivitis, wheeze and atopic eczema by modified ISAAC at 5yrs</td>
<td>Higher ratio of n-6:n-3 fatty acids during pregnancy associated with an increased risk of rhinoconjunctivitis in the offspring by 5 years of age OR=1.37; (95 % CI 1.07, 1.77) No association with increased total n-3 PUFA, n-3 PUFA (from fish or plants), DHA or EPA and eczema, rhinitis or wheeze</td>
<td>Hospital of birth, GA, maternal age, education, smoking during pregnancy, parental asthma &amp; rhinoconjunctivitis, delivery, sex, siblings, pets at home by 1 year of age, vitamin C, Zn, Se, vitamin D and E</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>------------------------------</td>
<td>--------------------</td>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Lumia et al., Finland, 2011 (106)</td>
<td>n=2679 Same population as (106)</td>
<td>Dietary fatty acid composition Total n-3 PUFA intake (g/d) Q1 (&lt;2.24); Q2 &amp; Q3 (2.24–3.84); Q4 (&gt;3.84) Total fish intake (g/d) Q1 (&lt;10.67); Q2 &amp; Q3 (10.67-31.99); Q4 (&gt;31.99) Mean Intake fish products=21g/d</td>
<td>Parent report of asthma by modified ISAAC combined with anti-asthmatic medication data from Finnish Social Insurance Institution at 5yrs</td>
<td>Low maternal intake of ALA and low total n-3-PUFA intake associated with an increased risk of asthma in the offspring, OR= 1.67; (95% CI, 1.12–2.48), OR=1.66; (95% CI, 1.11–2.48, p=0.036)</td>
<td>Maternal age, education, parity, mode of delivery, GA, BW, sex, area of birth, maternal smoking, parental asthma or rhinoconjunctivitis, pets, farming, contact with cow stable during the first year of life, BF duration</td>
<td></td>
</tr>
<tr>
<td>Nwaru et al., Finland, 2011 (106)</td>
<td>n=931 Same population as (106)</td>
<td>Diet including PUFA categorized. n-3 PUFA quartile data NR Mean Intake fish products=23g/d</td>
<td>Total and specific IgE analysis (egg, cow’s milk, fish, wheat, HDM, cat, timothy grass &amp; birch) at 5yrs</td>
<td>No significant association with n-3 PUFA intake and sensitisation</td>
<td>Place and season of birth, maternal age, smoking during pregnancy, education, parental asthma and rhino-conjunctivitis, siblings, sex, GA at birth</td>
<td></td>
</tr>
<tr>
<td>Study/Reference</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Jedrychowski et al., USA/Poland, 2011 (101)</td>
<td>n=469 Women recruited from ambulatory prenatal clinics 2001-2004</td>
<td>FFQ in second and third trimester (food items or intake timing NR)</td>
<td>Frequency of smoked, fried, roasted and grilled fish g/w T1 (&lt;90); T2 (91–205); T3 (&gt;205) Mean Intake fish=148g/w</td>
<td>Face to face interview regarding infant health and physician confirmed infantile eczema at 3, 6, 9 &amp; 12m</td>
<td>Maternal fish intake of &gt;205gm/week associated with a reduction in risk of eczema by 43% OR=0.57 (95% CI 0.35–0.93, p=0.047)</td>
<td>Maternal age, education, atopy, duration BF, siblings and damp or mouldy house.</td>
</tr>
<tr>
<td>Miyake et al., Japan, 2009 (108)</td>
<td>n=763 Women recruited from obstetric clinics to the Osaka Maternal and Child Health Study 2001-2003</td>
<td>150-item DHQ at any stage of pregnancy mean 17.7 weeks gestation (previous 4w)</td>
<td>Daily intake of fatty acids, cholesterol, fish and meat Total n-3 PUFA intake g/d Q1 (1.7); Q2 (2.2); Q3 (2.5); Q4 (3.0) Total fish intake g/d Q1 (23.4); Q2 (38.7); Q3 (51.7); Q4 (73.2) Mean intake n-3 PUFA=2.4 g/d, fish=48.4g/d</td>
<td>Parent reported symptoms of wheeze and eczema by ISAAC 16-24m</td>
<td>High maternal ALA intake associated with a reduced risk of wheeze in the offspring OR=0.52, (95% CI, 0.28-0.97), although the inverse exposure-response relationship was not statistically significant</td>
<td>Maternal age, GA, location, family income, maternal &amp; paternal education, asthma, atopic eczema and allergic rhinitis, maternal intake of vitamins D and E during pregnancy, changes in diet previous month, smoking, siblings, sex, BW, household smoking, BF</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Willers et al.</td>
<td>n=2832</td>
<td>Dietary questionnaire self-completed between 30th and 36th week of gestation (previous 4w)</td>
<td>Frequency of fish consumption; Rarely (never to 1-3/m, n=74%) Regularly (Once to &gt;4/w n=24%); Daily (≥ once/d, n=1)</td>
<td>Parent report of asthma symptoms by ISAAC 1-8yrs</td>
<td>No overall association between maternal fish consumption during pregnancy and childhood wheeze, dyspnoea, steroid use or asthma symptoms.</td>
<td>Child sex, maternal education, smoking, parental allergy, smoking in the home at 8 years, BF, siblings, BW, maternal overweight 1 year after pregnancy, maternal supplements in pregnancy, region, study arm</td>
</tr>
<tr>
<td>Chatzi et al.</td>
<td>n=468</td>
<td>35-item FFQ by face to face interview 3 months post-partum (referred to pregnancy period)</td>
<td>Frequency of fish intake categorized as never, (n=4.4%) times per year, monthly, weekly (≥ 1/w 85.8%) Other categories NR</td>
<td>Annual questionnaire by interviewer 1-6yrs SPT (HDM x2, grass pollen, olive tree, mixed graminae, parietaria) at 6.5yrs</td>
<td>Maternal fish intake ≥2.5/w inversely associated with persistent wheeze OR=0.34; (95% CI 0.13- 0.84) p=&lt;0.05</td>
<td>Maternal age, atopy, social class and education, maternal supplement use and smoking, maternal and paternal asthma, BF, lower track respiratory infections at 1 year, BW, birth order, GA, number of siblings and BMI at 6.5 years</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>---------------------</td>
<td>--------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Romieu et al., Spain, 2007</td>
<td>n=468 Same population as (88)</td>
<td>35-item FFQ including fatty fish by face to face interview 3 months post-partum (relating to pregnancy)</td>
<td>Frequency of fish intake categorized as never, (n=4.4%) times per year, monthly, weekly (≥ 1/w 85.8%) Other categories NR</td>
<td>Annual questionnaire by interviewer 1-6yrs Specific IgE (HDM x2 and mixed grass pollens) at 4yrs SPT (HDM x2, grass pollen, olive tree, mixed graminae, parietaria) at 6yrs</td>
<td>Fish intake 2.5/w vs once was protective against risk of eczema (OR=0.73; 95% CI 0.55–0.98) p=0.03 No significant association between fish intake and IgE levels at age 4 years Fish intake 2.5/w vs once was protective against positive SPT to HDM and atopic wheeze (OR=0.68; 95% CI 0.46–1.01) p=0.05, (OR=0.55; 95% CI 0.31–0.96) p=0.03</td>
<td>Maternal age, smoking and pre-pregnancy BMI, maternal and paternal atopy, asthma, social class, child sex, GA, BW, parity, BF, pets, child BMI at age 6.5 years, child's fish intake</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Willers et al. United Kingdom 2007 ([11])</td>
<td>n=1212 Women recruited from antenatal clinic. 1997-1999</td>
<td>150-item Semi-quantitative FFQ mailed to participants at 32 weeks gestation to record diet in previous 2-3 months</td>
<td>Total &amp; fatty fish consumption; Total Fish; Never (107); 1/w (255); &gt;1/w (831) Total Oily Fish; Never (629); 1/w (414); &gt;1/w (161) Mean intakes NR</td>
<td>ISAAC spirometry, broncho-dilator response, exhaled nitric oxide and SPT (cat, timothy grass, egg and HDM) at 5yrs</td>
<td>Maternal fish consumption ≥ 1/w vs never associated with a decreased risk of doctor-confirmed eczema, currently treated eczema and doctor confirmed hay fever OR=0.57 (95% CI, 0.35-0.92) p trend=0.08; OR=0.58(95% CI, 032-1.06) p trend=0.028; OR=0.28 (95%CI, 0.06-1.19) p trend=0.04 No consistent association between maternal fish consumption and atopic sensitisation, spirometry, bronchodilator response or exhaled nitric oxide</td>
<td>Maternal age, education, smoking during pregnancy, paternal social class maternal asthma &amp; atopy, child’s BW, sex, presence of older siblings, BF, smoking in the child’s home at 5 years</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure Assessment</td>
<td>Maternal Exposure Categories</td>
<td>Outcome Assessment</td>
<td>Results</td>
<td>Adjusted Variables</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Suasenthaler et al., Germany, 2006</td>
<td>n=2641 Healthy full-term infants from maternity hospitals in birth cohort study (LISA) 1997-1999</td>
<td>FFQ administere d after birth (median 3 days) (previous 4w)</td>
<td>High vs low fish intake; 5 categories ranging from; &lt;2/m or never to ≥4/w (individual categories NR) High intake (T1, n=122); Low intake (T2&amp;3, n=322) Mean intakes NR</td>
<td>Parental report of doctor diagnosed eczema at 6, 12, 18 &amp; 24m Total and specific IgE analysis (egg, cow milk, wheat, peanut, soybean, codfish, HDM, cockroach, cat dander, mixed moulds, seasonal allergens) at 2yrs</td>
<td>High vs low maternal fish intake inversely associated with doctor-diagnosed eczema OR=0.75; (95% CI, 0.57-0.98) p=&lt;0.05 No significant association between fish consumption and allergic sensitisation</td>
<td>Location, maternal age at delivery, maternal smoking, parental education, exclusive BF for 4m, parental history of atopic diseases, season of birth, child sex</td>
</tr>
</tbody>
</table>

**Abbreviations:** FFQ, Food Frequency Questionnaire; w, week; m, month; d, day; NR, Not Reported; BW, Birth Weight; GA, Gestational age; SES socio-economic status, NR, not reported; FFQ, food frequency questionnaire; OR, odds ratio; BMI, body mass index; n-3, omega-3; LCPUFA, long chain poly-unsaturated fatty acid; DHQ, diet history questionnaire; ISAAC, International Study of Asthma and Allergies in Childhood; EPA, eicosapentanoeic acid; DHA, docosahexaenoic acid; Q, quartile; T, Tertile; IgE, immunoglobulin E; HDM, house dust mite; ALA, alpha linolenic acid; SPT, skin prick test
All of the included RCTs recruited women during the antenatal period from clinical care settings. Four of the five RCTs enrolled women who were at risk of delivering a fetus with atopic disease (i.e. first degree relative with history of allergic disease)\textsuperscript{(112-117)} however, only one of these trials confirmed atopy in the mother by skin prick testing prior to enrolment \textsuperscript{(119)}. The remaining trial included women attending their routine 30 week midwife clinic visit regardless of atopy status \textsuperscript{(119)}. Characteristics and results of RCTs are presented in Table 2-2.
<table>
<thead>
<tr>
<th>Ref</th>
<th>Setting &amp; Participants</th>
<th>Intervention &amp; Timing</th>
<th>Outcomes</th>
<th>Follow Up</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan et al Australia, 2003 (118)</td>
<td>n=98 Atopic, non-smoking pregnant women recruited from antenatal clinic 1999-2001</td>
<td>Fish oil capsules, 4x1g/d 3700mg n-3 LCPUFA (56.0% DHA, 27.7% EPA) Control: Olive oil capsules 20w GA until delivery</td>
<td>Clinical examination and history by physician to determine incidence of asthma, atopic eczema and food allergy and SPT (hens egg, cow milk, peanut, HDM and cat)</td>
<td>12m</td>
<td>Sensitisation to egg at 12m lower in the intervention group (OR=0.34; 95% CI, 0.11-1.02, p= 0.05) No difference in frequency of eczema however the intervention group had less severe disease OR=0.09; (95% CI, 0.0-0.94, p=0.045) Recurrent wheeze, persistent cough and diagnosed asthma all lower in intervention group (10/40 vs.12/43), (5/40 vs. 11/43), (2/40 vs. 6/43) respectively, not statistically significant</td>
</tr>
<tr>
<td>Ref</td>
<td>Setting &amp; Participants</td>
<td>Intervention &amp; Timing</td>
<td>Outcomes</td>
<td>Follow Up</td>
<td>Results</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>-----------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Olsen et al.</td>
<td>Denmark, 2005 (119)</td>
<td>Fish oil capsules 4x1g/d 2700mg n-3 LCPUFA (23% DHA, 32% EPA)</td>
<td>Asthma related diagnosis extracted from the Danish National Patient Registry</td>
<td>16yrs</td>
<td>Lower incidence of diagnosis of ‘any asthma’ in the fish oil group (2/263 vs. 8/136, p=0.03 ) Lower incidence of diagnosis of ‘allergic asthma’ in the fish oil group (8/263 vs. 11/136, p=0.01)</td>
</tr>
<tr>
<td>Furuhjelm et al.</td>
<td>Sweden, 2009 (119)</td>
<td>Fish oil capsules, 9x0.5g/d 2700mg n-3 LCPUFA (1.6g EPA, 1.1g DHA)</td>
<td>Clinical examination by nurse (Paediatrician exam if food allergy or eczema) at 3, 6 &amp; 12m. SPT (cow’s milk, egg and wheat) IgE antibodies to egg, milk and wheat at 3 &amp; 12m. SPT (cow’s milk, egg and wheat)</td>
<td>6m</td>
<td>Incidence of IgE associated eczema 0-6m was lower in the intervention group (4/52, 8% vs. 13/65, p=0.06) not statistically significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: Soy oil capsules 25w GA until 3.5 months post-natal</td>
<td></td>
<td>12m</td>
<td>Incidence of IgE associated eczema, sensitisation to egg and period prevalence of “any positive SPT” was lower in the intervention group at 0-12m (4/52 vs. 15/63, p=0.02), (6/52 vs. 16/63, p=0.02), (8/52 vs. 20/63, p=0.04)</td>
</tr>
</tbody>
</table>

n=533 Women attending midwife clinic at routine 30 week assessment 1989-1990

Control 1: Olive oil capsules
Control 2: No supplement 30w GA until delivery

1989-1990

Control: Olive oil capsules

Control 2: No supplement 30w GA until delivery

1989-1990
<table>
<thead>
<tr>
<th>Ref</th>
<th>Setting &amp; Participants</th>
<th>Intervention &amp; Timing</th>
<th>Outcomes</th>
<th>Follow Up</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furuhjelm et al. Sweden, 2011 (112)</td>
<td>n=145 Same population as (113)</td>
<td>Clinical examination by paediatrician and SPT (cow’s milk, egg and wheat, cat, timothy &amp; birch)</td>
<td>24m 143/145 98%</td>
<td>Cumulative incidence (0-24m) of any IgE mediated disease, positive SPT to egg, any positive SPT and any IgE-associated eczema was lower in the intervention group (6/54 vs. 19/62, p = 0.01), (7/52 vs. 18/61, p = 0.04), (10/52 vs. 22/61, p=0.048), (5/54 vs. 15/63, p = 0.04) No difference between groups for ‘any asthma’, IgE associated asthma, ‘any eczema’, ‘any rhino-conjunctivitis’, IgE associated rhino-conjunctivitis at 24m or cumulative incidence 0-24 months</td>
<td></td>
</tr>
<tr>
<td>Noakes et al. United Kingdom, 2012 (114)</td>
<td>n=123 Women with low habitual oily fish intake with a fetus at high risk of atopy recruited from antenatal clinic, year NR</td>
<td>2x150gm salmon portions/w (1160mg DHA, 570mg EPA) Control: Habitual diet low in oily fish 20w GA until delivery</td>
<td>Total serum IgE from cord blood at birth Symptom diary cards, examination by nurse &amp; SPT (HDM, cat, dog tree mix, grass mix, egg, salmon, cow’s milk)</td>
<td>Birth 107/123 87% 6m 86/123 70%</td>
<td>No significant difference in total IgE at birth between groups No significant difference in total IgE, SPT, incidence of eczema, severity of eczema or wheeze between groups</td>
</tr>
<tr>
<td>Palmer et al. Australia, 2012 (118)</td>
<td>n=706 Women with a fetus at high risk of atopy recruited from antenatal clinics 2005-2007</td>
<td>Fish oil capsules 3x0.5g/day 900mg n-3 LCPUFA (800mg DHA, 100mg EPA) Control: Vegetable oil capsules 20w GA until delivery</td>
<td>Clinical examination by physician to determine incidence of asthma, eczema and food allergy. SPT (cow’s milk, egg, wheat, tuna, peanut, grass pollen, perennial ryegrass, olive tree pollen, <em>Alternaria tenuis</em>, cat, HDM)</td>
<td>12m 681/706 96%</td>
<td>No significant difference between groups of “any” IgE mediated disease Incidence of IgE associated eczema was lower in the intervention group (26/368 (7%) vs. 39/338 (12%), p=0.06) not statistically significant Sensitisation to egg at 12m was lower in the intervention group (34/368 (9%) vs. 52/338 (15%), p=0.02)</td>
</tr>
<tr>
<td>Palmer et al. Australia, 2013 (122)</td>
<td>n=706 Same population as (118)</td>
<td>Clinical examination by physician to determine incidence of asthma, eczema and food allergy SPT (cow’s milk, egg, wheat, tuna, peanut, cashew, sesame, grass pollen, perennial ryegrass, olive tree pollen, <em>Alternaria tenuis</em>, cat and HDM)</td>
<td>3yr 638/706 90%</td>
<td>No significant difference between “any” IgE mediated disease in the first 3 years of life 64/368 (17.3%) vs 76/338 (22.6%) p= 0.11 No significant difference between clinical outcomes of eczema, rhino-conjunctivitis or asthma with or without sensitisation</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations; n-3 LCPUFA, long chain poly-unsaturated fatty acid; EPA, eicosapentanoic acid; DHA, docosahexaenoic acid; IgE, immunoglobulin E; HDM, house dust mite; ALA, alpha linolenic acid; SPT, skin prick test; SCORAD, SCORing Atopic Dermatitis
Exposure/Intervention

Observation of maternal dietary n-3 PUFA intake during pregnancy in cohort studies was ascertained by the use of diet history questionnaires (DHQ) or food frequency questionnaires (FFQ). FFQs were either self-administered or interviewer administered by phone or face to face. Timing of administration of the questionnaire varied between the first, second and third trimester or any time point during pregnancy. All studies required the women to complete the FFQ retrospectively from 3 days to 3 months post-partum. Dietary fish exposure varied between 83gms/week and 46gms/day. Intensity of questioning in FFQs varied from 35 to 360 items, Table 2-1.

The intervention in four out of five RCTs was fish oil capsules. Capsules varied in composition and dose of n-3 LCPUFA ranging from 900mg to 3700mg of total n-3 LCPUFA per day. One RCT supplied women with 2 x 150mg portions of farmed salmon to consume twice per week, equivalent to 3450mg n-3 LCPUFA per week (the control group continued their habitual low fish diet). Placebos used in double blind RCTs consisted of olive oil, soy oil or vegetable oil that did not contain n-3 LCPUFA. One study had two control groups (olive oil and ‘no oil’). Timing of the intervention commenced between 20 and 30 weeks gestation and ceased at delivery of the infant in four of the included studies. One RCT continued supplementation of mothers for 3.5 months into the post-partum period.
Clinical Outcomes

Clinical indication of allergic disease in the offspring in observational studies and RCTs was diverse. Study outcomes reported included the following: symptoms of wheeze, eczema, and rhinitis by parent report using the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire (validated for use in 6-7 year old children) (97, 99, 100, 102, 105, 106, 121), non-validated health questionnaire, (104) parent report of physician diagnosis of asthma or eczema (96, 98, 103), asthma medication data from social insurance database (105) or patient registry (119) and clinical examination by nurse or physician (101, 112-114, 116, 118, 120). Five of the included observational studies and four of the RCTs assessed atopy outcomes, using either total and specific serum IgE analysis (107, 122), SPT (97, 98, 112, 113, 116, 118, 120) or a combination of both outcomes (96, 114). Individual or multiple outcomes were reported as cumulative incidence or point prevalence. Age of the child at assessment of study outcomes varied between 6 months and 8 years in observational studies and 6 months and 3 years in RCTs with the exception of one registry linkage trial reporting history of asthma diagnosis at 16 years (119).
Quality of Observational Studies

FFQ’s and DHQ’s are applicable to large cohorts and provide information on a wide range of foods, however there are many limitations including dietary misreporting which leads to dietary misclassification of intake and/or portion sizes. Detection of modest nutrient associations when using a FFQ for dietary assessment are unlikely (123, 124), in particular, reliable estimates of absolute amounts of dietary fats (125, 126). A number of studies did not capture fish type i.e. ‘fatty fish’ (Table 2-1) which may be problematic as there is significant variation in the amount of n-3 LCPUFA intake depending on type of fish consumed. Dietary intake over the preceding period of 4 weeks to 3 months was documented retrospectively and may be subject to recall bias. (Table 2-1) With the exception of two studies (101, 103) maternal diet was assessed only once (between 12 and 40 weeks gestation), which may not be indicative of diet throughout the whole of pregnancy, as demonstrated by one of the Japanese studies (109). They conducted dietary assessment at ‘any time’ between the 5th and 39th week of pregnancy and reported that 30% of respondents had substantial changes to their diet in the previous month.

Substantial geographic variation existed in cohort studies in relation to total fish intake, fish sub-groups and the number of types of fish consumed (127). Decreased variability between categories was evident in four included studies from the Netherlands (102, 106, 107, 110) a country where there is generally low fish consumption. In one study, of the 2760 participants, only 7% of the population consumed fish more 1.5 times per week (30gm/day) and only one woman ate fish daily (99). In contrast, the mean fish intake in two Japanese studies was 48gm/day with low variability between quartiles due to generalized high fish consumption.
Presence of allergic disease symptoms in the offspring were self-reported in the majority of studies using the ISAAC questionnaire (or a ‘modified’ version). The ISAAC is a standardised questionnaire and has been validated in 6-7 and 13-14 year old children, however most children in the included cohort studies were younger. (Table 2-1) The ability to differentiate IgE mediated allergic disease was diminished as only four of the 13 publications assessed atopy in the child (SPT or serum IgE) (96, 98, 108, 111). Attrition bias was possible in a number of studies with follow up rates <80% (100, 102, 103, 105, 106, 108-111). When comparing population characteristics of participants and non-participants, participants tended to display healthier lifestyle habits, higher maternal and paternal educational levels and higher income levels (103, 109). One study with three publications only included infants at risk of developing diabetes by the presence of human leucocyte antigen (HLA) in cord blood (105, 106, 108). Children with Type 1 diabetes have been reported to have a reduced incidence of asthma and allergic diseases compared with the general population therefore this genetic susceptibility may further decrease the generalisability of this study’s findings.
Quality of RCTS

A summary of the risk of bias associated with each RCT is shown in Table 2-3 (128). Two trials lacked sufficient clarity when reporting concealment of the allocation sequence (112-114) and were therefore assessed as unclear risk of bias. Blinding of participants was endeavoured by image matching capsules in four double blind RCTs (112, 113, 116, 118, 120) although one of these trials included a “no supplement” group which may have resulted in un-blinding of participants and personnel (119). All trials using fish oil capsules reported side effects of “fishy burps” potentially risking un-blinding of participants, however, objective outcome assessment such as SPT reduce the risk of performance bias. One single blind trial that supplied salmon portions to women in the intervention group and no salmon in the control group was assessed as high risk of bias as the participants could not be blinded (114). This trial also had a high attrition rate with only 62% of the control group, attending the 6 month follow up, well below the minimum follow up considered acceptable for minimising attrition bias (≥80%) (114). Risk of bias from incomplete outcome data reporting was high in two other trials (113, 118). One trial had significantly higher attrition in the treatment group (15%) than the control group (2%) due to side effects perceived to be associated with the study product (118). Another excluded 25 mothers (17%) from the analysis as they did not complete supplementation for the requested period (113). Such post-randomisation exclusions and attrition can contribute to systematic loss to follow up and increase the risk of bias in already small and underpowered samples. Other bias was unclear in one registry linkage study due to lack of clarity regarding reporting of standardisation of outcome assessment (119). Data was ascertained by searching the Danish National Patient Registry (NPR), a mandatory national hospital
discharge register for asthma and allergic disease related coding. Whilst the long term follow up rate for this study is outstanding, the overall rate of diagnosed asthma is much lower than reported in other studies. It is unknown how the diagnosis of asthma was made and likely that there was an underrepresentation of milder cases treated outside of the Danish hospital system. Of the five included RCTs, four studies used SPT as an outcome measure to determine atopy status of the offspring, \((113, 114, 116, 118)\), however, definitions of sensitisation were inconsistent. Two studies used standard clinical definition of wheal size ≥3mm as the diagnosis of ‘sensitisation’ \((114, 116)\). Another two studies conducting 6 month and 12 month follow up of infants included results of ≥2mm wheal size in the analysis of sensitisation \((113, 118)\). Reasons for choosing this criterion as opposed to the standard clinical definition are not mentioned. There is evidence that SPT reactions in the very young (<12months) are often diminished making interpretation difficult \((129)\).

**Table 2-3 Summary of risk of bias assessment for included RCTs**

<table>
<thead>
<tr>
<th>Ref</th>
<th>Random sequence generation</th>
<th>Allocation concealment</th>
<th>Blinding of participants/personnel</th>
<th>Blinding of outcome assessment</th>
<th>Incomplete outcome data</th>
<th>Selective reporting</th>
<th>Other bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>((113))</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>H</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>((114))</td>
<td>L</td>
<td>L</td>
<td>H</td>
<td>U</td>
<td>L</td>
<td>L</td>
<td>U</td>
</tr>
<tr>
<td>((116))</td>
<td>L</td>
<td>U</td>
<td>L</td>
<td>L</td>
<td>H</td>
<td>H</td>
<td>L</td>
</tr>
<tr>
<td>((118))</td>
<td>L</td>
<td>U</td>
<td>H</td>
<td>L</td>
<td>H</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>((119))</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
</tbody>
</table>

*Abbreviations: L, Low risk of bias; H, High risk of bias; U, Unclear risk of bias*
Results of Observational Studies

Table 2-1 summarizes all identified observational studies that investigated the association between maternal n-3 LCPUFA or fish intake and allergic disease outcomes in the offspring. Nine of the thirteen included studies found a protective association between increased n-3 LCPUFA or fish intake in the prenatal diet and incidence of one or more allergic disease symptoms in the child. (96-98, 101, 103-105, 121) One of these studies assessed maternal diet by phone interview and FFQ, although results were only significant using phone interview data (103). Four studies showed no association between maternal n-3 LCPUFA or fish intake and allergic disease outcomes (100, 106, 108, 111). One study reported an adverse association in one category of fish intake (109).

Eczema

The incidence of eczema was reported in eight studies with four of these showing that higher fish/n-3 LCPUFA exposure was associated with a reduction in the risk of eczema in the offspring between 27% and 43%. Fish intake of >205gms per week (101) and fish intake 2.5 times per week vs never (96) was protective against the risk of eczema at age 12 months. High (1-2 times per week) vs low fish intake and fish intake of ≥ once per week vs never was inversely associated with doctor diagnosed eczema at ages 24 months (104) and five years (111). Two separate studies from Japan found no association between increased maternal fish intake and eczema and one of the publications from the DIPP Study investigating dietary fatty acids found no association between increased total n-3 PUFA, DHA or EPA with eczema (100, 106, 121). One study from the Netherlands reported that the mid category of intake (35-69gm per week) was associated with the highest eczema
rate, whereas the lower and higher category of fish intake (1-34gm per week, >70gm per week) showed no association with overall risk of eczema (102).

Rhino-conjunctivitis/Hayfever

Three studies assessed symptoms of rhino-conjunctivitis (103, 106, 111). One study concluded that maternal fish intake ≥ once per week vs never was protective for ‘doctor confirmed hayfever’ at age five years (97). Two studies found no significant association between zero maternal fish intake vs high frequency intake and rhino-conjunctivitis (103) or between increased total n-3 PUFA, DHA or EPA and rhino-conjunctivitis (106).

Asthma/wheeze

Nine studies assessed incidence of asthma or symptoms of wheeze. Maternal fish consumption frequency was inversely related to parent report of doctor diagnosis of asthma and wheezing symptoms at ages 18 months and seven years, (103) atopic wheeze at age six years (96) and persistent wheeze at age 6.5 years (98). Two studies reported a protective association of an increased total maternal n-3 LCPUFA intake (but not fish) on wheeze at 24 months, (121) asthma at age five years (105). The three remaining studies (two from the Netherlands) found no significant association between increased fish or n-3 LCPUFA consumption and wheeze, (106) risk of asthma, (105) or asthma symptoms (111).
Sensitisation

Four studies reported outcomes of sensitisation. Three of these evaluated fish or n-3 PUFA intake on total and specific IgE at ages two years, (104) four years (96) and five years (107), all showing no significant association. One of these studies conducted further follow up at age six years of age with skin prick testing and reported that fish intake ≥2.5 times per week was protective against sensitisation to HDM (96). One study that conducted SPT at five years of age found no significant association between maternal fish intake and atopic sensitisation.

Results of RCTs

Table 2-2 summarizes all identified RCTs that investigated the effect of prenatal n-3 LCPUFA supplementation (or supplied fish) on allergic disease outcomes in the offspring. Five out of seven RCTs found a protective effect on one or more clinical outcomes of allergic disease or sensitisation.

Eczema

The incidence of IgE mediated eczema between children born to mothers supplemented with n-3 LCPUFA during pregnancy and control groups was assessed between 0 to 3 years of age in four RCTs and reported in six of the included publications (112-114, 116, 118, 120). One trial that conducted follow up of children over a two year period found a significant reduction in the cumulative incidence of atopic eczema at ages 12 and 24 months (112, 113). Another study reported a non-significant protective effect of the intervention at 12 months with no effect when re-assessed at age three years (116, 120).
The two remaining studies found no effect of n-3 LCPUFA supplementation on eczema, (114, 118) although one did report a significant reduction in severity of disease (118).

Results from RCTs reporting outcomes of ‘atopic eczema’ (eczema symptoms and positive SPT) at 12 months of age were combined in meta-analysis (113, 120) showing a significant reduction in the incidence of atopic eczema (RR; 0.53, 95% CI: 0.35, 0.81; p=0.004), Error! Reference source not found.. These two trials also reported outcomes of incidence of ‘any’ eczema (eczema symptoms with or without positive SPT) at age 12 months, showing no significant effect of the intervention (RR; 0.85; 95% CI: 0.67, 1.07; p=0.16), Error! Reference source not found..

Figure 2-2 Incidence of confirmed ‘atopic’ eczema (with sensitisation) at 12 months

Figure 2-3 Incidence of ‘any’ eczema (with or without sensitisation) 0-12months
Rhino-conjunctivitis

There was no difference between the groups in the cumulative incidence of ‘any rhino-conjunctivitis’ or ‘IgE mediated rhino-conjunctivitis’ in the two studies reporting this outcome at 0-24 months of age (112) and 0-3 years of age (116) (RR: 0.81; 95% CI: 0.44, 1.47; p=0.49), Error! Reference source not found.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental Events Total</th>
<th>Control Events Total</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furlan et al. 2011</td>
<td>1</td>
<td>54</td>
<td>2</td>
<td>65</td>
</tr>
<tr>
<td>Palmer 2013</td>
<td>18</td>
<td>366</td>
<td>20</td>
<td>338</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>422</td>
<td>403</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>19</td>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 0.05, df = 1 (P = 0.80); I² = 0%
Test for overall effect: Z = 0.70 (P = 0.46)

Figure 2-4 Cumulative incidence of IgE mediated rhino-conjunctivitis 0-3 years

Asthma

Asthma and/or asthma symptoms were assessed in five of the seven included publications at different ages and with a variety of outcome measures, preventing meaningful meta-analysis. There were no differences in asthma or wheeze with or without sensitisation at ages six months, (114) 24 months, 0-24 months (112) or three years (120). One registry based follow up RCT included asthma diagnosis as an outcome, reporting a significant reduction in ‘any asthma’ and ‘allergic asthma’ at 16 years of age in the fish oil supplemented group compared with the ‘olive oil’ control group. However, a significant positive effect on ‘any asthma’ and ‘allergic asthma’ was also evident in the second ‘no oil’ control group (119).
Sensitisation

Sensitisation to common allergens was assessed by SPT at ages six months (114), 12 months (113, 114, 116, 118), 24 months (130) and three years (120). Three studies with sensitisation outcomes at 12 months were combined in meta-analysis and showed a significant protective effect of the intervention on the period prevalence of 'any positive SPT' (RR: 0.68; 95% CI: 0.52, 0.89; P=0.006), Error! Reference source not found.. The trial with the salmon portion based intervention also measured total IgE at birth and age six months with no difference evident between the groups.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>n-3 LCPUFA Events</th>
<th>Total</th>
<th>Control Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio M.H, Fixed, 95% CI</th>
<th>Risk Ratio M.H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan 2013 (29)</td>
<td>6</td>
<td>35</td>
<td>14</td>
<td>37</td>
<td>13.1%</td>
<td>0.68 [0.34, 1.37]</td>
<td></td>
</tr>
<tr>
<td>Furuhata 2006 (25)</td>
<td>10</td>
<td>62</td>
<td>22</td>
<td>91</td>
<td>19.5%</td>
<td>0.59 [0.28, 1.02]</td>
<td></td>
</tr>
<tr>
<td>Fairley 2012 (20)</td>
<td>53</td>
<td>368</td>
<td>67</td>
<td>338</td>
<td>67.4%</td>
<td>0.73 [0.52, 1.01]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>455</td>
<td>436</td>
<td>100.0%</td>
<td></td>
<td>0.68</td>
<td>[0.52, 0.89]</td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>72</td>
<td>103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Chisq = 6.9, df = 2 (P = 0.03); I² = 0%
Test for overall effect: Z = 2.77 (P = 0.006)

Figure 2-5 Incidence of 'any positive skin prick test (SPT) 0-12 months
Meta-analysis of sensitisation to individual allergen extracts showed a significant reduction in ‘sensitisation to egg’ at 0-12 months of age (RR: 0.55; 95% CI: 0.39, 0.76; P=0.0004),

Figure 2-6 Sensitisation to egg between 0-12 months

Figure 2-7 Sensitisation to 'any food' at 12 months

, and ‘sensitisation to any food’ at age 12 months (RR: 0.59; 95% CI: 0.46, 0.76; P<0.0001), Error! Reference source not found..
**Chapter Two**

Figure 2-6 Sensitisation to egg between 0-12 months

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Events</th>
<th>Total</th>
<th>Control</th>
<th>Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio M.H. Fixed, 95% CI</th>
<th>Risk Ratio M.H. Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan 2003 (26)</td>
<td>10</td>
<td>35</td>
<td>25</td>
<td>37</td>
<td>19.7%</td>
<td>0.42</td>
<td>[0.24, 0.75]</td>
<td></td>
</tr>
<tr>
<td>Furuhjelm 2000 (29)</td>
<td>8</td>
<td>64</td>
<td>21</td>
<td>96</td>
<td>15.4%</td>
<td>0.46</td>
<td>[0.22, 0.95]</td>
<td></td>
</tr>
<tr>
<td>Palmer 2011 (29)</td>
<td>95</td>
<td>360</td>
<td>77</td>
<td>398</td>
<td>54.9%</td>
<td>0.58</td>
<td>[0.48, 0.71]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>457</td>
<td>1600</td>
<td>180</td>
<td>1000</td>
<td>100.0%</td>
<td>0.58</td>
<td>[0.45, 0.75]</td>
<td></td>
</tr>
</tbody>
</table>

Total events: 73

Heterogeneity: CH² = 2.18, df = 2 (p = 0.34); I² = 0%

Test for overall effect: Z = 4.19 (p < 0.0001)

![Figure 2-7 Sensitisation to 'any food' at 12 months](image)
Discussion

My systematic review and meta-analysis shows that increased prenatal intake of n-3 LCPUFA in observational studies and RCTs are both suggestive of benefits. However, due to the inconsistency of results, the hypothesis linking increased maternal n-3 LCPUFA intake to a protective association or effect on childhood allergic disease, cannot unequivocally be confirmed nor rejected. Despite the challenges of capturing and comparing outcomes of a dynamic disease, some of the consistency observed across the study designs employed is noteworthy \(^{(125)}\). It is widely acknowledged that causal inferences based on results from studies of nutritional epidemiology that have been confirmed by results from RCTs, represent more persuasive evidence than those elucidated from observational data alone \(^{(131)}\). Observational studies showed a trend towards a protective association of increased maternal n-3 LCPUFA during pregnancy on the incidence of eczema and wheeze or asthma. Similar results were evident in RCTs for eczema but not wheeze or asthma, perhaps due to the age of the child when outcome assessments were conducted (1 - 3 years). The most significant and consistent outcome in RCTs was a reduction in the incidence of sensitisation (positive skin prick test) in the n-3 LCPUFA group. Whilst the presence of an IgE mediated reaction to a food or inhalant allergen generally confirms that an individual is “atopic”, the relationship between sensitisation and symptoms of allergic disease is known to be complex and dynamic. This finding may have some correlation on the reduction in respiratory disease evident in observational studies in the school age child. It is well documented that early sensitisation to food allergens (especially egg) is shown to be a valuable predictor of subsequent sensitisation to Aero-allergens and allergic respiratory diseases \(^{(132, 133)}\).
Heterogeneity between study designs may in part be explained by the limited number of well powered high quality RCTs with long term follow-up of the child and consistent age appropriate outcomes. Whilst epidemiological studies have the potential to assess long term health effects of nutrient intake, the possibility for residual confounding cannot be discounted, particularly when assessing single nutrient exposure\(^{(123)}\). The protective associations seen in the offspring of women with increased intake of n-3 LCPUFA during pregnancy, may not be due to this exposure, but rather serve as a proxy indicator for a number of other maternal characteristics that are associated with the healthy development of their offspring\(^{(134)}\). These confounding factors are difficult to fully accommodate in data analysis even when acknowledged. In addition, dietary intake in epidemiological studies is notoriously difficult to measure and often results in variations in findings\(^{(135)}\). Flaws in standard tools for dietary assessment (FFQ or DHQ) are well documented and cannot be ignored\(^{(123, 126, 134-136)}\). This issue was emphasized in the large Danish birth cohort (n=28936) observing the association between fish intake and asthma diagnosis\(^{(137)}\). Self-completed FFQ data showed a null association between fish intake and asthma diagnosis at 18 months and 7 years, however, analysis of fish intake data collected by telephone interview showed a statistically significant inverse relationship.
The main strength of my integrated systematic review including both prospective observational studies and RCTs lies in the detailed search strategy, rigorous and transparent methods and the qualitative and quantitative synthesis of differing methodologies. Using well-established processes for conducting systematic reviews (128) we determined that observational studies and many RCTs had methodological weaknesses making it difficult to draw any strong inferences from this review.

There have been numerous studies investigating the effect of maternal dietary n-3 LCPUFA status on regulation of the fetal immune response (84-86, 138, 139). These studies report plausible biological mechanisms that may modulate the development of allergic disease in infants with a genetic risk. However, as evident in this and other reviews of increased maternal supply of n-3 LCPUFA and outcomes of allergy (90-92, 94, 140-143), results are promising but inconsistent. Continued supplementation of breast feeding mothers during the early postpartum period to compensate for the natural decline in maternal plasma n-3 LCPUFA may provide additional benefit to development and maturation of the infant immune system, and is worthy of further investigation (144).
It is generally agreed that increasing number of people affected by allergic disease is a consequence of our 21st century lifestyle. Almost certainly this rise is attributed to genetic and environmental factors, including, but not limited to diet. Diet is easily modifiable; perhaps this simple, low cost approach of increasing n-3 LCPUFA intake during pregnancy may offer the best opportunity for a primary prevention strategy, decreasing the burden of allergic disease for future generations. Further evidence from well-powered, high-quality trials of comparable methodology and standardised, objective outcome assessments are required to investigate optimal dose and tolerability and conclusively establish benefits. Due to the dynamic nature of allergic disease, it is essential that RCTs of maternal n-3 LCPUFA supplementation continue follow up of children throughout childhood. The 6 year follow up of children whose mothers took part in the DOMInO trial presented in this thesis aims to fill this research gap and investigate if the significant protective effects of maternal n-3 LCPUFA seen in early life persist until early school age. Results of this study will enable consistent, appropriate recommendations for pregnant women and potentially, improved pregnancy and fetal outcomes, and outcomes for children.
Methods and study design of the six year allergy follow up of children at high hereditary risk of atopy born to mothers supplemented with n-3 LCPUFA during pregnancy
Introduction

Previous epidemiological studies and RCTs have described benefits of an increased maternal intake of n-3 LCPUFA during pregnancy on the incidence of allergic disease in the offspring. To investigate the long term effects of prenatal n-3 LCPUFA supplementation a six year allergy follow-up of children born to mothers who participated in a double blind, multi-centre randomised controlled trial was conducted.

Aim

The aim of the six year allergy follow up study is to determine whether n-3 LCPUFA supplementation of pregnant women with a fetus at high hereditary risk of atopic disease will result in fewer children with allergic disease (eczema, wheeze or allergic rhinitis) with sensitisation (defined as a positive skin prick test) at 6 years of age.

Hypothesis

Fewer children at higher-hereditary risk of atopic disease that were supplemented with n-3 LCPUFA supplementation during prenatal life will have allergic disease (eczema, wheeze or allergic rhinitis) with sensitisation.
Participants & Methods

The Parent Trial

The DOMInO Trial (Docosahexaenoic Acid (DHA) to Optimise Mother Infant Outcome) was conducted in five perinatal centres around Australia and designed to examine the effect of increasing DHA availability during pregnancy on the mother and infant with a large, high quality RCT with a high dose of DHA in a population where DHA intake is low (145).

DOMInO Trial design and dietary treatments

Women allocated to the intervention group were asked to consume three 500mg capsules of n-3 LCPUFA-rich fish oil concentrate per day, providing 800 mg/d of docosahexaenoic acid (DHA) and 100 mg/d of eicosapentaenoic acid (EPA). The control group were asked to take three 500-mg vegetable oil capsules without n-3 LCPUFA. All capsules were matched in size, shape, and colour and were donated by Efamol, Surrey, UK. Women took capsules from 21 weeks’ gestation until delivery and reported their adherence to supplementation at telephone calls at 28 and 36 weeks’ gestation. In addition, the concentration of individual LCPUFA in plasma phospholipids from cord blood was assessed as an independent biomarker of adherence.
DOMInO Trial randomisation & Blinding

The randomisation schedule for the DOMInO Trial was produced by an independent statistician using ralloc.ado version 3.3 in Stata Release 9. Randomisation was performed using randomly permuted blocks of sizes 2, 4, 6 and 8 in proportions of 0.125, 0.375, 0.375 and 0.125 respectively. The randomisation was stratified by centre and parity (first pregnancy > 20 weeks gestation, subsequent pregnancy > 20 weeks gestation). A telephone randomisation service was used to allocate consenting women to receive DHA or placebo supplementation. Once randomised, each woman received a unique identification number and a corresponding treatment pack containing either intervention or placebo in pre-packaged form.

Participants, research staff and investigators in the original DOMInO trial were blinded to treatment group allocation. To facilitate blinding, the DHA and placebo capsules were identical in appearance. Although the blinding was broken during the analysis of the DOMInO trial, with the exception of the study statistician all research staff and investigators involved in the six year follow up were not aware of individual group allocations. Participating families were able to request details of group allocation at any stage after completion of the initial DOMInO trial. The number of families requesting to be un-blinded in each group will be reported as a trial quality outcome for this study.
Enrolment to 1 & 3 year allergy follow-up

At enrolment into the DOMInO Trial, parents residing in South Australia were screened to assess their atopy status and that of the child’s father and siblings by asking a series of structured questions;

*Has the mother ever been medically diagnosed with or had medications for the following?*

- **Asthma**
  - ❑ Yes ❑ No
- **Eczema**
  - ❑ Yes ❑ No
- **Hayfever**
  - ❑ Yes ❑ No

*Has the father ever been medically diagnosed with or had medications for the following?*

- **Asthma**
  - ❑ Yes ❑ No
- **Eczema**
  - ❑ Yes ❑ No
- **Hayfever**
  - ❑ Yes ❑ No

*Do you have other children that have ever been medically diagnosed with or had medications for the following?*

- Not Applicable
  - ❑ Yes ❑ No
- **Asthma**
  - ❑ Yes ❑ No
- **Eczema**
  - ❑ Yes ❑ No
- **Hayfever**
  - ❑ Yes ❑ No

Families who screened positive to allergy screening (i.e. reporting symptoms of at least one familial medically diagnosed allergic disease) indicating that their unborn child was at high hereditary risk of allergy, were invited to participate in a nested child allergy follow-up at 1 and 3 years of age (116, 120). Written informed consent for the 1 & 3 year nested follow up was sought before birth (n=706). Follow up appointments consisted of an allergy assessment including medical assessment and skin prick test (SPT) to determine IgE-mediated allergic disease (eczema,
and/or food allergy at 1 year of age and eczema, asthma, allergic rhinitis food allergy at 3 years of age) in the child. These assessments were completed in 2009 and results have been published previously (116, 120).

6 year allergy follow participation

In 2012, families who had consented to the 1 & 3 year allergy follow up cohort (minus deaths and withdrawals) were eligible to participate in the 6 year allergy follow study (n=668). Approval for the 6 year allergy follow-up was granted from the Women’s and Children’s Health Network (WCHN) Human Research Ethics Committee (HREC) on 9th January 2012 (REC 2434/12/14) and the Southern Adelaide Clinical (SAC) HREC on 11th April 2012 (146.12). The study was registered on the Australian New Zealand Clinical Trials Registry, ACTRN12615000498594.

Primary Outcome

The primary outcome of the 6 year allergy follow up is the incidence of IgE mediated allergic disease (eczema, wheeze, rhinitis, rhino-conjunctivitis symptoms) with sensitisation at 6 years of age (defined as positive SPT of ≥3mm).

Secondary Outcomes

A number of secondary outcomes were included in the statistical analysis to enable assessment of the effect of supplementation on individual allergic disease and sensitisation, Table 3-1.
Table 3-1 Secondary outcome descriptions

<table>
<thead>
<tr>
<th>Outcome</th>
<th>DHA supplementation of pregnant women with a fetus at high-risk of atopic disease will.....</th>
<th>CRF Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitisation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Sensitisation</td>
<td>Reduce the proportion of children with sensitisation with or without allergic disease</td>
<td>Sensitisation to at least one allergen (K1.4)</td>
</tr>
<tr>
<td>Sensitisation by extract</td>
<td>Reduce the proportion of children with sensitisation to individual allergen extracts</td>
<td>Sensitisation to individual allergens (K1.4)</td>
</tr>
<tr>
<td>Sensitisation no allergic disease</td>
<td>Reduce the proportion of sensitised children without symptoms of allergic disease</td>
<td>Sensitisation (K1.4) with no symptoms of ‘current wheeze’ (E2), ‘rhinitis’ (F2), ‘or eczema (G2 &amp; G3)</td>
</tr>
<tr>
<td>Individual IgE allergic disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eczema symptoms with sensitisation</td>
<td>Reduce the proportion of children with any eczema symptoms and sensitisation</td>
<td>Eczema symptoms (G2 and G3) with sensitisation (K1.4)</td>
</tr>
<tr>
<td>Current wheeze with sensitisation</td>
<td>Reduce the proportion of children with symptoms of current wheeze and sensitisation</td>
<td>Current wheeze (E2) with sensitisation (K1.4)</td>
</tr>
<tr>
<td>Rhinitis with sensitisation</td>
<td>Reduce the proportion of children with symptoms of allergic rhinitis with sensitisation</td>
<td>Evidence of rhinitis (F2) with sensitisation (K1.4) to ≥ aero-allergen</td>
</tr>
<tr>
<td>Rhino-conjunctivitis with sensitisation</td>
<td>Reduce the proportion of children with symptoms of rhino-conjunctivitis with sensitisation</td>
<td>Evidence of rhino-conjunctivitis (F2 &amp; F3) with sensitisation (K1.4) to ≥aeroallergen</td>
</tr>
</tbody>
</table>
Table 3-2 Additional secondary outcome descriptions

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA supplementation of pregnant women with a fetus at high-risk of atopic disease will.....</th>
<th>CRF Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parent reported allergic disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parent report eczema ‘ever’</td>
<td>Reduce the proportion of children with parent reported eczema</td>
<td>G7 = Yes</td>
</tr>
<tr>
<td>Parent reported asthma ever</td>
<td>Reduce parent report of asthma “ever”</td>
<td>E6 = Yes</td>
</tr>
<tr>
<td>Parent reported hay fever ‘ever’</td>
<td>Reduce the proportion of children with parent reported hay fever</td>
<td>F6 = Yes</td>
</tr>
<tr>
<td><strong>Any allergic disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any eczema symptoms</td>
<td>Reduce the proportion of children with a eczema symptoms</td>
<td>Eczema symptoms (G2 and G3)</td>
</tr>
<tr>
<td>Any current wheeze</td>
<td>Reduce the proportion of children with symptoms of current wheeze</td>
<td>Current wheeze’ (E2)</td>
</tr>
<tr>
<td>Ever had asthma and current wheeze</td>
<td>Reduce parent report of asthma with current symptoms</td>
<td>Current wheeze (E2) and asthma ever (E6) = Yes</td>
</tr>
<tr>
<td>Any allergic rhinitis symptoms</td>
<td>Reduce the proportion of children with symptoms of allergic rhinitis</td>
<td>Rhinitis ( F2)</td>
</tr>
<tr>
<td>Any Rhino-conjunctivitis</td>
<td>Reduce the proportion of children with symptoms of rhino-conjunctivitis</td>
<td>Evidence of rhino-conjunctivitis (F2 &amp; F3)</td>
</tr>
</tbody>
</table>
### Outcome

n-3 LCPUFA supplementation of pregnant women with a fetus at high-risk of atopic disease will…..

<table>
<thead>
<tr>
<th>Outcome</th>
<th>CRF Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eczema severity</strong></td>
<td></td>
</tr>
<tr>
<td>Eczema severity – persistent rash</td>
<td>Reduce the severity of symptoms of eczema</td>
</tr>
<tr>
<td>Eczema severity - awake at night</td>
<td>Reduce the severity of symptoms of eczema</td>
</tr>
<tr>
<td>Eczema requiring treatment last 12m</td>
<td>Reduce the proportion of children requiring treatment for eczema</td>
</tr>
<tr>
<td><strong>Severity of wheeze/asthma</strong></td>
<td></td>
</tr>
<tr>
<td>Frequency of current wheeze</td>
<td>Reduce the frequency of symptoms of current wheeze</td>
</tr>
<tr>
<td>Wheeze disturbs sleep</td>
<td>Reduce the severity of asthma symptoms</td>
</tr>
<tr>
<td>Severe wheeze limiting speech</td>
<td>Reduce the severity of asthma symptoms</td>
</tr>
<tr>
<td>Exercise wheeze</td>
<td>Reduce symptoms of asthma</td>
</tr>
<tr>
<td>Night cough</td>
<td>Reduce symptoms of asthma</td>
</tr>
<tr>
<td>Asthma -Activities affected at school</td>
<td>Reduce the proportion of children whose activities are affected at school by asthma symptoms</td>
</tr>
<tr>
<td>Outcome</td>
<td>n-3 LCPUA supplementation of pregnant women with a fetus at high-risk of atopic disease will.....</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sporting activities affected</td>
<td>Reduce the proportion of children whose sporting activities are affected by asthma symptoms</td>
</tr>
<tr>
<td>Salbutamol last 12m</td>
<td>Reduce the proportion of children requiring Salbutamol for asthma symptoms</td>
</tr>
<tr>
<td>Regular inhaled preventer</td>
<td>Reduce the proportion of children requiring preventer medication for asthma symptoms in the last 12 months</td>
</tr>
<tr>
<td>Oral steroids</td>
<td>Reduce the proportion of children requiring oral steroids for asthma symptoms in the last 12 months</td>
</tr>
<tr>
<td>Current asthma meds</td>
<td>Reduce the proportion of children requiring current asthma medication for asthma symptoms</td>
</tr>
<tr>
<td><strong>Severity of rhinitis/hayfever</strong></td>
<td></td>
</tr>
<tr>
<td>Severity of rhinitis symptoms</td>
<td>Reduce severity of allergic rhinitis symptoms</td>
</tr>
<tr>
<td>Hayfever requiring treatment last 12m</td>
<td>Reduce severity of allergic rhinitis symptoms</td>
</tr>
<tr>
<td>Snoring</td>
<td>Reduce the incidence of snoring</td>
</tr>
</tbody>
</table>
Trial Quality Outcomes

The hypothesis for trial quality outcomes is that there will be no difference between the groups in the following outcomes:

- Allergic disease without sensitisation
- Hospitalisations in last 12 months
- Serious adverse events
- Child Health Questionnaire
- SPT per protocol
- Completion of SPT
- Proportion of families un-blinded before the 6 year assessment
The Six Year Appointment

Study Invitation

At enrolment in to the DOMInO Trial, home and work contact details of both parents and four other contacts of the parents’ choice, e.g. grandparents, other relatives, friends were collected. Regular contact has been maintained with families over the years via newsletters, study results letters and greetings cards. Families were also alerted to the 6 year follow-up study through a letter advising them of the results of the analysis of the 1 year nested allergy follow-up in 2012.

Each mail-out offered the opportunity to update details of families whose mail was returned due to change of address. The purpose of this communication is to ensure that families involved in the DOMInO trial follow up studies are kept informed and in doing so, potentially minimising attrition. Families were invited to participate in the 6 year follow up as described;

A ‘Study Information Pack’ was sent to the care giver of the eligible child when the child was 5 years and 9 months of age. This mail out contained the following ethically approved documents;

- Participant Information Sheet (Appendix 1)
- Participant Consent Form (Appendix 2)
- Contact Details Form to enable families to update any name, address of telephone details (Appendix 3)
- Reply paid envelope
When the child was between 5 years 10 months of age and 6 years, the family was telephoned to confirm receipt of the study information pack and ascertain if they wanted to participate. At this call, further explanation about the study was provided and the parent/carer was given an opportunity for the family to ask any questions. Arrangements were then made for the child to attend the Child Nutrition Research Centre (CNRC) clinic at either WCH or FMC and a letter confirming the date, time and location of the appointment (including map and directions) was sent to the family, (Appendix 4). This letter also contained detailed information regarding preparation for the skin prick test i.e. no medication containing anti-histamines for 4 days prior to the appointment and no creams to the skin on the day of the appointment. An appointment reminder was sent one day prior to the appointment via short message service if a mobile phone number was available, or by telephone call if not. Every effort was undertaken to make attendance at the assessment as convenient as possible for the family to encourage attendance including, choice of clinic location, evening or weekend appointments, or a cab charge if no transport was available.
Locating families

In order to maximise follow-up and minimise bias due to attrition, a number of strategies were used in an attempt to notify parents of the 6 year follow up and ensure the maximum number of children completed the six year follow up. In the event that contact could not be made with either parent in a family, a number of alternative methods were used including:

- Telephone call to mobile phone, home phone, work phone
- SMS
- Email
- Letter
- Contacting listed alternate contacts by phone and letter
- Online White pages (Australia)
- Monthly checks of hospital medical records (OACIS)
- Evening and weekend calls to home and mobile numbers

Despite efforts described, contact attempts for a number of families proved futile (n=24). A HREC amendment request was submitted with a proposal to use social media (Facebook) to message these parents to inform them of the study and invite them to reply (Appendix 5). Unfortunately, this amendment request was rejected on the basis of privacy issues as it could not be confirmed that the person contacted via Facebook was in fact, the person consented to allergy follow up. A total of 4898 attempts at communication (including successful and unsuccessful) were made with participants throughout the study. The average amount of communication (including letters, phone calls, SMS) required per person to arrange and complete an assessment was 8 times.
During the 6 year Appointment

Six year appointments were scheduled for one hour time slots on a day of the week and a time that suited the family (including evenings and weekends). The assessment was completed in the following order;

- The parent/carer and child were showed to the clinic room and personal details cross checked with details that were already on file.
- The complete assessment was explained to the parent/carer and child and opportunity given for them to ask any questions.
- Informed consent was documented by signing of the study consent form by the parent/carer and student investigator (Appendix 2). A copy of the signed consent form was offered to parents and the original copy was filed in the child’s CRF.
- The skin prick test (SPT) was explained to the child in a language that they could understand and the procedure was commenced according to the internal standard operating procedure (Appendix 6) and as detailed in Chapter 3.4.2.
- Parent/carers completed the child health questionnaire whilst the SPT was being conducted. Once complete and while awaiting results of skin testing, CRF question were completed by interview.
- Once SPT results were measured and recorded in the CRF the child’s height and weight was obtained according to CNRC standard operating procedures.
- Parents were advised of results of the SPT and a copy of results provided for their records (Appendix 7)
• Verbal consent was obtained for the results of the SPT to be sent to the child’s general practitioner should any follow up be required.

• Following completion of the appointment the child was presented with a Certificate of Appreciation (Appendix 8) and got to choose some stickers for being so brave.

• Parents were given $20.00 reimbursement for travel and parking expenses and a petty cash form signed to document same.

After the 6 year appointment

• The CRF was checked for accuracy and completion and all documents filed including consent form, child health questionnaire and contact details form). The CRF was filed ready for double checking by another staff member before sending for data entry.

• The electronic study management system was updated to record that the child had attended the appointment.

• A template GP letter was completed (Appendix 9) with the child’s details and SPT results and sent to the GP with a copy filed in the CRF.

• The clinic area was tidied, cleaned and re-stocked.

Off-site Assessments

Original HREC approval was granted to conduct the six year assessments including SPT at WCH or FMC. During the process of contacting participants and attempting to schedule appointments it became apparent that a number of families were unable or unwilling to travel from their home to either FMC or WCH for the assessment. In an attempt to maximise follow up completion, in particular
completion of the SPT for the primary outcome, a HREC amendment request to conduct the assessment in the home of the child was prepared. The risk of anaphylaxis from skin prick testing is extremely rare and if occurs, is mostly due to testing with fresh food. Children participating in the DOMInO 6 allergy follow up had previously undergone a SPT as part of the 1 & 3 year nested allergy follow-up assessments. In total more than 1300 SPT’s were conducted at 1 and 3 years of age with no incidence of anaphylaxis. All of these appointments were conducted in a hospital setting although there is more recent evidence of the safety of this procedure in a non-hospital setting [24]. As an added precaution for in-home testing we planned to exclude all foods from the standard allergen panel if SPT was performed in the home. The amendment request was submitted on 27th August 2012, (Appendix 10) HREC approval was given pending review of the application by WCHN Risk Management. Following further discussion, the WCHN Clinical Risk Manager judged that they did not support conduct of the SPT in the home, as follows;

“This basis of this decision was related to the risk to a child that has a reaction to SPT rather than the prevalence of a reaction (or the risk of this occurring), and to ensure that the appropriate emergency equipment is available and that there are established processes to access a higher level of care if required.” Julie Paholski, Risk Manager.

An amended proposal was submitted to HREC in alignment with concerns raised by the WCHN Risk Manager and approval was granted to conduct the six year assessment including SPT in a health care environment that is a local hospital or SA Health GP Plus Clinic. The Manager of Insurance Services, SA Health, agreed with this amendment and further to this provided ‘once off’ authorisation that I
would be covered under the SA Health professional indemnity program to conduct the six year assessments, including SPT, interstate. This amendment to the protocol enabled completion of an additional 27 SPT’s (6% of total SPTs) and although time intensive due to the travel involved was worthwhile to completeness of outcomes and study quality.

Assessment by Correspondence

A number of families residing in Adelaide at the time of enrolment to the nested allergy cohort have since re-located interstate or overseas. Of the 668 participants eligible for participation in the 6 year follow up, 53 families (13%) no longer live in the Adelaide metropolitan area. In this instance the family was contacted to ascertain if they would be returning to Adelaide at any time prior to September 2014 so that we may be able to schedule an appointment. If not, the family was mailed an ‘External Correspondence Pack’ consisting of the following documents;

- Parent Case Report Form (CRF) for completion by the primary care giver
- Child Health Questionnaire (Appendix 12)
- Certificate of appreciation for the child (Appendix 8Error! Reference source not found.)
- Reply paid envelope
In addition to ex-Adelaide participants, a number of families living locally either refused or were unable to attend a clinic appointment and were also sent the ECP. ECP’s that were not returned within 2 weeks of mailing were followed up by phone call to the family to confirm receipt of same. On a number of occasions, forms were returned without a signature on the consent form or without the consent form included which meant that we were unable to use this data. At times, the forms were not returned at all despite reminder calls however parents were happy to be interviewed over the phone to obtain the information. On 21/05/13 an amendment request was submitted to HREC to request that verbal consent be allowed for use of the parent CRF and CHQ in accordance with Section 2.2.5 of the National Statement; (146)

Consent may be expressed orally, in writing or by some other means (for example, return of a survey, or conduct implying consent), depending on:

a) the nature, complexity and level of risk of the research; and

b) the participant’s personal and cultural circumstances

HREC approval was granted on to waive consent for these participants (Appendix 14).
Skin Prick Test

The skin prick test (SPT) has many advantages over other methods of determining sensitisation including; low cost, immediate results and relative sensitivity and specificity. SPT should cause minimal discomfort and has a low risk of adverse side effects and anaphylactic reaction when performed on patients who are well. The IgE mediated response in the skin results immediately in a wheal and flare reaction which is essentially induced by mast cell degranulation after allergen challenge (147). Sensitisation of the child is a component of the composite primary outcome of this follow up study and therefore important to determine quickly and accurately whether or not the child was sensitised to any of the allergen extracts tested.

The effectiveness of the SPT as a diagnostic tool for sensitisation depends upon a number of factors including; quality and standardisation of allergen extracts, equipment, and the technique of the practitioner. The most convenient and frequently used sites for SPT are the volar surface of the forearm or the back. In younger children, the back is preferable as there is a larger surface area of skin to utilise. An additional advantage of choosing the back is that it increases comfort of the procedure (lying prone) and may decrease psychological stress to the child as they do not observe the lancet. Droplets of allergen extract are placed directly onto the skin and penetrated with a lancet in a prick and lift action. This allows introduction of the allergen into the epidermis and superficial dermis and recognition of the allergen by the IgE antibodies on the mast cells located in the skin if the individual has been ‘sensitised’ (148).
It is important that this step of the procedure is performed correctly, Figure 3-1. The aim is to prick and lift the skin without drawing blood as bleeding indicates an over-vigorous technique and may lead to increased risk of adverse reaction, inaccurate results as well as increased discomfort for the child.

Figure 3-1 Skin Prick Test – Prick and lift action

SPT Procedure

I. Parents were questioned regarding recent general health of the child and use of any anti-histamine containing medications in the past 4 days. Adverse effects of SPT are more likely in an individual whom is unwell or has unstable asthma and should be avoided. Use of medications containing anti-histamine can interfere with skin tests and produce inaccurate results.

II. The procedure was also explained to the child in appropriate language and they were assisted to lie prone on the clinic bed with back exposed. A DVD player was provided for the child to distract them from the procedure and enhance compliance, Figure 3-2.
III. Skin on the back was inspected to ensure it was intact where testing was to be undertaken. SPT should not be performed on skin that is not intact e.g. eczema as it increases the risk of adverse reactions and reduces accuracy. Skin was then numbered 1 – 12 to correspond with allergen extracts, allowing 2cm between test areas.

![Figure 3-2 Child ('Cleo') undergoing SPT procedure whilst watching a movie](image)

IV. A small drop of allergen extract was squeezed onto the skin next to the corresponding marked number, ensuring that the dropper did not come into contact with the skin. Care was taken not to use excessively large droplets as they may run and contaminate other sites making results inaccurate. A new lancet was used for each droplet of extract to ensure there was no transfer of allergens.

V. Once all droplets were “pricked” the droplets were carefully blotted from the skin with a tissue after 2 minutes, ensuring that there was no wiping movement that may transfer extracts.
VI. The timer was set for 10 minutes to read the histamine reaction which reaches a peak in 8 to 10 minutes, then for another 5 minutes to read the remaining results. All skin tests were read in a standard manner to determine the mean diameter of the wheal (in mm). This was determined by measuring the longest diameter of the wheal and then measuring the diameter 90° to the longest diameter. The two measurements were then added together and divided by two, Figure 3-3. The presence of pseudopodia (i.e. a protruding process from the wheal) was not included in the measurement as this can falsely increase the size of the reaction.

![Figure 3-3 Diagram of SPT wheal measurement](image)

VII. The child’s skin was wiped with non-allergenic baby wipes and urea cream applied if the area was uncomfortably itchy. The child and parent were advised that the itch and wheal would settle over the next 2-4 hours.

VIII. All procedures were performed in accordance with WCHN infection control policies and Hand Hygiene Australia Guidelines.

IX. A response was considered positive if the mean of the horizontal and perpendicular wheal diameters of at least one allergen extract was 3mm or greater in size than the mean wheal of the negative control site at 15 minutes (sensitisation).
Safety

Although the risk of an adverse reaction was low in our population, measures were in place should they be required. All assessments were completed under a ‘buddy system’ i.e. there was always a second staff member to call upon for help during if additional support was needed. Adrenaline was always on hand for administration by a medical officer if the need arose and as the majority of assessments were conducted at the WCH or FMC, support of the emergency response team was available if required. Assessments that were conducted off site at GP Plus clinics, SA Country Hospitals or Interstate locations, had the support of clinical staff with recognised emergency procedures. In addition, training was completed in anaphylaxis management, auto-injector administration and annual infant, child and adult, basic life support.
Allergen extracts

Allergen extracts are aqueous solutions of proteins combined with 50% glycerol, which acts as a preservative. They are manufactured specifically for use in skin prick testing and supplied in multi-use dropper bottles, with a rubber teat and glass dropper. The allergen panel selected for this six year follow up consisted of three common food allergens (whole hen’s egg, peanut and cashew Nut) and seven common aero-allergens (ryegrass pollen, olive tree pollen, *D. pteronyssinus*, *D. farinae*, cat, *alternaria tenuis* and dog) which are linked to respiratory allergic diseases (asthma and rhino-conjunctivitis) at early school age. Two controls were included in the panel, a negative control consisting of saline and glycerol (i.e. no allergen) and positive control consisting of histamine phosphate, Figure 3-4.

*Figure 3-4 Labelled Allergen Extracts for the 6 year assessment*
The purpose of the negative control is to identify if the participant displays a wheal from the skin prick alone. This may lead to false positive reactions to allergens that the participant is not sensitised too. If the negative control results in wheal after 10 minutes, the reading is recorded and this measurement is subtracted from measurements of the remaining panel. The purpose of the positive control is to produce a wheal of ≥ 3mm to ensure reactivity of the participant to skin testing. A wheal size less than this may indicate that recent intake of medications containing antihistamine, incorrect technique or non-reactive skin.

Extracts are not currently manufactured in Australia and need to be sourced from overseas. As different manufacturer’s preparations may vary in their content and proportions of major allergenic proteins, consistency was maintained throughout the study. Due to expiry dates of allergen extracts between March 2012 and August 2014, it was necessary to use different batches per site. The following
Table 3-3 lists all allergen extracts used for SPT assessments at all locations throughout for the 6 year follow up study. One extract (Alternaria) became unavailable from our usual supplier (Alyostal) throughout the study and was therefore was sourced from Hollister-Stier.
### Table 3-3 List of allergen extracts used

<table>
<thead>
<tr>
<th>No.</th>
<th>Extract</th>
<th>Manufacturer</th>
<th>Conc.</th>
<th>Batch</th>
<th>Expiry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>Hollister-Stier</td>
<td>N/A</td>
<td>IC4405</td>
<td>03/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hollister-Stier</td>
<td>N/A</td>
<td>1F6109</td>
<td>07/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hollister-Stier</td>
<td>N/A</td>
<td>E1203528</td>
<td>09/2015</td>
</tr>
<tr>
<td>2</td>
<td>Whole Hens Egg</td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>105740</td>
<td>11/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>112607</td>
<td>02/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>9703049</td>
<td>02/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>9710858</td>
<td>06/2016</td>
</tr>
<tr>
<td>3</td>
<td>Peanut</td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>105811</td>
<td>11/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>9709394</td>
<td>04/2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>9712475</td>
<td>11/2016</td>
</tr>
<tr>
<td>4</td>
<td>Cashew Nut</td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>112074</td>
<td>01/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>9702609</td>
<td>01/2015</td>
</tr>
<tr>
<td>5</td>
<td>Ryegrass pollen</td>
<td>Alyostal</td>
<td>100IR/ml</td>
<td>105501</td>
<td>10/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100IR/ml</td>
<td>9700965</td>
<td>09/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100IR/ml</td>
<td>9706622</td>
<td>10/2015</td>
</tr>
<tr>
<td>6</td>
<td>Olive tree pollen</td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>113405</td>
<td>04/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9703935</td>
<td>04/2015</td>
</tr>
<tr>
<td>7</td>
<td>D. pteronyssinus</td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>113383</td>
<td>04/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9707373</td>
<td>12/2015</td>
</tr>
<tr>
<td>8</td>
<td>D. farinae</td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>113595</td>
<td>05/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9702602</td>
<td>01/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9707249</td>
<td>11/2015</td>
</tr>
<tr>
<td>9</td>
<td>Cat Fur</td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>113149</td>
<td>04/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9700749</td>
<td>09/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9703475</td>
<td>03/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9709512</td>
<td>04/2016</td>
</tr>
<tr>
<td>10</td>
<td>Alternaria tenuis</td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>113768</td>
<td>05/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>1000 IC/ml</td>
<td>113768</td>
<td>05/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hollister-Stier</td>
<td>1:10</td>
<td>K31E5633</td>
<td>10/2014</td>
</tr>
<tr>
<td>11</td>
<td>Dog Hair</td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>113742</td>
<td>05/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alyostal</td>
<td>100 IR/ml</td>
<td>9706643</td>
<td>10/2015</td>
</tr>
<tr>
<td>12</td>
<td>Histamine</td>
<td>Hollister Steer</td>
<td>N/A</td>
<td>7099ED</td>
<td>09/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hollister-Stier</td>
<td>N/A</td>
<td>3275201</td>
<td>09/2014</td>
</tr>
</tbody>
</table>
Allergen extracts should be stored in a temperature-monitored refrigerator between (+2°C and +8°C) to reduce the rate of potency loss. The potency of allergen extracts is affected by several factors including; the passage of time, temperature, concentration, number of allergens in a vial, volume of the storage vial and presence of stabilisers and preservatives. Two purpose specific refrigerators were purchased for storage of allergens for the study and one was located at each site (WCH and FMC).
Assessment of Allergic Disease

The incidence of ‘allergic disease with sensitisation’ is a composite primary outcome of the DOMInO 6 year follow up. A validated questionnaire, the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire was used to assess symptoms of wheeze, eczema and rhinitis/rhino-conjunctivitis in the children in a standardised way (41). The ISAAC questionnaire consists of three modules containing ‘core’ questions which have been used in previously published questionnaires and have found differences between populations (49). Symptom-based questions are recommended to avoid underestimation of the prevalence of disease and achieve comparable results in different populations. They are also designed to screen even patients with mild symptoms (149). Case definitions and severity are established by asking about cardinal symptoms, not by reference to labels or diagnoses. Enquiry about symptoms proceeds from the relatively mild to the relatively severe, and precedes enquiry about diagnosis. Descriptions and definitions of primary and secondary outcomes of allergic disease symptoms and sensitisation for the purpose of analysis were as follows.

Eczema

The eczema module of the ISAAC questionnaire was administered to determine the incidence of symptoms of eczema within the last 12 months. Questions have been specifically designed to discriminate typical mild-moderate atopic eczema from non-atopic eczema and other inflammatory skin disorders (42). The combination of a chronic itchy rash with flexural involvement had both high sensitivity (0.80) and specificity (0.97) when compared with eczema diagnosis by standardised dermatologist assessment (159). Additional questions were included
as indicators of severity including night waking and whether the rash had ever cleared completely in the last year. The 6 year eczema assessment comprised the following questions;

G1 Has your child EVER had an itchy rash which was coming and going for at least 6 months?

G2 Has your child had this itchy rash at any time in the last 12 months?

G3 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?

G4 At what age did this itchy rash first start to occur?

G5 Has this rash cleared completely at any time during the last 12 months?

G6 In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?

G7 Has your child EVER had eczema?

An answer of “YES” to both G2 and G3 indicated current (within last 12 months) symptoms indicative of atopic eczema and was used in analysis of the primary and secondary outcome/s.
Rhinitis & Rhino-conjunctivitis

Adult population surveys of rhinitis symptoms followed up by interview and SPT determined that the combination of rhinitis symptoms (sneezing, blocked or runny nose) with ocular symptoms (itching or watering eyes) had the best predictive values for a positive SPT (allergic rhino-conjunctivitis) (151). The ISAAC questionnaire was therefore developed to include these questions in addition to seasonality and a simple marker of severity (interference with daily activities). The rhinitis module of the ISAAC questionnaire was utilised for the 6 year allergy assessment and comprised the following questions:

F1 Has your 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?

F2 In the last 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?

F3 In the last 12 months, has this nose problem been accompanied by itchy watery eyes?

F4 In which of the last 12 months did this nose problem occur?

F5 In the last 12 months, how much did this nose problem interfere with your child's daily activities?

F6 Has your child EVER had hay fever?

An answer of ‘YES’ to F2 was considered positive for incidence of ‘rhinitis’ symptoms. An answer of ‘YES’ to both F2 and F3 was indicative of rhino-conjunctivitis symptoms for the purpose of analysis of primary and secondary outcomes.
Wheeze

The presence of ‘wheezing’ has been shown to be the most important symptom for the identification of individuals with asthma (59). Due to the intermittent nature of asthma, symptoms of wheezing occurring within the previous 12 months have been used to define current asthma symptoms or ‘current wheeze’. Responses to questions about self-reported or parent reported wheezing and/or whistling in the chest in the previous 12 month period have been shown to have good specificity (0.81) and sensitivity (0.85) compared with respiratory physician assessment (59).

The asthma module of the ISAAC questionnaire included in the DOMInO 6 CRF comprises the following questions;

E1 Has your child EVER had wheezing or whistling in the chest at any time in the past?

E2 Has your child had wheezing or whistling in the chest in the past 12 months?

E3 How many attacks of wheezing or whistling has your child had in the past 12 months?

E4 In the past 12 months, how often on average has your child's sleep been disturbed due to wheezing or whistling?

E5 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?

E6 Has your child EVER had asthma?

E7 In the past 12 months, has your child's chest sounded wheezy during or after exercise?
Chapter Three

E8 In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection?

A positive answer to question E2 was indicative of current symptoms and in the presence of a positive SPT to one of the aero-allergens tested, was indicative of atopic wheeze (asthma). In addition the incidence of current wheeze with sensitisation at 6 years, a number of longitudinal outcomes were also assessed. There is generally poor agreement on definitions of different phenotypes of wheezing disorders in young children. Population studies have shown that approximately one in three children has at least one episode of wheezing prior to their third birthday, and the cumulative incidence of wheeze is almost 50% at the age of 6 years \(^{(54)}\). A number of these wheezing illnesses are caused by viral infections and children will grow out of their wheeze by 6 years of age. Due to complexities of asthma diagnosis and in the absence of a clinician exam, I have reported secondary outcomes related to asthma by symptoms and category of wheeze.

- **Never wheeze** – Answered ‘No’ to questions about current wheeze at 1, 3 and 6 years.
- **Transient wheeze** – Answered ‘Yes’ to current wheeze at 1 year and/or 3 year but ‘No’ to current wheeze at 6 years.
- **Late onset wheeze** – Answered ‘No’ to current wheeze at 1 and 3 years but ‘Yes’ to current wheeze at 6 years.
- **Persistent wheeze** – Answered ‘Yes’ to current wheeze at 1 and/or 3 and ‘Yes’ to current wheeze at 6 years.
Sensitisation

Sensitisation was defined as a positive skin prick test (SPT) to at least one of the allergen extracts tested at the 6 year appointment. A response was considered positive if there was a mean of the horizontal and perpendicular wheal diameters of 3mm or greater in size than the mean wheal of the negative control site at 15 minutes \(^{(129)}\), see Chapter 0.

- Sensitisation for eczema was defined as a positive response to at least one allergen extract (Whole hens egg, Peanut, Cashew nut, Ryegrass pollen, Olive tree pollen, \textit{D. pteronyssinus}, \textit{D. farinae}, cat, \textit{Alternaria tenuis}, Dog).
- Sensitisation for wheeze and rhinitis was defined as a positive response to at least one aero- allergen extracts tested (Ryegrass pollen, Olive tree pollen, \textit{D. pteronyssinus}, \textit{D. farinae}, cat, \textit{Alternaria tenuis}, Dog).
Case Report Form (CRF)

The case report form (Appendix 11) was designed to capture all required data for each participant as defined in the HREC approved trial protocol. In addition to data collected and recorded in the CRF for assessment of primary and secondary outcomes, a number of additional questions were included to provide information required to assess possible confounding variables of the intervention. The CRF questions for the 6 year follow up assessment were completed via interview with the parent/carer. Information collected included information about the child’s socio-demographic characteristics, lifestyle, diet and environment, see Table 3-4.
### Table 3-4 Case report form (CRF) sections

<table>
<thead>
<tr>
<th>Section</th>
<th>Information collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Information</td>
<td>Whom the child lives with and their level education and employment details. Details obtained in this section were used to ascertain the child’s Social Economic Index for Areas (SEIFA) from the Australian Bureau of Statistics, (3) and occupation coding from the Australian &amp; New Zealand Coding of Occupations (ANZCO) (64). Both indexes were completed as a comparison of measurement of socio-economic status between the intervention and control groups.</td>
</tr>
<tr>
<td>Home Environment</td>
<td>Other occupants in the home, exposure to cigarettes, pets and fuels.</td>
</tr>
<tr>
<td>Dietary Information</td>
<td>Fish intake, vitamin/mineral supplement intake, intake of various food groups.</td>
</tr>
<tr>
<td>Family Health Data</td>
<td>Allergy history of mother, father and siblings.</td>
</tr>
<tr>
<td>Child Allergic Disease Symptoms</td>
<td>ISAAC core questions for eczema, asthma and rhinitis.</td>
</tr>
<tr>
<td>General Health Information</td>
<td>Physical activity, screen/sedentary time and paracetamol and ibuprofen use.</td>
</tr>
<tr>
<td>Section</td>
<td>Information collected</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>Child Assessment</td>
<td>Height, weight and skin prick test (SPT).</td>
</tr>
<tr>
<td>Child Health Questionnaire</td>
<td>CHQ-PF50 Quality of life questionnaire.</td>
</tr>
<tr>
<td>Serious Adverse Events</td>
<td>Hospital admission to intensive care or anaphylaxis in previous 12 months.</td>
</tr>
</tbody>
</table>

Comprehensive instructions regarding accurate completion of the CRF were detailed in the CRF Completion Instructions. (Appendix 13)
Anthropometrics

The child's height and weight were measured as an index of the nutritional well-being of the children and to enable calculation of body mass index (BMI). All measurements were conducted in accordance with departmental Standard Operating Procedures for height and weight. Height and weight were measured twice and the mean of these measurements were used for analysis. If the difference between the two measures was greater than 10%, a third measurement was taken and the two measurements in closest agreement were used to calculate the mean. If it was not possible to perform the anthropometric measures at the time of the appointment, caregivers were asked to forward recent measurements taken at home. Weight was measured using an electronic scale that was calibrated before the commencement of the study using standardised iron weights. Children's weight was measured to the nearest 100 grams whilst the child was wearing only minimal clothing. Height was measured with a stadiometer to the nearest 0.1 cm. Shoes were not worn for the height measurement and hair ornaments were removed so that the head could lie flat against the stadiometer.
Child Health Questionnaire (CHQ)

The HealthAct Child Health Questionnaire (CHQ-PF50) is a generic child quality-of-life evaluation instrument used to gauge paediatric health-related quality of life (HRQOL), (Appendix 12).

The CHQ-PF50 comprises 50 item questions that measure physical, psychological and social domains in all health conditions irrespective of the underlying disease and has been normed for use as a self-completed measure by parents/guardians and children, see Table 3-5. Each domain is scored from 0 to 100, with higher scores indicating better HRQOL. A four-week recall period is used for all scales except for the Change in Health (CH) and Family Cohesion (FC) items and the General Health (GH) scale. The recall stem for Change in Health is “compared to last year.” Since the FC item and GH scale ask about health and family relationships “in general,” no recall period is used.

The CHQ-PF50 is designed for self-completion by the parent/guardian of the child and has been validated for use with children at least five years of age or older. Every CHQ use requires authorization, a completed license and payment of licensing fees.
<table>
<thead>
<tr>
<th>Concepts</th>
<th>Low Score</th>
<th>High score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical functioning</td>
<td>Child is limited a lot in performing all physical</td>
<td>Child performs all types of physical activities, including the most vigorous, without limitations due to health</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Role/Social-Physical</td>
<td>Child is limited a lot in schoolwork or activities</td>
<td>Child has no limitations in schoolwork or activities with friends due to physical health</td>
</tr>
<tr>
<td>General Health Perceptions</td>
<td>Parent believes child’s health is poor and likely</td>
<td>Parent believes child’s health is excellent and will continue to be so</td>
</tr>
<tr>
<td>Bodily pain</td>
<td>Child has extremely severe, and frequent pain</td>
<td>Child has no pain or limitations due to pain</td>
</tr>
<tr>
<td>Parental Impact-time</td>
<td>Parent experiences a lot of limitations in time</td>
<td>Parent doesn’t experience limitations in time for personal needs due to child’s physical or psychosocial health</td>
</tr>
<tr>
<td>Parental Impact-emotional</td>
<td>Parent experiences a great deal of emotional worry/concern as a result of child’s physical or psychosocial health</td>
<td>Parent doesn’t experience feelings of emotional worry/concern due to child’s physical or psychosocial health</td>
</tr>
<tr>
<td>Concepts</td>
<td>Low Score</td>
<td>High Score</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Role/Social-Emotional</td>
<td>Child is limited a lot in schoolwork or activities with friends as a result of emotional problems</td>
<td>Child has no limitations in schoolwork or activities with friends due to behaviour problems</td>
</tr>
<tr>
<td>Role/Social-Behavioural</td>
<td>Child is limited a lot in schoolwork or activities with friends as a result of behaviour problems</td>
<td>Child has no limitations in schoolwork or activities with friends due to behaviour problems</td>
</tr>
<tr>
<td>Self Esteem</td>
<td>Child is very dissatisfied with abilities, looks, family/peer relationships and life overall</td>
<td>Child is very satisfied with abilities, looks, family/peer relationships and life overall</td>
</tr>
<tr>
<td>Mental Health (Well-being)</td>
<td>Child has feelings of anxiety and depression all of the time</td>
<td>Child feels peaceful, happy and calm all of the time</td>
</tr>
<tr>
<td>Behaviour (Getting along)</td>
<td>Child very often exhibits aggressive, immature, or delinquent behaviour</td>
<td>Child never exhibits aggressive, immature, or delinquent behaviour</td>
</tr>
<tr>
<td>Family activities</td>
<td>The child’s health very often limits and interrupts family activities or is a source of family tension</td>
<td>The child’s health never limits or interrupts family activities nor is a source of family tension</td>
</tr>
<tr>
<td>Family cohesion</td>
<td>Family’s ability to get along is rated “poor”</td>
<td>Family’s ability to get along is rated “excellent”</td>
</tr>
<tr>
<td>Change in health</td>
<td>Child’s health is much worse now than 1 year ago</td>
<td>Child’s health is much better now than 1 year ago</td>
</tr>
</tbody>
</table>

**Study Management**
Chapter Three

Steering Committee

A steering committee was formed in the design phase of the DOMInO 6 study. The purpose of the steering committee was to ensure expert contribution during all stages of the study from an investigative team with broad experience. The Steering Committee was comprised of the following members;

- Karen Best (Student Investigator).
- Prof Maria Makrides (PhD Primary Supervisor) has a background as a dietitian and has clinical trials expertise in infant nutrition.
- Dr James Martin is Director of Pulmonary Medicine at the Women’s and Children’s.
- Prof Declan Kennedy (PhD Co-supervisor) is a paediatric specialist in the area of respiratory medicine with expertise in the diagnosis and assessment of asthma.
- A/Prof Debra Palmer is an experienced paediatric clinical dietitian who has specialised in the area of nutritional interventions and allergic diseases research.
- A/Prof Michael Gold (PhD Co-supervisor) is a clinical Paediatric Allergist.
- Mr Thomas Sullivan a statistician with DMAC.
Meetings were arranged monthly to review and discuss progress of the 6 year follow-up and to consider strategies to maintain timely follow-up of all participants. Advice from the steering committee was sought in regard to interpretation of ISAAC questions and symptoms of allergic disease for the statistical analysis plan and support was given from members by identifying specialists to assist with resources for off-site assessments.

Web Based Management Information System

The DOMInO web based management information system (MIS) is a Java based platform purpose designed for CNRC by the Data Management and Analysis Centre (DMAC) at University of Adelaide. Regular planning and design meetings were held with DMAC staff prior to commencement of the study to establish requirements and discuss design details.

The aim of the MIS was to enable;

- Tracking of study processes
  - study information sent to participant
  - signed consent form received
- Status of Appointments
  - due/overdue/rescheduled
  - completed/uncompletable
- Participant information
  - contact details of participant and nominated contacts
  - participant status i.e. withdrawn, lost to follow up
  - due/overdue/re-scheduled
• Documentation of communication with participants
  o Record of all participant communication including, letters sent, call received etc.

• Data management
  o Location of CRF’s
  o Data queries received and sent
  o Resolution of data queries on screen

Study activity including any contact attempts with participants or their listed contacts was logged in real time into the MIS. This system provided an invaluable tool for tracking study milestones, monitoring participant status and overall study management. A comprehensive manual describing functions and use of the MIS was written in conjunction with the senior data manager at DMAC and was available for use by staff and updated regularly throughout the study.
Research Staff Assistance

Due to the large number of children participating in the study at varying locations, it was necessary to enlist the help of CNRC staff to assist with completion of some of the six year assessments (20%). As there is a risk of variability between practitioners performing the SPT procedure, the number of clinic staff recruited to assist with conducting the assessments was kept to a minimum. Three Registered Nurses (two with previous SPT experience) were trained in child skin prick testing and ongoing quality assurance measures were implemented to ensure that all SPT procedures (method and measurement) were conducted consistently, accurately and in accordance with the current best practice and the Child Nutrition Research Centre standard operating procedures (Appendix 6). In-service sessions on SPT technique and measuring were undertaken throughout the study and all SPT results per person were analysed monthly to identify any outliers. Staff were trained in CRF completion and Good Clinical Practice (GCP) guidelines for completion of clinical trial documents. A written booklet of detailed CRF completion instruction was also provided and updated at regular intervals throughout the study (Appendix 13). All completed CRFs were double checked by a second trained staff member for completeness and accuracy and then co-signed. Any missing or extraneous entries were followed up with the staff member who completed the original entry. Once all CRF’s were signed as complete by two staff members, they were checked a final time by the Student Investigator prior to signing the Investigator Statement.
Data Management

Data entry of CRF’s was managed by the Data Management & Analysis Centre (DMAC). Finalised CRFs were photocopied and the original copy of the CRF and CHQ were sent express post to DMAC. A CRF tracking function on the MIS was used to record and track the location of original CRFs. Following data entry, DMAC data entry personnel would forward data queries via the MIS if there was any missing responses or if a value entered was outside of the parameters set for a given question (i.e. child weight <20kg or >50kg). Queries would be resolved directly in the MIS and the CRF copy filed on-site was updated. The data query process commenced as soon as the first CRF was received at DMAC and continued until the end of the study. This ongoing process of query resolution eliminated an accumulation of data queries requiring resolution at study completion.
Statistical Analysis

706 children were enrolled in the 1-3 year nested allergy follow-up of the DOMInO trial (368 in the n-3 LCPUFA group and 338 in the control group). Sample size, was calculated as 95% power to detect a 40% relative reduction in the percentage of children with allergic disease at 6 years of age from 30% to 18% (alpha =0.05, 2-sided). The power remained high (91 or 90%) even with 85% or 80% successful follow-up of children, respectively. It was estimated that 30% of children in the placebo group would have allergies which was conservative given that this follow up study includes children with both single and double familial risk. Similarly, the postulated effect size of a 40% relative reduction in allergic disease was modest and realistic compared with the 67% and 59% relative reductions in child allergy outcomes reported by Furuhjelm et al (113) and Olsen et al (119), respectively, in response to fish oil treatment during pregnancy.

A detailed Statistical Analysis plan was drafted in consultation with steering committee members including statistician, Thomas Sullivan, (Appendix 15). In brief, the planned analysis of the two randomised groups was performed using an intention-to-treat (ITT) approach; participants were analysed according to the treatment they were randomised to receive irrespective of compliance with the protocol. Note the study design was consistent with the ITT principle since women and children were followed up regardless of compliance. The primary outcome of diagnosis of allergic disease (asthma, allergic rhinitis or eczema) with sensitisation at 6 years of age was compared between the treatment and control groups using log binomial regression models. Effects of treatment are described as relative risks with 95% confidence intervals. If any of the models fail to converge, a log Poisson regression model with robust variance estimation was used instead. If the number
and percentage of participants experiencing the binary outcome of interest was considered too small to reliably estimate the relative risk, a Fisher exact test was performed. All analyses were adjusted for the stratification variables of centre and parity as well as the baseline characteristic variables of infant sex and maternal history of allergic disease. In order to address each hypothesis, both unadjusted and adjusted analyses were performed. The adjusted analyses were used to draw conclusions about the effect of treatment, with unadjusted analyses performed for completeness and to confirm the results of the adjusted analyses.

Secondary outcomes

Secondary continuous outcomes were analysed using linear regression models, with treatment effects expressed as mean differences, while secondary binary outcomes were analysed using log binomial regression models, with treatment effects expressed as relative risks. For each outcome variable, statistical significance was assessed at the 0.05 level using a two-sided comparative test of treatment effect. All analyses were performed using SAS® version 9.3 or later, or Stata Release 12 or later.
Missing Data

To address missing outcome data, multiple imputation using chained equations was performed in Stata version 13. Separately for each treatment group, a total of 100 complete datasets were generated under an assumption that the data were missing at random. In addition to analysis model variables, imputation models included auxiliary variables that helped with the prediction of missing values and/or made the missing at random assumption more plausible. Following analysis of the 100 complete datasets, individual treatment effects estimates were combined using Rubin's rules. The primary analysis was based on imputed data and included all families that consented to participate in the allergy follow-up, excluding missing data due to two deaths in the control and one death in the n-3 LCPUFA group. Due to small number of cases, no imputed analysis was completed for egg sensitisation, cashew sensitisation, wheeze with disturbed sleep, anaphylaxis, Epipen, persistent wheeze, persistent wheeze with sensitisation or transient wheeze with sensitisation.
Results of the six year allergy follow up of children at high hereditary risk of atopy born to mothers supplemented with n-3 LCPUFA during pregnancy
Introduction

The primary outcome of the 6 year follow-up of children born to women supplemented with omega-3 LCPUFA during pregnancy, was the incidence of any IgE-mediated allergic disease (eczema, wheeze, rhinitis or rhino-conjunctivitis symptoms) with sensitisation defined as positive SPT of ≥3mm. Secondary outcomes included sensitisation to individual allergen extracts and the incidence and severity of individual allergic disease (eczema, wheeze, rhinitis or rhino-conjunctivitis symptoms) with or without sensitisation. A number of trial quality outcomes were also assessed. All analyses were adjusted for the stratification variables of centre and parity as well as the baseline characteristic variables of child sex and maternal history of allergic disease. In order to address each hypothesis, both unadjusted and adjusted analyses were performed. The following chapter details the results of the pre-planned primary and secondary analyses of the six year allergy follow-up as detailed in the Statistical Analysis Plan (Appendix 15).
**Sample and participant flow**

Pregnant women were enrolled to the DOMInO Trial from antenatal clinics at FMC and WCH during 2005 to 2008 (Chapter Three). Participating women completed an interviewer administered allergy screening questionnaire as part of the DOMInO Trial. Women residing in South Australia with a fetus at high risk of allergic disease (mother, father, sibling with medically diagnosed eczema, asthma or hay fever) were invited to participate in ‘1 & 3 year allergy follow-up’ (n=1080). Enrolment began on 20th March 2006 and ended on 8th May 2008, with a total of 706 infants recruited into the study. In total, 681/706 (96.5%) infants completed their one year allergy assessment and 638/706 (90.4%) children attended an assessment at three years of age.

In 2012, 668 children who consented to the ‘1& 3 year allergy follow-up’ remained eligible for invitation to participate in the six year allergy follow up (n=706 originally consented minus deaths and withdrawals since enrolment). In total 603/668 children (90.3% of available cohort) completed allergy assessments between April 2012 and August 2014, Figure 4-1.
Figure 4-1  Flow diagram of the nested allergy follow-up cohort of the DOMInO trial

Abbreviations; DOMInO=Docosahexaenoic Acid to Optimise Mother Infant Outcome; LCPUFA, long chain polyunsaturated fatty acids
Non completers

Despite varied and consistent efforts to locate all families, 24 were unable to be contacted and were classified as lost to follow up. 40 families that were contacted declined to participate in the six year follow up for a variety of reasons, Figure 4-2.

Figure 4-2 Reasons for refusal to participate in the 6 year allergy follow-up

In total, 605/668 children (90.2% of the eligible cohort) consented to take part in an allergy assessment at 6 years of age (608/706 or 85.7% of original cohort consented prior to birth). Two families withdrew from the study following consent as they were too busy to attend an appointment or complete questionnaires at home. There was no difference in completers vs non completers at 6 years of age. Based on the original cohort consented prior to birth (n=706) 289/338 (85.5%) in the control group vs. 314/368 (85.3%) in the n-3 LCPUFA group completed a six year assessment, Fisher exact $p$-value > 0.99.
Baseline Characteristics

Of the 706 women identified as having a fetus at risk of allergy (due to family history) and consented to the ‘1 & 3 years allergy follow up’ up of their child 493/706 (70%) reported maternal allergic disease and 385 (54%) reported paternal disease. A total of 206/706 (29%) families reported a history of allergic disease in both parents. There were no differences in baseline demographic and clinical characteristics between the intervention (n-3 LCPUFA) and control groups at enrolment to the nested ‘1 & 3 year follow up’, see Table 4-1.
Table 4-1 Baseline demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>n-3 LCPUFA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled - Flinders Medical Centre</td>
<td>134 (36)</td>
<td>122 (36)</td>
</tr>
<tr>
<td>Enrolled - Women’s &amp; Children’s Hospital</td>
<td>234 (64)</td>
<td>216 (63)</td>
</tr>
<tr>
<td>Maternal smoking during pregnancy</td>
<td>47 (13)</td>
<td>45 (13)</td>
</tr>
<tr>
<td>Maternal BMI (kg/m^2): median (IQ range)</td>
<td>26.1 (23-31)</td>
<td>26.5 (23-31)</td>
</tr>
<tr>
<td>Maternal age at trial entry: mean (sd)</td>
<td>29.6 (6)</td>
<td>29.5 (5.6)</td>
</tr>
<tr>
<td>Parity &gt;= 1</td>
<td>218 (59)</td>
<td>207 (61)</td>
</tr>
<tr>
<td>Mother completed secondary education</td>
<td>232 (63)</td>
<td>222 (66)</td>
</tr>
<tr>
<td>Father completed secondary education</td>
<td>196 (53.0)</td>
<td>182 (54)</td>
</tr>
<tr>
<td>Infant sex male</td>
<td>169 (46)</td>
<td>168 (50)</td>
</tr>
<tr>
<td>Maternal History of Allergic Disease</td>
<td>257 (69.8)</td>
<td>236 (69.8)</td>
</tr>
<tr>
<td>Eczema</td>
<td>92 (25.0)</td>
<td>63 (18.6)</td>
</tr>
<tr>
<td>Asthma</td>
<td>156 (42.4)</td>
<td>148 (43.8)</td>
</tr>
<tr>
<td>Allergic Rhinitis</td>
<td>133 (36.1)</td>
<td>121 (35.8)</td>
</tr>
<tr>
<td>Paternal History of Allergic Disease</td>
<td>207 (56.3)</td>
<td>178 (52.7)</td>
</tr>
<tr>
<td>Eczema</td>
<td>53 (14.4)</td>
<td>37 (10.9)</td>
</tr>
<tr>
<td>Asthma</td>
<td>122 (33.2)</td>
<td>86 (25.4)</td>
</tr>
<tr>
<td>Allergic Rhinitis</td>
<td>108 (29.3)</td>
<td>109 (32.2)</td>
</tr>
<tr>
<td>Both Parents with History of Allergic Disease</td>
<td>109 (29.6)</td>
<td>97 (28.7)</td>
</tr>
</tbody>
</table>

Abbreviations: IQ, inter-quartile; sd, standard deviation; LCPUFA, long chain polyunsaturated fatty acids. For n-3 LCPUFA and control groups, data are number of subjects (percentage)
Denominators based on numbers randomised to the 1 & 3 year follow up study
Compliance with the intervention

Participants in both groups of the original allergy follow up cohort were, on the whole, compliant with the intervention. At 28 weeks’ gestation, 284/368 (77%) of mothers in the n-3 LCPUFA group and 270/338 (80%) of mothers in the control group reported that they had missed zero to three capsules a week (from 21 capsules a week). Fewer than 2% of mothers in each group chose not to take any capsules. Concentrations of DHA and EPA in the plasma phospholipids of cord blood from women in the n-3 LCPUFA group were greater than those for the control group (DHA: median 7.5% v 6.2% total phospholipid fatty acids, P<0.001; EPA: median 0.54% v 0.27% total phospholipid fatty acids, P<0.001). The concentration of total n-3 LCPUFA in the cord blood was higher in women in the n-3 LCPUFA group (median 8.8% v 7.2% total phospholipid fatty acids, P<0.001), whereas the concentration of arachidonic acid in cord blood was lower in women in the n-3 LCPUFA group compared with control (mean 14.6% v 16.4%, P<0.001 based on a comparison of means).
SPT Completion

Skin prick testing was completed as a component of the six year allergy assessment in 482/603 children (80%) who completed a six year assessment. Skin prick testing was unable to be completed for 113 children, Figure 4-3. Seven children who attended the clinic were uncooperative and refused the SPT. 46 parents consented for their child to take part in the allergy follow up but refused the SPT component of the assessment. Remaining families were too busy to attend a clinic appointment or were unable to attend due to their regional, interstate or overseas location. All of these families completed the case report form by correspondence i.e. no SPT. Of the children who were consented to the six year allergy follow, there was no difference between the n-3 LCPUFA (250/314, 79.62%) and control (232/289, 80.28%) groups for those who did or did not have a SPT.

Figure 4-3 Reasons for non-completion of skin prick test at the 6 year assessment
Assessment Location

A total of 455 six year assessments were conducted as per original ethics approved protocol at the Child Nutrition Research Centre clinic at the Women’s and Children’s Hospital or Flinders Medical Centre. Following amendment approval by the Women’s and Children’s Health Network Human Research Ethics Committee (Appendix 14), an additional 27 assessments including SPT were completed off-site in metropolitan, regional and interstate clinics, see Table 4-2.

Table 4-2 Locations where 6 year assessments were completed

<table>
<thead>
<tr>
<th>Assessment Location</th>
<th>Number of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women’s &amp; Children’s Hospital</td>
<td>273</td>
</tr>
<tr>
<td>Flinders Medical Centre</td>
<td>182</td>
</tr>
<tr>
<td>Noarlunga SA Health GP Plus</td>
<td>8</td>
</tr>
<tr>
<td>Modbury SA Health GP Plus</td>
<td>4</td>
</tr>
<tr>
<td>Morphett Vale SA Health GP Plus</td>
<td>1</td>
</tr>
<tr>
<td>Ceduna Health Service</td>
<td>3</td>
</tr>
<tr>
<td>Southern Area Health Service, Victor Harbor</td>
<td>2</td>
</tr>
<tr>
<td>Murray Bridge Hospital</td>
<td>1</td>
</tr>
<tr>
<td>Balaklava Hospital</td>
<td>1</td>
</tr>
<tr>
<td>Angaston Hospital</td>
<td>1</td>
</tr>
<tr>
<td>Eudunda Hospital</td>
<td>1</td>
</tr>
<tr>
<td>Strathalbyn Hospital</td>
<td>2</td>
</tr>
<tr>
<td>Port Pirie Health Service</td>
<td>1</td>
</tr>
<tr>
<td>Perth, Western Australia</td>
<td>1</td>
</tr>
<tr>
<td>Melbourne, Victoria</td>
<td>1</td>
</tr>
</tbody>
</table>

SA, South Australia; GP, General Practitioner
Primary Outcome Analysis

There was no difference in the composite primary outcome of incidence of IgE mediated allergic disease symptoms (eczema, wheeze, rhinitis or rhino-conjunctivitis) with sensitisation at six years of age between the n-3 LCPUFA and control group; 116/367 (31.48%) vs 106/336 (31.46%), RR: 1.00 (0.78, 1.25, p=0.99); aRR: 1.04 (0.82, 1.33, p=0.73), Figure 4-4.

![Allergic disease symptoms with sensitisation](image)

*Figure 4-4 Incidence of IgE-mediated allergic disease symptoms with sensitisation defined as positive skin prick test ≥3mm at 6 years*
Sensitivity analysis

To explore the validity of the primary analysis, sensitivity analyses on the primary outcome were performed by imputing only to families that consented to 6 year follow-up and by considering missing not at random mechanisms. When the adjusted odds of allergic disease with sensitisation were allowed to differ between participants with missing data and participants with observed data (i.e. when the missing at random assumption was relaxed), estimated treatment effects varied. Allowing the odds ratio for allergic disease with sensitisation (missing vs. observed) to vary between 0.5 and 2, all treatment effect estimates remained non-significant (confidence intervals all crossed the null value of 1). The most extreme treatment effect estimate was observed when the odds of allergic disease with sensitisation were 2 times higher in participants with missing data in the control group only; but this treatment effect was still not statistically significant, Error!

Reference source not found..
The sensitivity analysis was performed using a logistic regression rather than a log binomial model due to software constraints. Despite this, it seems pretty clear that the null effect is fairly robust to varying (and realistic) assumptions about the missing data.
Secondary Outcomes

Incidence of Eczema Symptoms

The overall prevalence of eczema at six years of age was 15.6%. Of these, 55% of children tested were identified as having atopic eczema, i.e. a positive SPT to one of more of the allergens tested (8.65%). There were no differences between the n-3 LCPUFA and control groups on the incidence of ‘eczema symptoms with sensitisation at six years’, ‘any eczema symptoms at six years’ or ‘parent reported eczema ever’, 36/367 (9.90%) vs 36/336 (10.64%), aRR 0.77, 0.95 (0.59, 1.53), p=0.83; 53/367 (14.53%) vs 58/336 (17.26%), aRR 0.84 (0.58, 1.22), p=0.36; 108/367 (29.42%) vs 115/336 (34.24%), aRR; 0.87 (0.69, 1.10), p=0.25, respectively, see Figure 4-6.

Figure 4-6 Symptoms of eczema with and without sensitisation at 6 years and parent reported eczema ever
Severity of Eczema Symptoms

The severity of eczema symptoms was determined by being ‘kept awake at night’, ‘persistence of symptoms’ (i.e. not clearing within past 12 months) and/or ‘requiring treatment’ in past 12 months. There were no differences between the n-3 LCPUFA and control groups on outcomes of eczema severity, Table 4-3.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent symptoms</td>
<td>26/367 (7.09%)</td>
<td>20/336 (5.98%)</td>
<td>1.19 (0.64, 2.21)</td>
<td>0.60</td>
<td>1.17 (0.63, 2.17)</td>
<td>0.62</td>
</tr>
<tr>
<td>Eczema kept awake at night</td>
<td>23/367 (6.17%)</td>
<td>25/336 (7.35%)</td>
<td>0.84 (0.47, 1.51)</td>
<td>0.56</td>
<td>0.84 (0.46, 1.50)</td>
<td>0.55</td>
</tr>
<tr>
<td>Eczema requiring treatment</td>
<td>53/367 (14.42%)</td>
<td>52/336 (15.43%)</td>
<td>0.93 (0.64, 1.37)</td>
<td>0.73</td>
<td>0.94 (0.64, 1.38)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; aRR, adjusted relative risk.
For n-3 LCPUFA and control groups, data are number of subjects (percentage).
*All data are based on analysis of 100 imputed datasets.
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease.
Wheeze

Current wheeze was defined as symptoms of ‘wheezing’ or ‘whistling’ in the chest within the past 12 months. The incidence of ‘current wheeze with sensitisation’ at 6 years was 12.7% and ‘any current wheeze’ (i.e. with or without sensitisation) was 25.2%. There was no difference between the n-3 LCPUFA and control groups on the incidence of ‘current wheeze with sensitisation’ or ‘any current wheeze’, 60/367 (16.36%) vs 45/336 (13.53%), aRR 1.24 (0.83, 1.85), p=0.30; 98/367 (26.63%) vs 82/336 (24.50%), aRR 1.08 (0.82, 1.42), p=0.58 respectively. The overall prevalence of ‘parent reported asthma ever’ was 21.3% and there was no difference in incidence between the n-3 LCPUFA and control group on this outcome, 79/367 (21.41%) vs 73/336 (21.74%), aRR; 1.01 (0.75, 1.37), p=0.92, Figure 4-7.

Figure 4-7 Symptoms of wheeze with and without sensitisation at 6 years and parent reported asthma ever
Severity of wheeze and asthma

The severity of wheeze symptoms were assessed by the frequency of wheeze attacks, the severity of episodes (i.e. unable to speak) and presence of night waking. There were no differences between the n-3 LCPUFA and control group on measures of severity of wheeze symptoms in the past 12 months, Table 4-4.

Severity of asthma symptoms as reported by the parent were assessed according to interruption in the child’s school or sporting activities and medication use and frequency of use. There were no differences between the n-3 LCPUFA and control group on measures of severity of asthma, Table 4-4.
### Table 4-4 Severity of wheeze and parent reported asthma at 6 years

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current wheeze with frequent attacks</td>
<td>27/367 (7.30%)</td>
<td>26/336 (7.82%)</td>
<td>0.93 (0.53, 1.65)</td>
<td>0.82</td>
<td>0.93 (0.53, 1.65)</td>
<td>0.79</td>
</tr>
<tr>
<td>Wheeze with disturbed sleep**</td>
<td>7/314 (2.23%)</td>
<td>11/288 (3.82%)</td>
<td>-</td>
<td>-</td>
<td>0.58 (0.23, 1.59)</td>
<td>0.26</td>
</tr>
<tr>
<td>Wheeze limiting speech</td>
<td>13/367 (3.57%)</td>
<td>12/336 (3.58%)</td>
<td>0.90 (0.43, 2.33)</td>
<td>0.10</td>
<td>1.00 (0.43, 2.33)</td>
<td>0.10</td>
</tr>
<tr>
<td>Exercise wheeze</td>
<td>32/367 (8.76%)</td>
<td>29/336 (8.51%)</td>
<td>1.03 (0.61, 1.74)</td>
<td>0.91</td>
<td>1.02 (0.61, 1.74)</td>
<td>0.94</td>
</tr>
<tr>
<td>Night cough</td>
<td>69/367 (18.91%)</td>
<td>63/336 (18.69%)</td>
<td>1.01 (0.72, 1.41)</td>
<td>0.94</td>
<td>1.01 (0.73, 1.41)</td>
<td>0.93</td>
</tr>
<tr>
<td>Ever had asthma and current asthma</td>
<td>56/367 (15.17%)</td>
<td>54/336 (16.13%)</td>
<td>0.94 (0.65, 1.37)</td>
<td>0.75</td>
<td>0.97 (0.67, 1.41)</td>
<td>0.86</td>
</tr>
<tr>
<td>Asthma affected activities at school</td>
<td>35/367 (9.66%)</td>
<td>31/336 (9.24%)</td>
<td>1.05 (0.63, 1.73)</td>
<td>0.86</td>
<td>1.07 (0.65, 1.74)</td>
<td>0.79</td>
</tr>
<tr>
<td>Asthma affected sporting activities</td>
<td>29/367 (8.00%)</td>
<td>32/336 (9.59%)</td>
<td>0.83 (0.50, 1.40)</td>
<td>0.50</td>
<td>0.85 (0.51, 1.41)</td>
<td>0.54</td>
</tr>
<tr>
<td>Salbutamol for asthma</td>
<td>69/367 (18.75%)</td>
<td>59/336 (17.49%)</td>
<td>1.07 (0.76, 1.43)</td>
<td>0.70</td>
<td>1.10 (0.78, 1.57)</td>
<td>0.60</td>
</tr>
<tr>
<td>Inhaled preventer for asthma</td>
<td>25/367 (6.90%)</td>
<td>33/336 (9.83%)</td>
<td>0.70 (0.41, 1.20)</td>
<td>0.20</td>
<td>0.70 (0.41, 1.21)</td>
<td>0.19</td>
</tr>
<tr>
<td>Oral steroids for asthma</td>
<td>22/367 (6.04%)</td>
<td>25/336 (7.40%)</td>
<td>0.82 (0.45, 1.49)</td>
<td>0.51</td>
<td>0.82 (0.45, 1.49)</td>
<td>0.52</td>
</tr>
<tr>
<td>Currently used asthma medication</td>
<td>41/367 (11.12%)</td>
<td>38/336 (11.30%)</td>
<td>0.98 (0.62, 1.55)</td>
<td>0.95</td>
<td>1.00 (0.63, 1.57)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; aRR, adjusted relative risk

*All data are based on analysis of 100 imputed datasets unless otherwise indicated. For n-3 LCPUFA and control groups, data are number of subjects (percentage)

†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease

**Adjusted analyses not done owing to rarity of outcomes
Categories of wheeze

Due to the heterogeneous nature of asthma and wheeze, we sought to categorise wheeze symptoms to specific phenotypes (54, 55). ‘Transient wheeze’ was defined as wheeze symptoms at one and/or three years but no wheeze at six years of age. ‘Late onset wheeze’ was defined as no symptoms of wheeze at one or three years but wheeze at six years and ‘Persistent wheeze’ included children who had wheeze symptoms at one and/or three and also had wheeze at six years. There was no difference between the n-3 LCPUFA and control group on any of the described wheezing phenotypes, see Table 4-5.
### Table 4-5 Categories of wheezing phenotypes with and without sensitisation

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient wheeze with sensitisation**</td>
<td>2/342 (0.58%)</td>
<td>3/318 (0.94%)</td>
<td>0.62 (0.10, 3.69)</td>
<td>0.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Late onset wheeze with sensitisation</td>
<td>53/367 (14.33%)</td>
<td>39/336 (11.64%)</td>
<td>1.23 (0.79, 1.92)</td>
<td>0.35</td>
<td>1.25 (0.80, 1.95)</td>
<td>0.32</td>
</tr>
<tr>
<td>Persistent wheeze with sensitisation**</td>
<td>3/347 (0.86%)</td>
<td>2/322 (0.62%)</td>
<td>1.39 (0.23, 8.28)</td>
<td>0.72</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Transient wheeze</td>
<td>21/367 (5.81%)</td>
<td>21/336 (6.23%)</td>
<td>0.93 (0.51, 1.71)</td>
<td>0.82</td>
<td>0.94 (0.51, 1.72)</td>
<td>0.84</td>
</tr>
<tr>
<td>Late onset wheeze</td>
<td>88/367 (23.20%)</td>
<td>72/336 (20.34%)</td>
<td>1.11 (0.82, 1.50)</td>
<td>0.49</td>
<td>1.10 (0.82, 1.48)</td>
<td>0.53</td>
</tr>
<tr>
<td>Persistent wheeze**</td>
<td>8/346 (2.31%)</td>
<td>9/320 (2.81%)</td>
<td>0.82 (0.32, 2.10)</td>
<td>0.68</td>
<td>0.86 (0.34, 2.18)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; aRR, adjusted relative risk
*Data are based on analysis of 100 imputed datasets unless otherwise indicated. For n-3 LCPUFA and control groups, data are number of subjects (percentage)
**Adjusted analyses not done owing to rarity of outcomes
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Rhinitis & Rhino-conjunctivitis

The prevalence of rhinitis and rhino-conjunctivitis symptoms with sensitisation to one or more aero-allergens at six years of age was 39% and 22% respectively. There was no difference in symptoms of ‘rhinitis’ or ‘rhino-conjunctivitis’ with sensitisation between the n-3 LCPUFA and control group 75/367 (20.39%) vs 72/336 (21.29%), aRR; 0.98 (0.72, 1.35), p=0.92, 47/367 (12.72%) vs 39/336 (11.49%), aRR; 1.12 (0.72, 1.73), p=0.61 respectively. There was a suggestion of a reduction in the incidence of parent reported ‘hayfever ever’ in the n-3 LCPUFA group, 81/367 (22.05%) vs 98/336 (29.05%) aRR 0.77 (0.59, 1.01), p=0.055 see Figure 4-8.

![Figure 4-8 Symptoms of rhinitis and rhino-conjunctivitis with and without sensitisation at 6 years and parent reported hayfever ever](image-url)
Severity of Rhinitis/Rhino-conjunctivitis

The severity of rhinitis or rhino-conjunctivitis as determined by symptoms interfering with daily activity or requiring treatment did not differ between the groups, see Table 4-6.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms interfere with daily activities</td>
<td>34/367 (9.26%)</td>
<td>29/336 (8.75%)</td>
<td>1.06 (0.64, 1.75)</td>
<td>0.83</td>
<td>1.07 (0.65, 1.77)</td>
<td>0.80</td>
</tr>
<tr>
<td>Hayfever required treatment last 12 months</td>
<td>60/367 (16.25%)</td>
<td>71/336 (21.23%)</td>
<td>0.77 (0.55, 1.07)</td>
<td>0.12</td>
<td>0.77 (0.56, 1.08)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; aRR, adjusted relative risk
*Data are based on analysis of 100 imputed datasets unless otherwise indicated. For n-3 LCPUFA and control groups, data are number of subjects (percentage)
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Sensitisation

An allergen extract panel consisting of three common food and seven common inhalant allergens was used to skin prick test the child to determine sensitisation, Figure 3-4. Imputed analysis of sensitisation to individual allergen extracts showed a statistically significant reduction in the incidence of sensitisation to one HDM species, (*D. farinae*) in the n-3 LCPUFA group, 49/367 (13.42%) vs 68/336 (20.30%), aRR 0.67 (0.44, 1.00), *p* = 0.049, Figure 4-9.

![Graph showing sensitisation at 6 years of age](image)

*Figure 4-9 Sensitisation to individual allergen extracts at 6 years of age*

There was also 43% reduction in peanut sensitisation and a 48% reduction in cashew sensitisation at six years of age in the n-3 LCPUFA group although these results did not reach statistical significance due to small numbers. Sensitisation to other individual allergen extracts or ‘any sensitisation’ (sensitisation to any allergen extract) showed no statistically significant difference between the n-3 LCPUFA and control group, Table 4-7.
Table 4-7 Sensitisation to individual allergen extracts at 6 years of age

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>aRR (95% CI)†</th>
<th>P-value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any sensitisation</td>
<td>185/367 (50.49%)</td>
<td>163/336 (48.61%)</td>
<td>1.04 (0.87, 1.24)</td>
<td>0.67</td>
<td>1.04 (0.90, 1.28)</td>
<td>0.43</td>
</tr>
<tr>
<td>Egg sensitisation**</td>
<td>6/248 (2.42%)</td>
<td>5/232 (2.16%)</td>
<td>-</td>
<td>0.85</td>
<td>1.18 (0.37, 3.78)</td>
<td>0.79</td>
</tr>
<tr>
<td>Peanut sensitisation</td>
<td>27/367 (7.23%)</td>
<td>39/336 (11.68%)</td>
<td>0.62 (0.35, 1.09)</td>
<td>0.10</td>
<td>0.64 (0.36, 1.13)</td>
<td>0.13</td>
</tr>
<tr>
<td>Cashew sensitisation**</td>
<td>9/249 (3.61%)</td>
<td>16/231 (6.93%)</td>
<td>-</td>
<td>0.11</td>
<td>0.55 (0.25, 1.21)</td>
<td>0.14</td>
</tr>
<tr>
<td>Ryegrass sensitisation</td>
<td>97/367 (26.29%)</td>
<td>87/336 (25.89%)</td>
<td>1.02 (0.76, 1.36)</td>
<td>0.92</td>
<td>1.04 (0.78, 1.39)</td>
<td>0.78</td>
</tr>
<tr>
<td>Olive sensitisation</td>
<td>55/367 (14.89%)</td>
<td>41/336 (12.15%)</td>
<td>1.23 (0.77, 1.96)</td>
<td>0.39</td>
<td>1.27 (0.79, 2.03)</td>
<td>0.38</td>
</tr>
<tr>
<td><em>D. Pteronyssinus</em></td>
<td>75/367 (20.41%)</td>
<td>83/336 (24.68%)</td>
<td>0.83 (0.59, 1.15)</td>
<td>0.26</td>
<td>0.84 (0.60, 1.17)</td>
<td>0.30</td>
</tr>
<tr>
<td>D.Farinae sensitisation</td>
<td>49/367 (13.42%)</td>
<td>68/336 (20.30%)</td>
<td>0.66 (0.44,0.99)</td>
<td>0.046</td>
<td>0.67 (0.44, 1.00)</td>
<td>0.049</td>
</tr>
<tr>
<td>Cat sensitisation</td>
<td>46/367 (12.47%)</td>
<td>40/336 (11.95%)</td>
<td>1.04 (0.66,1.64)</td>
<td>0.85</td>
<td>1.07 (0.68, 1.68)</td>
<td>0.77</td>
</tr>
<tr>
<td>Alternaria sensitisation</td>
<td>68/367 (18.56%)</td>
<td>62/336 (18.37%)</td>
<td>1.01 (0.69, 1.47)</td>
<td>0.96</td>
<td>1.03 (0.71, 1.49)</td>
<td>0.88</td>
</tr>
<tr>
<td>Dog sensitisation</td>
<td>45/367 (12.32%)</td>
<td>40/336 (12.03%)</td>
<td>1.02 (0.63, 1.66)</td>
<td>0.92</td>
<td>1.07 (0.67, 1.72)</td>
<td>0.78</td>
</tr>
</tbody>
</table>
| Sensitisation no symptoms    | 72/367 (19.55%) | 56/336 (16.60%) | 1.18 (0.82, 1.69) | 0.37    | 1.17 (0.82, 1.68) | 0.39     

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; ARR, adjusted relative risk

*Data are based on analysis of 100 imputed datasets unless otherwise indicated. For n-3 LCPUFA and control groups, data are number of subjects (percentage)

**Adjusted analyses not done owing to rarity of outcomes

†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Trial Quality Outcomes

Skin Prick Test

Data was collected on skin prick test (SPT) completion in accordance with defined procedures and protocol. There was no difference between the n-3 LCPUFA and control group in the number of SPT’s that were completed per-protocol or the number of children that did not undergo SPT, see Table 4-8.
### Table 4-8 Six year follow up study quality outcomes – SPT Completion

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>ARR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed SPT</td>
<td>250/314 (79.62%)</td>
<td>232/289 (80.28%)</td>
<td>0.99 (0.92, 1.07)</td>
<td>0.84</td>
<td>0.99 (0.91, 1.07)</td>
<td>0.80</td>
</tr>
<tr>
<td>SPT completed per protocol**</td>
<td>246/250 (98.40%)</td>
<td>230/232 (99.14%)</td>
<td>0.99 (0.97, 1.01)</td>
<td>0.46</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; ARR, adjusted relative risk.
*For n-3 LCPUFA and control groups, data are number of subjects (percentage)
**Adjusted analyses not done owing to rarity of outcomes
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Child Health Questionnaire

The Child Health Questionnaire (CHQ) measures 14 unique physical and psychosocial concepts and was used to determine global health of the children. Scale scores were combined to derive two component summary scores, an overall physical and psychosocial score. There was no difference between the n-3 LCPUFA and control group in the CHQ physical score although there was an unexpected significant difference in favour of the control group on the psychosocial score, Table 4-9 Child Health Questionnaire Table 4-9. Mean summary scores of both the intervention and control groups were however, within normal limits and this difference between the groups was not considered clinically significant. Following review of the data this finding can be further explained by an overweighting of physically and/or mentally challenged children in the intervention group (3 vs 0) unrelated to the intervention. A score of 50 represents the mean in the general US population; scores above/below 50 are above/below the average in the US reference population. Research of the comparison reliability, validity, structure and norms of the CHQ in an Australian population suggest caution when using summary scores in a general population and suggest they may only be relevant for children with more severe illness than the general population (159).
Table 4-9 Child Health Questionnaire

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA mean (SD)</th>
<th>Control mean (SD)</th>
<th>Adjusted mean difference (95% CI) †</th>
<th>P-value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHQ physical score</td>
<td>55.17 (7.63)</td>
<td>55.91 (5.56)</td>
<td>-0.77 (-1.90, 0.36)</td>
<td>0.18</td>
</tr>
<tr>
<td>CHQ psychosocial score</td>
<td>49.99 (9.51)</td>
<td>51.83 (7.92)</td>
<td>-1.84 (-3.32, -0.36)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Abbreviations: CHQ, Child Health Questionnaire; SD, standard deviation; n-3 LCPUFA, long chain polyunsaturated fatty acid; † Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Safety Outcomes

Hospitalisation/Serious Adverse Events

This is a follow-up study with no active intervention nevertheless, all hospital admissions were documented as possible adverse events. There was no difference in hospitalisations within last 12 months between the n-3 LCPUFA and control groups 20/314 (6.37%) vs 20/288 (6.94%), ARR 0.92 (0.51, 1.68), p=0.79. There were no serious adverse events (admission to intensive care, anaphylaxis or death within the past 12 months) in this study.
Quality Outcomes

Blinding

The intervention phase of the DOMInO trial was completed in 2007 and since this time a number of women have elected to find out which arm of the trial they participated in. An un-blinding protocol is in place whereas women in this cohort can obtain this information independently of staff involved in any continuing research or outcome assessments. There were significantly more women in the control group who had elected to be un-blinded prior to the six year assessment, see Table 4-10. Whilst this outcome is unexpected and there is a potential for families to modify care of the child once un-blinded, post randomisation characteristics at six years were comparable between the groups. There were no differences in any of the child’s dietary measures assessed at six years including intake of DHA in the diet or by supplementation, it is therefore unlikely that un-blinding affected the environment of the child.
### Table 4-10 Six year follow up study quality outcomes - Blinding

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants un-blinded (prior to 6 year assessment)</td>
<td>31/314 (9.87%)</td>
<td>44/289 (15.22%)</td>
<td>0.65 (0.42, 1.00)</td>
<td>0.05</td>
<td>0.63 (0.41, 0.96)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; ARR, adjusted relative risk

*For n-3 LCPUFA and control groups, data are number of subjects (percentage)

†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease

Analysis performed on raw data, i.e. no imputed analysis
Post Randomisation Characteristics

Socio-demographic Characteristics

Assessment of socio-economic status was performed using a number of methods including years of schooling and completion of further education and occupation of the primary and secondary carer of the child. Occupation was classified according to the Australian & New Zealand Coding of Occupations (ANZCO) (154). Classification definitions are based on the skill level and specialisation usually necessary to perform the tasks of the specific occupation, with lower numbers representing more highly skilled occupations. The postcode where the child resided the majority of the time was also used and classified according to the Socio-Economic Indexes for Areas (SEIFA). SEIFA is a product developed by the Australian Bureau of Statistics (ABS) that ranks areas in Australia according to relative socio-economic advantage and disadvantage (10). The indexes are based on information from the five-yearly Census with a low score indicating relatively greater disadvantage in general. The intervention and control groups were comparable for all measures of socio-economic status, see Table 4-11.
### Table 4-11 Socio-economic status, carer education and occupation

<table>
<thead>
<tr>
<th>Post Randomisation Characteristic</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEIFA relative advantage and disadvantage quintile:</td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>. 1 (greater disadvantage)</td>
<td>51 (15.0)</td>
<td>50 (15.8)</td>
<td>.</td>
</tr>
<tr>
<td>. 2</td>
<td>97 (28.5)</td>
<td>93 (29.3)</td>
<td>.</td>
</tr>
<tr>
<td>. 3</td>
<td>71 (20.9)</td>
<td>59 (18.6)</td>
<td>.</td>
</tr>
<tr>
<td>. 4</td>
<td>69 (20.3)</td>
<td>57 (18.0)</td>
<td>.</td>
</tr>
<tr>
<td>. 5</td>
<td>52 (15.3)</td>
<td>58 (18.3)</td>
<td>.</td>
</tr>
<tr>
<td>Primary carer occupation:</td>
<td></td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>. ANZSCO 1-2 (highly skilled)</td>
<td>99 (31.6)</td>
<td>80 (27.8)</td>
<td>.</td>
</tr>
<tr>
<td>. ANZSCO 3-8</td>
<td>128 (40.9)</td>
<td>125 (43.4)</td>
<td>.</td>
</tr>
<tr>
<td>. Other</td>
<td>86 (27.5)</td>
<td>83 (28.8)</td>
<td>.</td>
</tr>
<tr>
<td>Secondary carer occupation:</td>
<td></td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>. ANZSCO 1-2 (highly skilled)</td>
<td>113 (41.1)</td>
<td>94 (37.2)</td>
<td>.</td>
</tr>
<tr>
<td>. ANZSCO 3-8</td>
<td>150 (54.5)</td>
<td>146 (57.7)</td>
<td>.</td>
</tr>
<tr>
<td>. Other</td>
<td>12 ( 4.4)</td>
<td>13 ( 5.1)</td>
<td>.</td>
</tr>
<tr>
<td>Primary carer completed secondary school</td>
<td></td>
<td></td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>221 (70.4)</td>
<td>214 (74.3)</td>
<td></td>
</tr>
<tr>
<td>Secondary carer completed secondary school</td>
<td></td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>175 (63.2)</td>
<td>161 (63.6)</td>
<td></td>
</tr>
<tr>
<td>Primary carer further education:</td>
<td></td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>Certificate/diploma</td>
<td>133 (42.4)</td>
<td>130 (45.6)</td>
<td>.</td>
</tr>
<tr>
<td>Degree</td>
<td>74 (23.6)</td>
<td>67 (23.5)</td>
<td>.</td>
</tr>
<tr>
<td>Higher Degree</td>
<td>29 ( 9.2)</td>
<td>20 ( 7.0)</td>
<td>.</td>
</tr>
<tr>
<td>No further study</td>
<td>78 (24.8)</td>
<td>68 (23.9)</td>
<td>.</td>
</tr>
<tr>
<td>Secondary carer further education:</td>
<td></td>
<td></td>
<td>0.61</td>
</tr>
<tr>
<td>Certificate/diploma</td>
<td>135 (49.3)</td>
<td>132 (52.6)</td>
<td>.</td>
</tr>
<tr>
<td>Degree</td>
<td>58 (21.2)</td>
<td>44 (17.5)</td>
<td>.</td>
</tr>
<tr>
<td>Higher Degree</td>
<td>17 ( 6.2)</td>
<td>12 ( 4.8)</td>
<td>.</td>
</tr>
<tr>
<td>No further study</td>
<td>64 (23.4)</td>
<td>63 (25.1)</td>
<td>.</td>
</tr>
</tbody>
</table>

*For n-3 LCPUFA and control groups, data are number of subjects (percentage)

Abbreviation; ANZCO, Australian and New Zealand Standard Classification of Occupations; SEIFA, Socio-Economic Indexes for Areas; n-3 LCPUFA, long chain polyunsaturated fatty acid
Environmental Characteristics

A number of environmental factors were considered regarding the child’s living conditions, including the number of people in the home, schooling and the child’s exposure to fuel (gas), pollutants (smoking), animals (farm animals, pets) and to common aero-allergens in the home (i.e. dust mite protection). There was no difference between the n-3 LCPUFA and control group in any environmental characteristics of the child at 6 years, see Table 4-12.
Table 4-12 Environment Characteristics at 6 years

<table>
<thead>
<tr>
<th>Post Randomisation Characteristic</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of adults in home:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>44 (14.0)</td>
<td>47 (16.3)</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>232 (73.9)</td>
<td>211 (73.0)</td>
<td>.</td>
</tr>
<tr>
<td>3+</td>
<td>38 (12.1)</td>
<td>31 (10.7)</td>
<td>.</td>
</tr>
<tr>
<td>Number other children in home:</td>
<td></td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>0</td>
<td>44 (14.0)</td>
<td>36 (12.5)</td>
<td>.</td>
</tr>
<tr>
<td>1</td>
<td>158 (50.3)</td>
<td>130 (45.1)</td>
<td>.</td>
</tr>
<tr>
<td>2</td>
<td>66 (21.0)</td>
<td>77 (26.7)</td>
<td>.</td>
</tr>
<tr>
<td>3+</td>
<td>46 (14.6)</td>
<td>45 (15.6)</td>
<td>.</td>
</tr>
<tr>
<td>Position of child in family:</td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>1</td>
<td>127 (40.4)</td>
<td>114 (39.7)</td>
<td>.</td>
</tr>
<tr>
<td>2</td>
<td>94 (29.9)</td>
<td>103 (35.9)</td>
<td>.</td>
</tr>
<tr>
<td>3+</td>
<td>93 (29.6)</td>
<td>70 (24.4)</td>
<td>.</td>
</tr>
<tr>
<td>Smoker in household</td>
<td>97 (30.9)</td>
<td>78 (27.1)</td>
<td>0.30</td>
</tr>
<tr>
<td>Cat as pet</td>
<td>105 (33.4)</td>
<td>100 (34.6)</td>
<td>0.76</td>
</tr>
<tr>
<td>Dog as pet</td>
<td>155 (49.4)</td>
<td>162 (56.1)</td>
<td>0.10</td>
</tr>
<tr>
<td>Exposure to farm animals</td>
<td>25 ( 8.0)</td>
<td>31 (10.7)</td>
<td>0.24</td>
</tr>
<tr>
<td>Gas fuel used in home</td>
<td>245 (78.0)</td>
<td>211 (73.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Dust mite protector mattress</td>
<td>61 (19.4)</td>
<td>65 (22.5)</td>
<td>0.36</td>
</tr>
<tr>
<td>Dust mite protector pillow</td>
<td>58 (18.5)</td>
<td>65 (22.5)</td>
<td>0.32</td>
</tr>
<tr>
<td>Year commenced schooling:</td>
<td></td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>2010</td>
<td>1 ( 0.3)</td>
<td>1 ( 0.3)</td>
<td>.</td>
</tr>
<tr>
<td>2011</td>
<td>74 (23.6)</td>
<td>64 (22.1)</td>
<td>.</td>
</tr>
<tr>
<td>2012</td>
<td>114 (36.3)</td>
<td>108 (37.4)</td>
<td>.</td>
</tr>
<tr>
<td>2013</td>
<td>120 (38.2)</td>
<td>113 (39.1)</td>
<td>.</td>
</tr>
<tr>
<td>2014</td>
<td>3 ( 1.0)</td>
<td>2 ( 0.7)</td>
<td>.</td>
</tr>
<tr>
<td>Not commenced</td>
<td>2 ( 0.6)</td>
<td>1 ( 0.3)</td>
<td>.</td>
</tr>
</tbody>
</table>

Abbreviations: n-3 LCPUFA, long chain polyunsaturated fatty acid
*For n-3 LCPUFA and control groups, data are number of subjects (percentage)
DHA Intake

The child’s dietary intake of DHA was assessed as fish meals consumed within the last month (one serve being 60-80gms of fish) and the number of DHA enriched foods consumed within the last month. Dietary intake of DHA was assessed over the previous month as an indication of usual intake. There was no difference in current DHA intake via fish meals or supplement use between the n-3 LCPUFA and control group at 6 years of age, Table 4-13.

**Table 4-13 Child’s Current DHA Intake at 6 years**

<table>
<thead>
<tr>
<th>Post Randomisation Characteristic</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>P-valuei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any fish meals within last month</td>
<td>284 (91.0)</td>
<td>250 (87.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>Number of fish meals in last month: median (IQ range)</td>
<td>4.0 (2.0-5.0)</td>
<td>4.0 (2.0-6.0)</td>
<td>0.53¥</td>
</tr>
<tr>
<td>Any fortified foods within last month</td>
<td>62.0 (24.5)</td>
<td>62.0 (25.9)</td>
<td>0.71</td>
</tr>
<tr>
<td>Number of fortified foods in last month: median (IQ range)</td>
<td>20.0 (10.0-30.0)</td>
<td>30.0 (12.0-30.0)</td>
<td>0.11¥</td>
</tr>
<tr>
<td>DHA intake via supplements</td>
<td>99.0 (31.6)</td>
<td>89.0 (30.9)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Abbreviations: IQ, Inter-quartile; n-3 LCPUFA, long chain polyunsaturated fatty acid; DHA, docosahexaenoic acid

*For n-3 LCPUFA and control groups, data are number of subjects (percentage)

iContinuous characteristics compared using independent samples t-tests, categorical characteristics compared using chi-square tests

¥Compared using Wilcoxon test
Dietary characteristics

Dietary characteristics of the child at 6 years were assessed by questioning frequency of intake of common foods as recommended by supplementary ISAAC environmental questions. Parents were questioned regarding frequency of intake of foods, on average, in the last 12 months as an indication of usual intake. There were no differences in dietary characteristics between the n-3 LCP UFA and control groups, see Table 4-14.
Table 4-14 Child Dietary Characteristics at 6 years

<table>
<thead>
<tr>
<th>Post Randomisation Characteristic</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>P-Value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>10 (3.2)</td>
<td>3 (1.1)</td>
<td>0.19</td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>41 (13.3)</td>
<td>41 (14.7)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>257 (83.4)</td>
<td>235 (84.2)</td>
<td></td>
</tr>
<tr>
<td>Fruit:</td>
<td></td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>6 (1.9)</td>
<td>6 (2.2)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>4 (1.3)</td>
<td>5 (1.8)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>298 (96.8)</td>
<td>268 (96.1)</td>
<td></td>
</tr>
<tr>
<td>Vegetables:</td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>16 (5.2)</td>
<td>9 (3.2)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>26 (8.4)</td>
<td>25 (9.0)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>266 (86.4)</td>
<td>245 (87.8)</td>
<td></td>
</tr>
<tr>
<td>Pulses:</td>
<td></td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>99 (32.2)</td>
<td>91 (32.7)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>131 (42.7)</td>
<td>119 (42.8)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>77 (25.1)</td>
<td>68 (24.5)</td>
<td></td>
</tr>
<tr>
<td>Cereal:</td>
<td></td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>3 (1.0)</td>
<td>2 (0.7)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>14 (4.5)</td>
<td>10 (3.6)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>291 (94.5)</td>
<td>267 (95.7)</td>
<td></td>
</tr>
<tr>
<td>Pasta:</td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>16 (5.2)</td>
<td>6 (2.2)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>202 (65.6)</td>
<td>176 (63.1)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>90 (29.2)</td>
<td>97 (34.8)</td>
<td></td>
</tr>
<tr>
<td>Rice:</td>
<td></td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>51 (16.6)</td>
<td>37 (13.3)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>198 (64.3)</td>
<td>182 (65.2)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>59 (19.2)</td>
<td>60 (21.5)</td>
<td></td>
</tr>
<tr>
<td>Butter:</td>
<td></td>
<td></td>
<td>0.64</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>142 (46.3)</td>
<td>138 (49.6)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>44 (14.3)</td>
<td>34 (12.2)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>121 (39.4)</td>
<td>106 (38.1)</td>
<td></td>
</tr>
</tbody>
</table>
### Post Randomisation Characteristic

<table>
<thead>
<tr>
<th>Post Randomisation Characteristic</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>P-Value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margarine:</td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>113 (36.9)</td>
<td>84 (30.2)</td>
<td>.</td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>32 (10.5)</td>
<td>36 (12.9)</td>
<td>.</td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>161 (52.6)</td>
<td>158 (56.8)</td>
<td>.</td>
</tr>
<tr>
<td>Nuts:</td>
<td></td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>165 (53.6)</td>
<td>166 (59.5)</td>
<td>.</td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>111 (36.0)</td>
<td>89 (31.9)</td>
<td>.</td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>32 (10.4)</td>
<td>24 ( 8.6)</td>
<td>.</td>
</tr>
<tr>
<td>Potatoes:</td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>34 (11.0)</td>
<td>22 ( 7.9)</td>
<td>.</td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>160 (51.9)</td>
<td>147 (52.7)</td>
<td>.</td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>114 (37.0)</td>
<td>110 (39.4)</td>
<td>.</td>
</tr>
<tr>
<td>Milk:</td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>13 ( 4.2)</td>
<td>7 ( 2.5)</td>
<td>.</td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>22 ( 7.1)</td>
<td>13 ( 4.7)</td>
<td>.</td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>273 (88.6)</td>
<td>259 (92.8)</td>
<td>.</td>
</tr>
<tr>
<td>Eggs:</td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>63 (20.5)</td>
<td>70 (25.3)</td>
<td>.</td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>159 (51.6)</td>
<td>140 (50.5)</td>
<td>.</td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>86 (27.9)</td>
<td>67 (24.2)</td>
<td>.</td>
</tr>
<tr>
<td>Fast food:</td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>156 (50.6)</td>
<td>122 (43.7)</td>
<td>.</td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>149 (48.4)</td>
<td>153 (54.8)</td>
<td>.</td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>3 ( 1.0)</td>
<td>4 ( 1.4)</td>
<td>.</td>
</tr>
</tbody>
</table>

Abbreviations: IQ, Inter-quartile; n-3 LCPUFA, long chain polyunsaturated fatty acid; DHA, docosahexaenoic acid

*For n-3 LCPUFA and control groups, data are number of subjects (percentage)

¹Continuous characteristics compared using independent samples t-tests, categorical characteristics compared using chi-square tests
Anthropometrics & Activity

The amount of time the child spent engaging in physical or sedentary activity was collected in addition to anthropometric measures of height and weight, BMI was calculated post hoc. There was no difference between the n-3 LCPUFA and control group in anthropometric measures or physical activity levels, Table 4-15.

*Table 4-15 Anthropometrics and Physical Activity*

<table>
<thead>
<tr>
<th>Post Randomisation Characteristic</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>P-Value(^i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child height (cm): mean (sd)</td>
<td>117.9 (5.3)</td>
<td>118.2 (5.4)</td>
<td>0.55</td>
</tr>
<tr>
<td>Child weight (kg): mean (sd)</td>
<td>22.5 (4.1)</td>
<td>22.7 (3.9)</td>
<td>0.67</td>
</tr>
<tr>
<td>Child BMI (kg/m(^2)): median (IQ range)</td>
<td>15.8 (14.9-16.9)</td>
<td>15.9 (14.9-17.2)</td>
<td>0.56(^¥)</td>
</tr>
<tr>
<td>Times per week engaging in physical activity:</td>
<td></td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>. Never or occasionally</td>
<td>9 (2.9)</td>
<td>5 (1.7)</td>
<td></td>
</tr>
<tr>
<td>. Once or twice per week</td>
<td>43 (13.7)</td>
<td>38 (13.2)</td>
<td></td>
</tr>
<tr>
<td>. Three or more times per week</td>
<td>262 (83.4)</td>
<td>245 (85.1)</td>
<td></td>
</tr>
<tr>
<td>Hours per day watching television:</td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>. &lt;= 1 hour</td>
<td>92 (29.3)</td>
<td>72 (25.0)</td>
<td></td>
</tr>
<tr>
<td>. &gt; 1 - &lt; 3 hours</td>
<td>189 (60.2)</td>
<td>185 (64.2)</td>
<td></td>
</tr>
<tr>
<td>. &gt;= 3 - &lt; 5 hours</td>
<td>24 (7.6)</td>
<td>28 (9.7)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; n-3 LCPUFA, long chain polyunsaturated fatty acid; IQR, inter quartile range; SD, standard deviation.
*For n-3 LCPUFA and control groups, data are number of subjects (percentage)
\(^i\)Continuous characteristics compared using independent samples t-tests, categorical characteristics compared using chi-square tests
\(^¥\)Compared using Wilcoxon test
Chapter Four

Other Child Characteristics

Age of administration and frequency of use was collected for paracetamol and ibuprofen. There was no difference between the n-3 LCPUFA or control group in age of the child at first dose or frequency of use of both paracetamol and ibuprofen within the past 12 months, see Table 4-16.

**Table 4-16 Paracetamol and Ibuprofen use at 6 years**

<table>
<thead>
<tr>
<th>Post Randomisation Characteristic</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>P-Valuei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol ever:</td>
<td>310 (98.7)</td>
<td>284 (99.0)</td>
<td>0.79</td>
</tr>
<tr>
<td>Age first received paracetamol:</td>
<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>. &lt;= 12 months</td>
<td>287 (92.9)</td>
<td>257 (91.8)</td>
<td></td>
</tr>
<tr>
<td>. &gt; 1 - &lt; 3 years</td>
<td>21 ( 6.8)</td>
<td>20 ( 7.1)</td>
<td></td>
</tr>
<tr>
<td>. &gt;= 3 - &lt; 5 years</td>
<td>1 ( 0.3)</td>
<td>2 ( 0.7)</td>
<td></td>
</tr>
<tr>
<td>. &gt;= 5 years</td>
<td>0 ( 0.0)</td>
<td>1 ( 0.4)</td>
<td></td>
</tr>
<tr>
<td>Paracetamol last 12 months:</td>
<td></td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>. At least once per fortnight</td>
<td>4 ( 1.3)</td>
<td>0 ( 0.0)</td>
<td></td>
</tr>
<tr>
<td>. At least once per month</td>
<td>31 (10.0)</td>
<td>29 (10.3)</td>
<td></td>
</tr>
<tr>
<td>. At least once per week</td>
<td>4 ( 1.3)</td>
<td>2 ( 0.7)</td>
<td></td>
</tr>
<tr>
<td>. Less than once per month</td>
<td>242 (78.1)</td>
<td>227 (80.5)</td>
<td></td>
</tr>
<tr>
<td>. Never, in the last 12 months</td>
<td>29 ( 9.4)</td>
<td>24 ( 8.5)</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen ever</td>
<td>222 (70.7)</td>
<td>183 (64.0)</td>
<td>0.0795</td>
</tr>
<tr>
<td>Age first received ibuprofen:</td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>. &lt;= 12 months</td>
<td>75 (33.9)</td>
<td>77 (42.5)</td>
<td></td>
</tr>
<tr>
<td>. &gt; 1 - &lt; 3 years</td>
<td>99 (44.8)</td>
<td>74 (40.9)</td>
<td></td>
</tr>
<tr>
<td>. &gt;= 3 - &lt; 5 years</td>
<td>34 (15.4)</td>
<td>18 ( 9.9)</td>
<td></td>
</tr>
<tr>
<td>. &gt;= 5 years</td>
<td>13 ( 5.9)</td>
<td>12 ( 6.6)</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen last 12 months:</td>
<td></td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>. At least once per fortnight</td>
<td>0 ( 0.0)</td>
<td>1 ( 0.5)</td>
<td></td>
</tr>
<tr>
<td>. At least once per month</td>
<td>15 ( 6.8)</td>
<td>6 ( 3.3)</td>
<td></td>
</tr>
<tr>
<td>. At least once per week</td>
<td>3 ( 1.4)</td>
<td>1 ( 0.5)</td>
<td></td>
</tr>
<tr>
<td>. Less than once per month</td>
<td>149 (67.1)</td>
<td>123 (67.2)</td>
<td></td>
</tr>
<tr>
<td>. Never, in the last 12 months</td>
<td>55 (24.8)</td>
<td>52 (28.4)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: n-3 LCPUFA, long chain polyunsaturated fatty acid

*For n-3 LCPUFA and control groups, data are number of subjects (percentage).

iContinuous characteristics compared using independent samples t-tests, categorical characteristics compared using chi-square tests
Discussion

The six year allergy follow-up of children at high hereditary risk of allergy was designed to resolve uncertainties surrounding the use of maternal n-3 LCPUFA supplementation during pregnancy as an allergic disease preventative strategy. The aim of this large RCT was to ascertain if beneficial effects seen in early childhood would translate to lower aero-allergen sensitisation and fewer children with allergic disease at early school age, a time when respiratory allergic diseases are prominent. These results show that a maternal dose of 900mg/day of n-3 LCPUFA during pregnancy had no effect on the composite primary outcome of IgE associated allergic disease symptoms (eczema, wheeze, rhinitis or rhino-conjunctivitis) with sensitisation at 6 years of age. However results from analysis of secondary outcomes show a significant reduction in sensitisation to HDM (*D. farinae*) and suggest a reduction in ‘parent reported hayfever ever’ in the n-3 LCPUFA group.

The lack of significant effect of n3-LCPUFA supplementation on the composite primary outcome of any allergic disease with sensitisation is perhaps not surprising due to the dynamic nature of the condition and the difficulty capturing temporality of combined symptoms. The pattern of sensitisation and allergic disease symptoms is known to differ with the age of the child however the timing of onset, remission or persistence and the nature of sensitisation to specific allergens is heterogeneous. The atopic march has been supported by a number of cross-sectional and longitudinal studies ([26, 28, 31, 154, 156, 157]) , however, the risk of developing atopic disease is complex and strongly influenced by both genetic and environmental factors indicating that the temporal pattern expressed in the atopic march may not be a simple progression ([28]). Recent reports challenge the generalisability of the atopic
march and suggest that IgE antibody responses do not reflect a single phenotype of atopy, but rather multiple different atopic vulnerabilities \(^{155, 158}\). However, despite the lack of any significant effect combined outcomes, it does seem that prenatal n-3 LCPUFA supplementation does have an effect on certain aspects of allergic disease, in particular, sensitisation. The prevalence of allergic disease symptoms and sensitisation patterns were consistent with previous studies and the atopic march hypothesis with 50% of children sensitised to one or more of the allergen extracts tested at six years of age. RCTs investigating n-3 LCPUFA supplementation during pregnancy and outcomes allergic disease in early childhood (≤3 years) have demonstrated a protective effect of the intervention on the incidence of allergic disease symptoms commonly occurring at the start of the atopic march (sensitisation to egg \(^{112, 116, 118}\) and eczema \(^{113}\)). At six years of age, there was no significant effect of n-3 LCPUFA supplementation on the incidence of atopic eczema symptoms although rates were low (10.2%) indicating a number of children had outgrown this condition. A total of 11.6% of children were sensitised to one or more food allergens (4.5% to egg) and 34.5% of children were sensitised aero-allergens which is consistent with the atopic march and age of assessment. There was statistically significant 33% reduction in HDM (\textit{D.farinae}) sensitisation in the n-3 LCPUFA group. HDM is the most common indoor aero-allergen detected in atopic individuals and plays a principal role in the pathogenesis of allergic airways disease including asthma and hayfever across all age groups \(^{159}\).
At six years of age 25.5% of children assessed had suffered hay fever in the past according to parent report, with a significant reduction (a RR; 0.77) in the in the n-3 LCPUFA group. Symptoms of rhinitis and rhino-conjunctivitis assessed via the symptom based ISAAC questionnaire however, showed no effect of the intervention. Previous studies have reported that the highest positive predictive value for atopy among children with rhinitis was ‘reported hay fever’ (70%) corroborating findings of the 6 year follow up (151) Cohort studies observing associations between increased fish intake and respiratory allergic disease outcomes in the school age child have found a reduction in hay fever (111) and asthma (103) though no RCTs have assessed effects of prenatal n-3 LCPUFA supplementation in this age group. The only RCT to assess respiratory allergic disease outcomes >3 years of age, showed reduction in asthma and atopic asthma at 16 years of age (119). However, asthma outcomes from this trial were obtained by linkage to a national registry of doctors’ visits where the expected event rates were low and it is unclear if asthma diagnosis was standardised. The prevalence of wheeze at 6 years was 25%, however only half of these cases were sensitised to one or more aero-allergens that were tested (atopic wheeze). Wheezing is common in early childhood however it is mostly transient, associated with diminished airway function. 60% of children with wheezing before the age of 3 years of age outgrow their condition by the age of 6 and have no increased risk of allergies or asthma (160). Wheezing still present at 6 years of age can be further classified into two categories; ‘persistent wheezing’ (wheeze in early childhood and continuing to wheeze at 6 years) and ‘late onset wheeze’ (no wheeze in early childhood but wheeze at 6 years). Both categories are frequently sensitised to common aeroallergens at 6 years of age with persistent wheezing also having a direct relationship with IgE levels in infancy (160). There were no differences between
the n-3 LCPUFA and control group on the incidence of wheeze or categories of wheeze (persistent or late-onset wheeze) although the incidence of persistent wheeze most likely associated with atopic asthma was low (2.5%) making it difficult to make any assumptions regarding any effect of the intervention.

One of the strengths of this 6 year follow up of the DOMInO Trial is the double blind design with concealed allocation. It is a large RCT with low risk of bias being one of the highest levels of evidence for assessing the clinical evidence for interventions (161). Follow up of children at 6 years was completed with minimal attrition (90% completed) and the integrity of the randomisation was maintained at 6 years. This is the only RCT to conduct follow up of maternal n-3 LCPUFA supplementation and allergy outcomes in the school age child and furthermore, maternal demographic characteristics were comparable to Australian women who gave birth in 2006-7 (162) suggesting generalisability of the results to the wider population.

A limitation of this study is that based on evidence from previous RCTs (113, 118, 119), it was powered to detect a 33% relative reduction in overall allergic disease and therefore was possibly under powered for the composite primary outcome. Interestingly, these trials used higher doses of n-3 LCPUFA ranging from 2700 mg/day (113, 119) to 3700 mg/day (118) and the trial with the greatest effect of the intervention continued supplementation beyond birth of the child until 3.5 months post-partum (113). Perhaps the provision of additional n-3 LCPUFA to the infant’s maturing immune system post birth results in greater effects. These RCTs were not specifically designed and powered to assess clinical outcomes therefore introducing the possibility of bias or random error, however results suggest an implication of
timing, dose and duration of n-3 LCPUFA supplementation on outcomes and are important considerations worthy of further investigation.

Another potential limitation of this study is the observation that more mothers in the control group vs the n-3 LCPUFA had sought un-blinding 44/289 (15.22%) vs 31/314 (9.87%). As this was a post-randomisation variable, I could not adjust for it in the statistical analyses; however, sensitisation assessment by SPT is an objective marker so it is unlikely that un-blinding would have an effect. In further exploratory analysis, I found no relation between blinded and un-blinded children who attended assessments and underwent skin prick testing (163) indicating that response bias related to this was unlikely. Un-blinding was conducted prior to the six year allergy follow up and performed by an independent data management office to ensure all staff who may be in contact with participants remained blinded.

An unexpected finding in this study was the significant difference in the psychosocial score of the CHQ (CHQ-PF50) in favour of the control, Table 4-9. Allergic disease is known to have a significant effect on the emotional and social health of patients and their families, however the CHQ-PF50 is a generic quality of life instrument, not responsive enough to detect changes in general health states such as allergic disease (164). Although this difference is statistically significant, mean summary scores of both the intervention and control groups were within normal limits and not considered clinically significant. Following further review of the data this finding can be further explained by an overweighting of physically and/or mentally challenged children in the intervention group (3 vs 0) unrelated to the intervention.
This study did not show an overall reduction in IgE associated allergic disease at 6 years of age, although the data suggests that ‘sensitisation to HDM’ and parent reported ‘hayfever ever’ are reduced by n-3 LCPUFA supplementation of the mother during pregnancy. HDM is the most common indoor aeroallergen in many regions, the strong association between HDM sensitisation and respiratory allergic diseases (asthma and allergic rhinitis) emphasise the potential public health implications to these findings. Likewise, the reduction in incidence of ‘parent reported hay fever’ supports the necessity to further investigate relationships between an increased n-3 LCPUFA supply to women bearing a fetus at risk of atopy. Although hay fever alone is not a life threatening disease, the burden on individuals suffering the disease and their families can be immense. Asthma and hayfever are often co-morbidities with asthma more severe in individuals with hayfever compared with those without (165). In a recent longitudinal birth cohort study childhood allergic rhinitis was associated with a 7-fold increased risk of asthma in adult life (166).

An additional non-significant finding that warrants further investigation is the reduction in sensitisation to peanut (18/250 (7.2%) vs 29/232 (12.5%) and cashew 9/249 (3.6%) vs 16/231 (6.9%) in the n-3 LCPUFA group. These results need to be treated with caution as numbers were low, however the non-significant risk reductions of 43% and 48% may still be of public health significance as the burden life threatening food allergy continues to increase.
There are plausible mechanisms by which increasing maternal dietary n-3 LCPUFA intake may modulate the fetal immune system and subsequent development of allergic disease in infants at risk of atopic disease. However because of inconsistencies and a paucity of RCT evidence, the hypothesis linking maternal n-3 LCPUFA intake to childhood allergic disease cannot unequivocally be confirmed nor rejected. Long term follow of children into adolescence and beyond is essential when assessing a dynamic condition such as allergic disease that changes over time. Clearly, further evidence from well-powered, high-quality trials of comparable methodology and standardised, objective outcome assessments are needed to definitively determine whether prenatal n-3 LCPUFA supplementation is beneficial as a primary prevention strategy for allergic disease.
Longitudinal analysis of childhood allergy outcomes of children at high hereditary risk of atopy born to mothers supplemented with n-3 LCPUFA during pregnancy
Chapter Five

Introduction

This longitudinal data analysis synthesizes results from three assessment time points from a nested allergy follow-up of children born to mothers who participated in a double blind, multi-centre randomised controlled trial of n-3 supplementation during pregnancy (DOMInO Trial) \(^{[145]}\). Data included in this analysis is from the same cohort of children that participated in the 1 & 3 year allergy follow up of the DOMInO Trial (previously published) \(^{[116, 120]}\) and the six year follow up detailed in Chapter 3 of this thesis.

Aim

The aim of this post-hoc analysis was to investigate the effects of n-3 LCPUFA supplementation of pregnant women with a fetus at high risk of hereditary disease on the incidence of IgE mediated allergic disease symptoms (eczema, rhinitis & rhino-conjunctivitis) over time, from 1 – 6 years of age.

Hypothesis

Fewer children at higher-hereditary risk of atopic disease that were supplemented with n-3 LCPUFA supplementation during prenatal life will have IgE mediated allergic disease symptoms (eczema, rhinitis, rhino-conjunctivitis) with sensitisation between the age of 1-6 years.
Methods

The study population consisted of children born to mothers who participated in the DOMInO Trial (Docosahexaenoic Acid (DHA) to Optimise Mother Infant Outcome) between October 2005 and January 2008 (145). Detailed information on trial design and dietary treatments have been reported in Chapter 3. In brief, women taking part in the DOMInO trial, residing in South Australia, were invited to participate in a 1 & 3 year child allergy follow up if their fetus was at high risk of allergic disease (mother, father or sibling/s reporting medically diagnosed allergic disease). Written informed consent was sought before birth and included consent for the offspring to take part in the 1 and 3 year of age allergy assessment (n=706). Families were contacted again when the child was nearing their 6th birthday, and invited to take part in the 6 year allergy follow up (n=668) see Figure 4-1.

Approval for the ‘1 & 3 year follow up’ and the 6 year follow up was granted by the Human Research Ethics Committees of the Women’s and Children’s Hospital, Adelaide and Southern Adelaide Health Service. All trial follow up was registered on the Australian New Zealand Clinical Trials Registry; DOMInO trial ACTRN12605000569606, ‘1 & 3 year allergy follow up’ ACTRN12610000735055, 6 year follow up ACTRN12615000498594
Women allocated to the intervention group were asked to consume three 500 mg capsules of fish oil concentrate, providing 800 mg of DHA and 100 mg of eicosapentaenoic acid (EPA) (900mg total n-3 LCPUFA) and women in the control group were asked to take three 500 mg vegetable oil capsules without n-3 LCPUFA daily. Capsules were matched in size, shape and colour and taken from 21 weeks gestation until delivery. Women reported their adherence to supplementation at telephone calls at 28 and 36 weeks’ gestation and the concentration of individual LCPUFA in plasma phospholipids from cord blood was assessed as an independent biomarker of adherence.

Primary Outcome

The primary outcome of the longitudinal allergy follow up is the incidence of overall IgE mediated allergic disease (eczema, wheeze or rhino-conjunctivitis symptoms) with sensitisation (defined as positive SPT of ≥3mm) and the incidence of individual allergic diseases and sensitisation to allergen extracts over time, from 1 – 6 years of age.

Secondary Outcome

The effect of prenatal n-3 LCPUFA supplementation on known associations between egg sensitisation and HDM sensitisation.
Assessment of allergic disease

Sensitisation

Children attended a clinic assessment and underwent a skin prick test to determine their sensitisation status at 1, 3 and 6 years of age. Skin prick testing was performed in a standardised manner and operators were limited to specifically trained study staff. Sensitisation (IgE response) was defined as a positive skin prick test reaction to at least one of the allergen extracts assessed. A test was considered positive if the mean of the horizontal and perpendicular weal diameters was ≥3 mm or greater in size than that of the negative control site at 15 minutes. Allergen extracts were selected according to the age of the child (i.e. common foods per age) and aero-allergens were selected based on common allergens in the geographical area of South Australia, Table 5-1.
Table 5-1 Allergen extracts tested at 1, 3 & 6 years

<table>
<thead>
<tr>
<th></th>
<th>1 year</th>
<th>3 year</th>
<th>6 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow's milk</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Whole hen's egg</td>
<td>Whole hen's egg</td>
<td>Whole hen's egg</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>Wheat</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tuna</td>
<td>Tuna</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peanut</td>
<td>Peanut</td>
<td>Peanut</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Cashew</td>
<td>Cashew</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Sesame</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Grass pollen</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Perennial rye grass</td>
<td>Perennial rye grass</td>
<td>Perennial rye grass</td>
<td></td>
</tr>
<tr>
<td>Olive tree pollen</td>
<td>Olive tree pollen</td>
<td>Olive tree pollen</td>
<td></td>
</tr>
<tr>
<td>Alternaria tenuis</td>
<td>Alternaria tenuis</td>
<td>Alternaria tenuis</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>Cat</td>
<td>Cat</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Dog</td>
<td>-</td>
</tr>
<tr>
<td>D. pteronyssinus (HDM)</td>
<td>D. pteronyssinus (HDM)</td>
<td>D. pteronyssinus (HDM)</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>D. farinae (HDM)</td>
<td>D. farinae (HDM)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HDM, house dust mite, D. pteronyssinus, Dermatophagoides pteronyssinus; D. farinae, Dermatophagoides farinae

Glycerin and histamine (10mg/mL) were used as negative and positive controls at each time point. Only allergen extracts that were assessed at 2 or more time points were included in the longitudinal analysis.
Allergic Disease Symptoms

The study design of the 1 & 3 year allergy follow up included assessment by a physician for the diagnosis of allergic disease. In addition to a medical assessment, an interviewer-administered questionnaire, based on core modules from the International Study of Asthma and Allergies in Childhood (ISAAC) was completed. At six years of age parents were interviewed regarding allergic disease symptoms of their child again using ISSAC core modules (consistent with the 1 & 3 year follow up), with no medical assessment. For the purpose of this analysis, only ISAAC data on allergic symptoms from the 1 and 3 year assessment have been used to ensure consistency with the six year follow up and reported outcomes. Outcomes of allergic disease symptoms include eczema at 1 year and eczema, rhinitis and rhino-conjunctivitis at 3 and 6 years, see Table 5-2.

Table 5-2 Allergic disease symptom questions at 1, 3 & 6 years

<table>
<thead>
<tr>
<th>Allergy Symptom</th>
<th>Age (y)</th>
<th>Outcome Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td>1, 3 &amp; 6</td>
<td>Presence of history of an itchy rash distributed to the facial, flexural or extensor surface of the skin.</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>3 &amp; 6</td>
<td>History of sneezing or a runny or blocked nose when there have not been symptoms to suggest an upper respiratory tract infection.</td>
</tr>
<tr>
<td>Rhino-conjunctivitis</td>
<td>3 &amp; 6</td>
<td>History of sneezing or a runny or blocked nose accompanied by itchy-watery eyes when there have not been symptoms to suggest an upper respiratory tract infection.</td>
</tr>
</tbody>
</table>
IgE-associated eczema was defined as a positive response to allergic disease symptoms outlined in Table 5-2 and a positive SPT response to at least one of the allergen extracts tested. IgE-associated respiratory allergic diseases (rhinitis and rhino-conjunctivitis) were defined as a positive response to allergic disease symptoms and a positive SPT response to at least one of the aero-allergens tested.

Due to the heterogeneous nature wheeze in early childhood this outcome was not included in this analysis but reported as phenotypes of wheeze according to onset and clinical course as described in Chapter 4, Table 4-5
Statistical Analysis

Binary outcomes were measured separately at 1, 3 and 6 years (or just at 3 and 6 years) and the groups were compared using log binomial generalised estimating equations. An independence working correlation matrix was used to adjust for the dependence within subjects due to repeated measurements over time. In the models the effect of treatment group, time and the interaction between treatment group and time was assessed. The association between time and outcome was difficult to establish based on only 3 time periods of measurement, therefore time was included in the models as a categorical predictor variable. Separate estimates of treatment effect were obtained at each time point, independent of whether the interaction effect was statistically significant or not. The effect of treatment, whether at an individual time point or across all time points, was described using relative risks and 95% confidence intervals. Fisher exact tests at each time point were performed if the number and percentage of participants experiencing the binary outcome of interest was considered too small to reliably estimate the relative risk at any of the time points. The analytic data set was based on 100 imputed complete data sets and included all children that were consented to participate in the allergy follow-up. For outcomes at 3 and 6 years, the imputed datasets had n=367 and n=336 in the n-3 LCPUFA and control groups respectively. At 1 year the complete datasets had n=368 and n=337 in the n-3 LCPUFA and control groups, the difference being due to the two deaths in the control group and one death in the n-3 LCPUFA group. Due to small number of cases, no imputed analysis was completed for egg sensitisation, cashew sensitisation or cat sensitisation.
Results

Sample and participant flow

Enrolment to the nested allergy follow up began in 2006. A total of 706 children were enrolled into the 1 & 3 nested allergy follow up between 2006 and 2008. Of these 666 infants enrolled, 94.3% completed a physician assessment, allergy symptom questionnaires and skin prick testing at 1 year of age and 587/706 (83.1%) at 3 years of age. Following completion of the 3 year allergy assessments in 2009, children born to mothers in the DOMInO trial were involved in additional follow up at 4 and 5 years of age investigating development & growth. During this period some families withdrew their consent to continue to participate in future follow up and were withdrawn from the DOMInO Trial cohort.

In 2012, children in the allergy cohort were due to turn 6 years of age. Following the exclusion of withdrawals a total of 668 families were eligible to participate in the 6 year allergy follow up of which 605 consented (90%), see Figure 4-1, Chapter 4. Six year assessments were completed in August 2014, 485/603 (80%) assessments included a skin prick test to investigate IgE-mediated response to allergen extracts (sensitisation).
Baseline characteristics

The incidence of maternal and paternal allergic diseases (allergic disease history) as assessed by questionnaire at enrolment into the DOMInO trial was comparable in both the intervention and control groups. A number of clinical and socio-demographic characteristics were assessed including maternal smoking, BMI, parity and age of the mother at delivery. The baseline enrolment characteristics of the women and their offspring who were consented to participate in allergy follow-up were comparable between the n-3 LCPUFA and control groups, see Table 4-1, Chapter 4.

Allergic disease symptom prevalence

The prevalence of any allergic disease symptoms with sensitisation increased with the age of the child from 3.6% at 1 year, 12.2% at 3 year and 28.5% 6 years. Individual relative risks and confidence intervals for allergic disease symptoms with sensitisation at 1, 3 and 6 years are presented in Table 5-3.
Table 5-3 Effect of n-3 LCPUFA supplementation on individual allergic disease symptoms at 1, 3 & 6 years

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time</th>
<th>n-3 LCPUFA*</th>
<th>Control*</th>
<th>RR</th>
<th>P-value</th>
<th>aRR (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic disease symptoms with sensitisation</td>
<td>1 year</td>
<td>10/368 (2.82%)</td>
<td>15/337 (4.58%)</td>
<td>0.61 (0.28, 1.34)</td>
<td>0.22</td>
<td>0.62 (0.29, 1.36)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>3 year</td>
<td>43/367 (11.71%)</td>
<td>44/336 (13.03%)</td>
<td>0.90 (0.59, 1.36)</td>
<td>0.61</td>
<td>0.91 (0.60, 1.37)</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>6 year</td>
<td>116/367 (31.48%)</td>
<td>106/336 (31.46%)</td>
<td>1.00 (0.78, 1.28)</td>
<td>0.99</td>
<td>1.04 (0.82, 1.33)</td>
<td>0.74</td>
</tr>
<tr>
<td>Eczema symptoms with sensitisation</td>
<td>1 year</td>
<td>10/368 (2.82%)</td>
<td>15/337 (4.58%)</td>
<td>0.61 (0.28, 1.34)</td>
<td>0.22</td>
<td>0.62 (0.29, 1.36)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>16/367 (4.37%)</td>
<td>19/336 (5.79%)</td>
<td>0.75 (0.39, 1.47)</td>
<td>0.40</td>
<td>0.76 (0.39, 1.47)</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>6 years</td>
<td>36/367 (9.90%)</td>
<td>36/336 (10.64%)</td>
<td>0.93 (0.58, 1.50)</td>
<td>0.77</td>
<td>0.94 (0.58, 1.52)</td>
<td>0.80</td>
</tr>
<tr>
<td>Rhinitis symptoms with sensitisation</td>
<td>3 years</td>
<td>26/367 (7.21%)</td>
<td>27/336 (8.03%)</td>
<td>0.90 (0.52, 1.54)</td>
<td>0.71</td>
<td>0.91 (0.53, 1.57)</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>6 years</td>
<td>75/367 (20.39%)</td>
<td>72/336 (21.29%)</td>
<td>0.96 (0.70, 1.31)</td>
<td>0.79</td>
<td>0.98 (0.71, 1.34)</td>
<td>0.88</td>
</tr>
<tr>
<td>Rhino-conjunctivitis symptoms with sensitisation</td>
<td>3 years</td>
<td>12/367 (3.14%)</td>
<td>17/336 (5.07%)</td>
<td>0.62 (0.29, 1.33)</td>
<td>0.22</td>
<td>0.62 (0.29, 1.33)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>6 years</td>
<td>47/367 (12.72%)</td>
<td>39/336 (11.49%)</td>
<td>1.11 (0.72, 1.71)</td>
<td>0.64</td>
<td>1.11 (0.72, 1.72)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; ARR, adjusted relative risk.
*All data are based on analysis of 100 imputed datasets unless otherwise indicated.
For n-3 LCPUFA and control groups, data are number of subjects (percentage)
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Allergic disease symptoms with sensitisation over time

Longitudinal analysis with interactive P value was calculated for symptoms of allergic disease with sensitisation. Based on the adjusted analysis, there was not enough evidence to conclude that the groups behaved differently over time for symptoms of eczema, rhinitis or rhino-conjunctivitis with sensitisation (n-3 LCPUFA vs. control), Table 5-10.

Table 5-4 Longitudinal analysis of treatment effect (n-3 LCPUFA vs control) on individual allergic disease symptoms across all years

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interaction P-value</th>
<th>Adjusted Interaction P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema symptoms with sensitisation</td>
<td>0.58</td>
<td>0.58</td>
</tr>
<tr>
<td>Rhinitis symptoms with sensitisation</td>
<td>0.81</td>
<td>0.80</td>
</tr>
<tr>
<td>Rhino-conjunctivitis symptoms with sensitisation</td>
<td>0.14</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Abbreviations: n-3 LCPUFA, long chain polyunsaturated fatty acid
Longitudinal analysis performed on raw data (no imputation)
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Risk of allergic disease symptoms with sensitisation across all years

After excluding the group by time interaction effect, there was not enough evidence to conclude that n-3 LCPUFA was associated with the risk of eczema, rhinitis or rhino-conjunctivitis with sensitisation across all years, Table 5-5.

**Table 5-5 Risk of allergic disease with sensitisation between n-3 LCPUFA and control groups across 1-6 years**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time</th>
<th>RR</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema symptoms with sensitisation</td>
<td>All years</td>
<td>0.82 (0.53, 1.27)</td>
<td>0.37</td>
<td>0.82 (0.53, 1.27)</td>
<td>0.37</td>
</tr>
<tr>
<td>Rhinitis symptoms with sensitisation</td>
<td>All years</td>
<td>0.94 (0.70, 1.28)</td>
<td>0.71</td>
<td>0.96 (0.71, 1.30)</td>
<td>0.79</td>
</tr>
<tr>
<td>Rhino-conjunctivitis symptoms with sensitisation</td>
<td>All years</td>
<td>0.97 (0.64, 1.46)</td>
<td>0.87</td>
<td>0.97 (0.64, 1.47)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Abbreviations: n-3 LCPUFA, long chain polyunsaturated fatty acid; RR, Relative risk; aRR, adjusted relative risk; CI, confidence interval

†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
Sensitisation pattern

Allergen extracts used in SPTs at allergy assessments at 1, 3 and 6 years were selected according to age of the child and known sensitisation patterns, see Table 5-1. The percentage of children with ≥1 positive SPT reaction to any allergen increased with age from 17.1% at 1 year, 25.9% at 3 years and 50% at 6 years. The allergen sensitisation pattern across 1 – 6 years was consistent with the typical sequence of initial food allergen sensitisation which decreases in prevalence with age while aero-allergen sensitisation prevalence continues to rise. Figure 5-1 depicts the percentage of children with positive skin prick tests per individual allergen extract at each assessment time point.
**Figure 5-1 Sensitisation pattern 1-6 years**

Data represented for 1 and 3 year sensitisation are based on analysis of 50 imputed datasets. Data represented for 6 year sensitisation are based on analysis of 100 imputed datasets with the exception of cashew and egg (raw data). Cashew and D. farinae were not assessed at 1 year.
Sensitisation point prevalence at 1, 3 & 6 years

Stand-alone results for the one year allergy follow up demonstrated a statistically significant reduction in the incidence of sensitisation to egg and a decreased incidence of atopic eczema in the intervention group (116). When these children were assessed again at 3 years of age a number of children had out grown their sensitisation to food allergens but many had not yet acquired sensitisation to the aeroallergens tested. There was a chance finding of an increased number of children sensitised to cat in the n-3 LCPUFA group (6.8% vs 3.4% in the control group) however, numbers were small and this difference did not reach statistical significance (adjusted relative risk 1.95; 95% CI 0.98–3.89; P = 0.06), Table 5-6.

There were no statistically significant results found from the 3 year follow up which is consistent with other studies of children in this age group (157). At 6 years of age there was a statistically significant reduction in the incidence of sensitisation to HDM (D. farinae) and a borderline reduction in parent reported hayfever in the n-3 LCPUFA group. There was also a 43% reduction in peanut sensitisation and a 48% reduction in cashew sensitisation in the intervention group although these results did not reach statistical significance, Table 5-6.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time</th>
<th>n-3 LCPUFA</th>
<th>Control</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n-3 LCPUFA</td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitisation</td>
<td>1 year</td>
<td>53/368 (14.40%)</td>
<td>67/338 (19.82%)</td>
<td>0.73 (0.52, 1.02)</td>
<td>0.07</td>
<td>0.75 (0.53, 1.04)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>91/368 (24.60%)</td>
<td>88/338 (26.10%)</td>
<td>0.94 (0.72, 1.23)</td>
<td>0.67</td>
<td>0.96 (0.74, 1.25)</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>6 years</td>
<td>185/367 (50.49%)</td>
<td>163/336 (48.61%)</td>
<td>1.04 (0.87, 1.24)</td>
<td>0.67</td>
<td>1.08 (0.91, 1.28)</td>
<td>0.39</td>
</tr>
<tr>
<td>Egg sensitisation</td>
<td>1 year</td>
<td>34/368 (9.74%)</td>
<td>52/338 (15.38%)</td>
<td>0.61 (0.40, 0.91)</td>
<td>0.02</td>
<td>0.62 (0.41, 0.93)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>11/368 (3.10%)</td>
<td>16/338 (4.70%)</td>
<td>0.65 (0.30, 1.41)</td>
<td>0.28</td>
<td>0.68 (0.32, 1.45)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>6 years**</td>
<td>6/248 (2.42%)</td>
<td>5/232 (2.16%)</td>
<td>1.16 (0.36, 3.73)</td>
<td>0.81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peanut sensitisation</td>
<td>1 year</td>
<td>15/368 (4.29%)</td>
<td>22/338 (6.82%)</td>
<td>0.62 (0.33, 1.18)</td>
<td>0.15</td>
<td>0.63 (0.34, 1.19)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>13/368 (3.74%)</td>
<td>20/338 (6.31%)</td>
<td>0.59 (0.29, 1.20)</td>
<td>0.14</td>
<td>0.64 (0.31, 1.29)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>6 years</td>
<td>27/367 (7.23%)</td>
<td>39/336 (11.68%)</td>
<td>0.62 (0.35, 1.09)</td>
<td>0.10</td>
<td>0.64 (0.36, 1.13)</td>
<td>0.13</td>
</tr>
<tr>
<td>Cashew sensitisation</td>
<td>3 years</td>
<td>5/338 (1.40%)</td>
<td>12/338 (3.53%)</td>
<td>0.41 (0.14, 1.18)</td>
<td>0.10</td>
<td>0.44 (0.15, 1.26)</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>6 years**</td>
<td>9/249 (3.61%)</td>
<td>16/231 (6.93%)</td>
<td>0.55 (0.25, 1.25)</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alternaria Tenuis</td>
<td>1 year**</td>
<td>4/349 (1.14%)</td>
<td>1/317 (0.31%)</td>
<td>N/A</td>
<td>0.38</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>32/368 (8.70%)</td>
<td>23/338 (6.80%)</td>
<td>1.29 (0.76, 2.19)</td>
<td>0.34</td>
<td>1.31 (0.78, 2.22)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>6 years</td>
<td>68/367 (18.56%)</td>
<td>62/336 (18.37%)</td>
<td>1.01 (0.69, 1.47)</td>
<td>0.96</td>
<td>1.03 (0.71, 1.49)</td>
<td>0.88</td>
</tr>
<tr>
<td>Cat sensitisation</td>
<td>1 year**</td>
<td>7/349 (2.01%)</td>
<td>5/317 (1.58%)</td>
<td>1.30 (0.42, 4.00)</td>
<td>0.65</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>25/368 (6.80%)</td>
<td>12/338 (3.55%)</td>
<td>1.97 (0.98, 3.95)</td>
<td>0.06</td>
<td>1.95 (0.97, 3.89)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>6 years</td>
<td>46/367 (12.47%)</td>
<td>40/336 (11.95%)</td>
<td>1.04 (0.66, 1.64)</td>
<td>0.85</td>
<td>1.07 (0.68, 1.68)</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>1 year**</td>
<td>3 years</td>
<td>6 years</td>
<td>3 years</td>
<td>6 years</td>
<td>3 years</td>
<td>6 years</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Ryegrass sensitisation</td>
<td>1/349 (0.30%)</td>
<td>22/368 (5.97%)</td>
<td>97/367 (26.29%)</td>
<td>0.72 (0.41, 1.26)</td>
<td>0.25</td>
<td>1.02 (0.76, 1.36)</td>
<td>0.92</td>
</tr>
<tr>
<td>Olive Tree</td>
<td>0/349 (0.00%)</td>
<td>14/368 (3.80%)</td>
<td>55/367 (14.89%)</td>
<td>0.38 (0.58, 3.24)</td>
<td>0.46</td>
<td>1.23 (0.77, 1.96)</td>
<td>0.39</td>
</tr>
<tr>
<td>D. farinae sensitisation</td>
<td>16/368 (4.50%)</td>
<td>23/368 (6.25%)</td>
<td>45/367 (12.32%)</td>
<td>0.92 (0.46, 1.82)</td>
<td>0.80</td>
<td>1.23 (0.77, 1.96)</td>
<td>0.39</td>
</tr>
<tr>
<td>D. pteronyssinus</td>
<td>17/338 (4.90%)</td>
<td>25/336 (7.40%)</td>
<td>40/336 (12.03%)</td>
<td>0.66 (0.44, 0.99)</td>
<td>0.046</td>
<td>1.02 (0.63, 1.66)</td>
<td>0.92</td>
</tr>
<tr>
<td>Dog Sensitisation</td>
<td>25/338 (7.40%)</td>
<td>3/367 (20.41%)</td>
<td>1.02 (0.63, 1.66)</td>
<td>0.92</td>
<td>1.07 (0.67, 1.72)</td>
<td>0.78</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; aRR, adjusted relative risk
For n-3 LCPUFA and control groups, data are number of subjects (percentage)
Denominators based on numbers randomised in 1 & 3 years follow up (368/338 at 1 and 3 years, 367/336 at 6 years to account for x3 deaths)
1 & 3 year data based on analysis of 50 imputed datasets, 6 year data are based on analysis of 100 imputed datasets unless otherwise indicated.
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
**Adjusted analyses not done owing to rarity of outcomes, analysis on raw data only
Sensitisation associations

Exploratory analyses were conducted to investigate the association of egg sensitisation and house dust mite sensitisation D. farinae in children who completed a SPT at 6 years and also had SPT results from the 1 year assessment (n=464). Analyses were conducted on the overall association of egg sensitisation to D. farinae and association per randomisation group.

Overall Association

Of the 62 children with egg sensitisation at 1 year, 25 (40.3%) reported dust mite sensitisation at 6 years. In comparison, of the 402 children without egg sensitisation at 1 year, 53 (13.2%) developed dust mite sensitisation at 6 years, Table 5-7. The difference in the risk of dust mite sensitisation at 6 years was found to be statistically significant between those with or without egg sensitisation at 1 year (13.2% vs. 40.3%, Fisher exact test P-value < 0.0001).

Table 5-7 Overall association of egg sensitisation at 1 year to D. farinae sensitisation at 6 years

<table>
<thead>
<tr>
<th>Egg sensitisation at 1 year</th>
<th>D. farinae sensitisation at 6 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (86.82%)</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>Yes (13.18%)</td>
<td>53</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>402</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No (59.68%)</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Yes (40.32%)</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>62</td>
</tr>
</tbody>
</table>

These exploratory analyses are based on un-imputed data
n-3 LCPUFA group

Within the n-3 LCPUFA group, the difference in the risk of dust mite sensitisation at 6 years was found to be statistically significant between the two groups defined by egg sensitisation at 1 year (9.7% vs. 37.5%, Fisher exact test $P$-value = 0.0008), Table 5-8.

Table 5-8 Association of egg sensitisation at 1 year to *D. farinae* sensitisation at 6 years in the n-3 LCPUFA group

<table>
<thead>
<tr>
<th>Egg sensitisation at 1 year</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Total</td>
</tr>
<tr>
<td>No</td>
<td>196 (90.32%)</td>
<td>21 (9.68%)</td>
<td>217</td>
</tr>
<tr>
<td>Yes</td>
<td>15 (62.50%)</td>
<td>9 (37.50%)</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>211</td>
<td>30</td>
<td>241</td>
</tr>
</tbody>
</table>

*These exploratory analyses are based on un-imputed data*
Within the control group, the difference in the risk of dust mite sensitisation at 6 years was found to be statistically significant between the two groups defined by egg sensitisation at 1 year (17.3% vs. 42.1%, Fisher exact test $P$-value = 0.002), Table 5-9.

**Table 5-9 Association of egg sensitisation at 1 year to *D. farinae* sensitisation at 6 years in the control group**

<table>
<thead>
<tr>
<th>Egg sensitisation at 1 year</th>
<th><em>D farinae</em> sensitisation at 6 years</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>153</td>
<td>32</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>82.70%</td>
<td>17.30%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>16</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57.89%</td>
<td>42.11%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>48</td>
<td>223</td>
<td></td>
</tr>
</tbody>
</table>

*These exploratory analyses are based on un-imputed data*
Sensitisation group by time interaction

Longitudinal analysis with interactive P value was calculated for allergen extracts that were used in skin prick testing at 2 or more time points at 1, 3 or 6 years (egg, peanut, cat, cashew and D.Farinae). Longitudinal analysis was not performed for sensitisation to ryegrass, olive, D. pteronyssinus and alternaria tenuis due to insufficient cases at 1 or 3 years. Based on the adjusted analysis, there was not enough evidence to conclude that the relative risk of ‘any sensitisation’ or sensitisation to individual allergen extracts (n-3 LCPUFA vs. control) changed over time, Table 5-10.

Table 5-10 Longitudinal analysis of treatment effect (n-3 LCPUFA vs control) on any sensitisation and sensitisation to individual allergen extracts across all years

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interaction P-value</th>
<th>Adjusted Interaction P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitisation</td>
<td>0.19</td>
<td>0.13</td>
</tr>
<tr>
<td>Egg sensitisation**</td>
<td>0.54</td>
<td>-</td>
</tr>
<tr>
<td>Peanut sensitisation</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Cat sensitisation**</td>
<td>0.12</td>
<td>-</td>
</tr>
<tr>
<td>Cashew sensitisation**</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>D.Farinae sensitisation</td>
<td>0.38</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Abbreviations: n-3 LCPUFA, long chain polyunsaturated fatty acid
Longitudinal analysis performed on raw data (no imputation)
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
**Adjusted analyses not done owing to rarity of outcomes
Longitudinal analysis not performed for sensitisation to ryegrass, olive, D. pteronyssinus and alternaria tenuis due to insufficient cases at 1 and 3 years
Risk of sensitisation across all years

After excluding the group by time interaction effect from the model, there was not enough evidence to conclude that treatment with n-3 LCPUFA was associated with the risk of ‘any sensitisation’ or sensitisation to individual allergen extracts across all years, Table 5-11. However, although not statistically significant, there were a number of risk reductions in sensitisation to individual allergen extracts in the n-3 LCPUFA group including; peanut (36%), egg (33%) and D. farinae 23%.

Table 5-11 Risk of sensitisation between n-3 LCPUFA and control groups across 1-6 years

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time</th>
<th>RR</th>
<th>P-value</th>
<th>aRR† (95% CI)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Sensitisation</td>
<td>All years</td>
<td>0.97 (0.82, 1.15)</td>
<td>0.73</td>
<td>1.00 (0.85, 1.18)</td>
<td>0.98</td>
</tr>
<tr>
<td>Peanut</td>
<td>All years</td>
<td>0.61 (0.38, 0.99)</td>
<td>0.047</td>
<td>0.64 (0.40, 1.03)</td>
<td>0.065</td>
</tr>
<tr>
<td>D. farinae</td>
<td>All years</td>
<td>0.71 (0.47, 1.05)</td>
<td>0.09</td>
<td>0.71 (0.48, 1.06)</td>
<td>0.09</td>
</tr>
<tr>
<td>Egg**</td>
<td>All years</td>
<td>0.65 (0.42, 1.01)</td>
<td>0.05</td>
<td>0.67 (0.43, 1.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>Cat**</td>
<td>All years</td>
<td>1.30 (0.80, 2.13)</td>
<td>0.29</td>
<td>1.34 (0.83, 2.18)</td>
<td>0.23</td>
</tr>
<tr>
<td>Cashew**</td>
<td>All years</td>
<td>0.48 (0.21, 1.08)</td>
<td>0.08</td>
<td>0.50 (0.22, 1.14)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; n-3 LCPUFA, long chain polyunsaturated fatty acid; aRR, adjusted relative risk
†Adjusted for enrolling centre, parity, child sex and maternal history of allergic disease
All data are based on longitudinal imputed analysis of 100 datasets unless otherwise indicated
**Analyses performed on raw (non-imputed) data due to rarity of outcome
Longitudinal analysis not performed for sensitisation to ryegrass, olive, D. pteronyssinus and alternaria tenuis due to insufficient cases at 1 and 3 years
Discussion

Atopy in early life is heterogeneous in timing of onset, remission or persistence and in the nature of sensitisation to specific allergens \(155\). Longitudinal RCT data with observations of the same outcomes, on the same participants over long periods of time are essential, particularly when assessing a dynamic condition such as allergic disease. The purpose of this analysis was to investigate whether an increased prenatal supply of n-3 LCPUFA would decrease the incidence of allergic disease symptoms with sensitisation over time (1-6 years).

The prevalence of positive skin prick tests (to food and aeroallergens) and allergic disease symptoms at 1, 3 & 6 years of age in this analysis were consistent with the recognised sequence of sensitisation and symptoms that occurs in atopic individuals (atopic march). The prevalence of ‘any sensitisation’ increased with the age of the child from 17.0% at 1 year, 25.8% at 3 years and 49.5% 6 years.

Results of this post hoc analysis show that there was not enough evidence to conclude that n-3 LCPUFA supplementation was associated with a change in the relative risk of allergic disease with sensitisation or sensitisation to individual allergen extracts over time. After excluding the group by time interaction, there were no statistically significant effects of prenatal n-3 LCPUFA supplementation on the risk of allergic disease symptoms with sensitisation or sensitisation to individual allergen extracts across all years however there was a 37% reduction in risk of peanut sensitisation and 33% reduction in risk of egg sensitisation which may have some clinical significance.
Although collectively, results of this longitudinal analysis did not reach statistical significance, results seen at discrete time points and their relationship with the atopic march warrant further mention. It is well documented that early sensitisation to food protein (egg/cow’s milk) predicts later sensitisation to aeroallergens and allergic disease, especially in children with atopic heredity or early atopic symptoms \(^{28, 30, 132, 133}\). At 1 year of age, there was a significant reduction in the incidence of sensitisation to egg followed by a significant reduction in sensitisation to *D. farinae* and a reduction in parent reported hayfever ever at 6 years of age. Although it is questionable that effects of n-3 LCPUFA supplementation on the fetal immune system during pregnancy would persist until 6 years of age, allergic disease is a cascade and there is a distinct possibility that the significant reduction in the sensitisation to food protein (egg) seen at 1 year of age modified the trajectory of disease. Analysis of associations between egg sensitisation at 1 year and *D. Farinae* sensitisation at 6 years showed a statistically significant difference between those with or without egg sensitisation overall and between groups. An unexpected finding at 3 years of age was an increase in sensitisation to cat in the n-3 LCPUFA group, despite no differences in cat ownership between the groups in the first 3 years of life. This finding is not consistent with previous studies, did not persist at 6 years and was most likely an aberration associated with the atopic march.

The protective effect of maternal n-3 LCPUFA supplementation during pregnancy on sensitisation outcomes in the offspring at 1 and 6 years and lack of significant impact on overall allergic disease symptoms raises the question of relevance of sensitisation and manifestation of disease. Whilst there was a borderline significant reduction in parent reported hayfever at 6 years, the reduction in ‘medically diagnosed eczema’ at 1 year did not reach statistical significance. The presence of an IgE mediated reaction to food
or inhalant allergens detected by SPT or serum IgE generally confirms that an individual is “atopic”, although the relationship between sensitisation and symptoms of allergic disease is known to be complex and dynamic. Recent reports challenge the generalisability of ‘atopic disease’ and suggest that IgE antibody responses do not reflect a single phenotype of atopy. A number of studies suggest multiple different atopic vulnerabilities that differ in their relationship with clinical expression of disease acing to atopy phenotype (155, 158, 169). Further investigation and classification of atopy phenotypes may further inform generalisability of results and a targeted preventative treatment approach, not to mention a better understanding of the pathogenesis of the atopic march (169).

The major strength of this analysis is the use of data from a well conducted RCT with a low risk of bias (145). Results of the 1 & 3 year allergy follow up have been published previously. Assessment of the allergic disease component of the allergy assessment was determined by a medical assessment for diagnosis of eczema and food allergy at 1 year and eczema, asthma and rhinitis at 3 years as opposed to questionnaire responses to determine allergic disease symptoms at 6 years. However, data in this longitudinal analysis was analysed using consistent allergic disease symptom questions based on ISAAC core modules that were collected at all three time points (1, 3 & 6) and skin prick test were conducted according to the same standardised procedure and protocols. A limitation of this analysis may be that the ISAAC questionnaire has been validated in children 6-7 years and of interest, is the inconsistency of ISAAC questionnaire data and medical diagnosis at 1 and 3 years of age. Eczema symptoms (with sensitisation) were underreported at 1 year when using ISAAC questionnaire data (25/706) compared to medical diagnosis of eczema (65/706). At 3 years, rhinitis symptoms (with sensitisation) were more likely to be reported by parents via ISAAC
questionnaire (53/706) than by medical diagnosis (38/706). A second limitation of this analysis is the over prediction of effect size as discussed in Chapter 3, and potential under-powering of the study at each time point, making it difficult to realise any longitudinal benefits.

The longitudinal nature of the data presented in this analysis serves to inform the field regarding the effect of n-3 LCPUFA on the pattern of sensitisation and allergic disease outcomes over the first 6 years of the child’s life. This is the first report of allergic disease assessment over the life of the child from 1-6 years of age following an RCT of prenatal n-3 LCPUFA supplementation and is a prerequisite for the continuous search for the prevention of allergic disease.

The plausible mechanisms (discussed in Chapter 1) leading to modulation of the developing immune system to a less allergic phenotype in the first year of life (following prenatal n-3 LCPUFA supplementation) may have the potential to interrupt the trajectory of the atopic march and may have benefits of decreased sensitisation throughout the life of the individual. Further follow-up of sensitisation patterns and incidence of disease as the child approaches adolescence may provide a more accurate account of the true effect of n-3 LCPUFA supplementation during pregnancy on the atopic march.
General Discussion
Summary of the rationale for, and results of, my study

The onset of allergic disease typically presents in early life, suggesting that the fetal immune system may be primed for the development of allergic disease before birth. There is an increasing body of evidence reporting modulation of immune biomarkers in the infant following prenatal n-3 LCPUFA supplementation which further supports the hypothesis that maternal diet may play a role in the programming of the fetal immune system development, immune cell function and subsequent allergic disease (67, 83, 88, 96, 98, 101, 104, 111, 117, 139, 170-174).

Results from cohort studies indicate that fish consumption 2-3 times per week (vs never) during pregnancy reduces the risk of atopic eczema at 1 (96, 101), 2 (104) and 5 years (111), hayfever at 5 years (111) asthma at 18 months and at 7 years (137) a positive SPT for house dust mite at 6 years (86), atopic wheeze at age 6 years (96, 98). Although the apparent consistency of these associations is promising, it is not possible to infer a causal link between increased n-3 LCPUFA exposure (via fish) in pregnancy and the reduction of allergic disease as it not possible to exclude the presence of residual confounding from environmental factors or that there may be constituents of fish other than n-3 LCPUFA driving these associations. RCTs in early childhood have demonstrated that prenatal supplementation with n-3 LCPUFA reduces the risk of atopic eczema at 1 year (113, 116) and 2 years (175), and sensitisation to egg at 1 year (113, 116, 118) and 2 years (175). The only RCT to assess outcomes beyond 3 years of age, a registry based follow up, found a reduced risk of atopic asthma (119). The noticeable consistent effects of these results indicate some modulation towards the development of IgE-mediated allergic disease, however small sample sizes and a lack of consistently controlled outcome assessments cannot exclude the possibility of bias or random error.
I systematically reviewed the literature and identified five RCTs investigating the effect of n-3 LCPUFA supplementation of pregnant women on outcomes of allergic disease and eleven prospective observational studies observing dietary intake of n-3 LCPUFA during pregnancy and outcomes of allergic disease. The results of my systematic review and meta-analysis showed that increased prenatal intake of n-3 LCPUFA in observational studies and RCTs are both suggestive of benefits of a reduction in allergic disease symptoms and sensitisation in early childhood. However, apart from the registry based RCT at 16 years, there was no evidence to see if these effects persisted beyond early childhood.

My study is the first RCT designed to investigate the effects of prenatal n-3 LCPUFA supplementation on outcomes of allergic disease in the offspring at six years of age (early school age). Results of this 6 year nested allergy follow up confirm that prenatal n-3 LCPUFA supplementation to women carrying a fetus at high risk of allergy has no effect on the composite primary outcome of; symptoms of allergic disease (eczema, wheeze, rhinitis, rhino-conjunctivitis) with sensitisation. However, results from analysis of secondary outcomes suggest a significant reduction in sensitisation to HDM (D. farinae) and a reduction in ‘parent reported hayfever’ in the n-3 LCPUFA group.
Situating my study in the context of other findings of n-3 LCPUFA in pregnancy and allergic disease outcomes

There are data to indicate a modulation of the neonatal immune response towards a less allergic phenotype in response to n-3 LCPUFA supplementation (118, 172). A small number of n-3 LCPUFA intervention studies during pregnancy have demonstrated protective effects of supplementation on the incidence of atopic eczema (113, 116, 118), wheeze (118) and sensitisation (113, 116, 118) although all were conducted in early childhood and some were not specifically designed and powered to assess these clinical outcomes (113, 118). A RCT by Dunstan et al designed to determine the feasibility, safety and effectiveness of maternal fish oil supplementation in modifying neonatal n-3 LCPUFA status showed a significant reduction in sensitisation to egg at 12 months and down regulation of a range of cord blood mononuclear cell cytokine responses to allergens in the intervention group (118). These data are consistent with the trends from Swedish study (113) showing fewer infants with IgE associated eczema, sensitisation to egg and period prevalence of “any positive SPT” at 12 months in response to n-3 LCPUFA supplementation from the 25th week of gestation. However, the lack of clarity regarding randomisation and allocation procedures and the small numbers in both studies cannot exclude the possibility of bias or random error. Results from the largest RCT of prenatal n-3 LCPUFA supplementation (powered to assess clinical outcomes of allergic disease) found consistent results with other RCTs at 1 year of age with a reduction in eczema and sensitisation to egg (116). This cohort of children are those who have participated in my 6 year allergy follow up study detailed in this thesis. At 6 years I found a reduction in sensitisation to HDM and a reduction in the incidence of parent reported hayfever, however when these children were assessed at 3 years of age, there were no significant results. This
highlights the importance of longer term follow of RCTs investigating allergic
disease outcomes in children and the complexity of assessing the impact of a
pregnancy intervention in a dynamic disease that may change clinical presentation
over time. The only other RCT to conduct follow up beyond early childhood was a
Danish, registry-based 16 year asthma follow up of offspring whose mothers were
allocated to fish oil, olive oil or no treatment during the last 10 weeks of pregnancy.
Although this RCT showed a significant reduction in asthma and atopic asthma
diagnosis (from medical records), there were surprisingly low percentage rates of
asthma prevalence across all groups and a lack of consistently controlled outcome
assessments which necessitate further research.

To my knowledge my longitudinal analysis is the only long term follow up study
assessing the effects of n-3 LCPUFA supplementation on allergic disease
symptoms and sensitisation over time between the ages of 1 and 6 years. These
results showed that there was no significant effect of n-3 LCPUFA
supplementation over time, however there are known associations with egg
sensitisation and subsequent HDM sensitisation in atopic individuals (133).
Exploratory analysis to investigate the association of egg sensitisation at 1 year of
age and subsequent HDM sensitisation at 6 years of age showed significant
increased risk of HDM sensitisation at 6 years if the child was sensitised to egg at
1 year. These results are consistent with the atopic march however it is a plausible
assumption that the reduction in egg sensitisation seen at 1 year in this cohort
may translate to reduction in HDM seen at 6 years. Further analysis and future
follow up of this cohort is important to ascertain the clinical implications of a
reduction in sensitisation on progression of the atopic march including symptoms
of allergic disease (respiratory disease in particular) and sensitisation.
Limitations of my study and directions for future research

Prenatal n-3 LCPUFA supplementation of children at risk of atopy did not affect overall symptoms of IgE mediated allergic disease in my study, although secondary outcomes suggest beneficial effects of supplementation on sensitisation to HDM and hay fever. There is a distinct possibility that these beneficial effects were due to chance, however, the fact that these outcomes were obtained from a large, well designed and executed RCT in addition to corroboration with outcomes from previous RCTs leads one to believe they are true, however further evidence would be needed to unequivocally determine this. The null effect of n-3 LCPUFA supplementation on the primary outcome at six years of age may be due to a number of factors including dilution of effect over time or a lack of adequate sample to assess the true effect of supplementation, particularly as the effects were smaller than originally hypothesized. Sample size calculations for the DOMInO trial nested allergy study were based on pilot data from Dunstan et al (118). With >328 children per treatment group, the DOMInO trial allergy follow up was powered to detect an absolute reduction of 10% (relative reduction of 33%) in the cumulative incidence of IgE-mediated allergic disease from 30% to 20% with >80% power (a = 0.05). Whilst some results at 1 year were consistent with this study by Dunstan, we did not see the same reductions in relative risk. It is a possibility that the effect size used in power calculations was over estimated resulting in the possibility of a type II error. Compared with the DOMInO trial allergy follow up, other RCTs have supplemented women with higher doses of n-3 LCPUFA ranging from 2700 mg/day (113, 119) to 3700 mg/day (118). The trial with the greatest effect on outcomes continued supplementation until 3.5 month post-partum which may be an important influencing factor on results. It seems that dose, timing and duration of n-3 LCPUFA supplementation may be
important considerations and worthy of further investigation. Future studies may consider continuation of prenatal n-3 LCPUFA supplementation into the postnatal period through supplementation of breast feeding mothers. There is evidence that maternal stores of n-3 LCPUFA are not easily replenished following pregnancy (176, 177). Additional post-partum n-3 LCPUFA supplementation may augment the anti-inflammatory effects seen during pregnancy while the infant's immune system continues to develop.

Further well powered RCTs are needed to assess the implications that a reduction in sensitisation may have on the clinical expression of disease, particularly in at risk populations (i.e. infants at hereditary risk). The reduction of sensitisation to peanut and cashew at 6 years of age in my study warrant further investigation. Based on my imputed results, to detect a reduction in peanut sensitisation from 11.7% to 7.2% at 6 years, a future trial would need 930 participants per group in order to have 90% power (or 706 per group for 80% power) and to detect a reduction in cashew sensitisation from 6.9% to 3.6% would require 1523 participants per group to have 90% power (or 1157 per group for 80% power).

The evidence is tantalisingly suggestive that prenatal n-3 LCPUFA supply has an effect on the intrauterine environment which in turn, seems to impact on the risk of developing allergic disease symptoms, in particular, sensitisation however the lack of consistent results cannot be ignored. The heterogeneity of atopy is not well characterised and recent reports suggest that IgE antibody responses do not reflect a single phenotype of atopy, but rather multiple different atopic vulnerabilities (155, 158). Exploration and categorisation of specific phenotypes of atopic disease and subsequent response to n-3 LCPUFA may provide answers to variations in effect and enable a targeted prevention strategy.
Concluding remarks and recommendations

Findings from my project indicate that prenatal supplementation with 900mg of n-3 LCPUFA from 18-20 weeks gestation until birth does not reduce the overall incidence of IgE mediated allergic disease at 6 years of age. However, my results did show a significant reduction in sensitisation to HDM (D. farinae) and a reduction in parent reported hayfever ever in the n-3 LCPUFA group. The work in this thesis and the current literature surrounding n-3 LCPUFA supplementation during pregnancy remain suggestive of benefits although there is insufficient evidence to definitively determine beneficial effects. On the basis of this evidence, routine supplementation of pregnant women with n-3 LCPUFA cannot be recommended as a primary preventative strategy for allergic disease. Whilst this work adds significantly to the body evidence, further high quality trials of n-3 LCPUFA supplementation in pregnancy with well-defined and consistently assessed allergic disease outcomes are needed to inform public health policy and support translation to clinical practice.
Bibliography


3. Economics A. The economic impact of allergic disease in Australia: Not to be sneezed at. Report for Australasian Society of Clinical Immunology and Allergy. 2007.


12. Jeeves. Irvine College of Medicine, Medical Immunology Core Notes. 2013 [cited 2013 10/05/2015]. Medical Immunology Core Notes. Available from: http://jeeves.mmg.uci.edu/immunology/CoreNotes/CoreNotes.htm


143. Saadeh D, Salameh P, Baldi I, Raherison C. Diet and allergic diseases among population aged 0 to 18 years: myth or reality? Nutrients. 2013 Sep;5(9):3399-423.


152. NOTE FOR GUIDANCE ON GOOD CLINICAL PRACTICE (CPMP/ICH/135/95), (2000).


162. UNIT PO. Pregnancy Outcome in South Australia. 2008.


168. Warner JO, Pohunek P, Marguet C, Clough JB, Roche WR. Progression from allergic sensitization to asthma. Pediatric Allergy and Immunology. 2000;11:12-4.


Appendix 1: Participant Information Sheet

6 year Allergy Follow-Up of children who participated in the DOMInO study

SCIENTIFIC TITLE: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children?

Dear (Mother/Carer),

We thank you and your child for your on-going commitment to the DOMInO study through your participation in the allergy follow-up at 1 and 3 years of age.

You may remember that the allergy follow-up involved 706 families who had at least one family member with a history of asthma, eczema or hayfever. We are interested in determining whether taking fish oil supplements during pregnancy can reduce the development of allergies in children and how long these effects may last. At 1 year of age, we found that there were fewer children with atopic eczema in the group whose mothers took fish oil supplements during pregnancy than those in the control group that took vegetable oil capsules, but there were no differences in the number of children with food allergies between the two groups. As your child is now approaching 6 years of age, we are writing to invite you and your child to participate in another allergy follow-up to investigate any changes in allergies as your child reaches school age. At this age, we expect asthma and hayfever to become more common and for many children to have outgrown their eczema and food allergies. By examining all children at 6 years of age, we will be able see whether the effects of fish oil supplementation during pregnancy are long standing or are only seen in early childhood.

What does the 6 year follow-up involve?

You and your child will be asked to attend one appointment at the Women’s and Children’s Hospital when your child is 6 years of age. At this appointment, your child will have a skin prick test and we will measure their height and weight and head circumference. You will be asked to complete two questionnaires to determine whether your child has any allergies and to assess their general health and wellbeing. The questionnaires should take around 30 minutes to complete. We will also ask you general questions regarding your child’s pre-school/school activities, illnesses, medications, hospitalisations and diet. If your child has been hospitalised since we last saw you, we will need to access your child’s medical records to document the reasons for hospitalisation and the treatments given. At 6 years of age, we will again ask you for information about smoking, pets and heaters in the home environment, dust mite protection on
your child’s bedding, maternal occupation/education and whether you have any other children that suffer from allergies.

Generally, the whole appointment at the hospital will last for about one hour. You will be given $20 to offset expenses associated with attending the appointment (such as travel and car parking). There is no need for any dietary supplements to be taken as a part of this 6 year follow-up study.

**Skin Prick Test**

The skin prick test will involve droplets of test solutions (containing different allergens) being placed onto the skin on your child’s back. The skin beneath each droplet will then be gently scratched with a sterile needle. Several allergens are tested at the same time on different areas of skin, requiring several scratches. This is usually painless, as just the very surface of the skin is scratched. The skin is then observed for a reaction to the allergen. If a reaction occurs, it usually happens within 20-30 minutes and gradually fades over an hour or so. **During this time your child may develop redness and a bump at the site where the allergen droplet was applied.** The size of any bumps will be recorded to determine the results of the test. Oral antihistamine syrups and tablets interfere with skin prick testing and in preparation for the test, we will ask that your child stops taking these 4 days before the test. In addition, on the day of the test, moisturisers should not be applied to the skin, prior to testing.

**Any Risks?**

There are many published reports on the use of skin prick testing in infants and children and the risks associated with the skin prick tests are very low. If your child has an allergic response to any of the test solutions applied, a small bump may develop and cause some temporary localised discomfort such as itching, accompanied by temporary redness around the area. Any unexpected severe allergic reactions will be treated immediately within the Hospital.

**Your rights**

If you decide to participate you are free to withdraw from the follow-up study at any time without any explanation of why you have chosen to do so and without prejudice to you or your child’s future care.

All information gathered will be treated with confidence and no information that could identify you or your child will be released to any person not associated directly with the study except in the case of a legal requirement to pass on personal information to authorised third parties. This requirement is standard and applies to information collected both in research and non-research
situations. Such requests to access information are rare; however we have an obligation to inform you of this possibility. Results from the study will be published in medical journals and at professional meetings, but neither you nor your child will be identified in any way. If you would like further information about the study please contact our research staff on (08) 8161 8045.

If you would like to take part in this ‘6 year Allergy follow-up study’ please complete the enclosed consent form and participant details form and return both to us in the reply paid envelope provided. One of our research staff will then telephone you to explain the study, answer any questions you may have and discuss arrangements for the appointment.

6 Year Allergy Follow-Up Study Questions

If at any time during the study you have any problems or questions, please ring our office on 8161 8045 and leave a message on our answering machine; one of our nurses will return your call as soon as possible. If you have an urgent problem and would like to talk to us immediately please ring 8161 7000 and ask for pager 5864, and one of our research nurses will answer your call.

Women’s and Children’s Health Network (WCHN) Human Research Ethics Committee Questions

This study has been reviewed and approved by the Women’s and Children’s Health Network (WCHN) Human Research Ethics Committee, approval number (2435/12/14). Should you wish to discuss matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the executive secretary of the Human Research Ethics Committee, Ms Brenda Penny, WCHN (8161 6521).

Yours Sincerely

The DOMino team

Child Nutrition Research Centre
Women’s and Children’s Hospital
72 King William Road
North Adelaide. 5007
Tel: 8161 8045
Appendix 2: Participant Consent Form

WOMEN’S & CHILDREN’S HEALTH NETWORK (WCHN)
HUMAN RESEARCH ETHICS COMMITTEE (HREC)

CONSENT FORM

6 year Allergy Follow-Up of children who participated in the DOMInO study

SCIENTIFIC TITLE: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children?

I (insert name)_______________________________________________________

hereby consent to my child's involvement in the research project entitled:

6 year Allergy Follow-Up of children who participated in the DOMInO study

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it and agree to taking part.

2. I understand that my child may not directly benefit by taking part in this study.

3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.

4. I understand that I can withdraw from the study at any stage and that this will not affect medical care or any other aspects of my child's relationship with this healthcare service.

5. I understand that I will be reimbursed a total of $20 for attendance at the appointment when my child is 6 years of age, to offset expenses associated with attending the appointment (such as travel and car parking).
6. I have had the opportunity to discuss taking part in this research project with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.

7. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.

8. I consent to my child having a skin prick test at 6 years of age, as explained in the Information Sheet.

9. I consent to my child having his/her height/weight/head circumference measured, as explained in the Information Sheet.

10. I consent to answering the questionnaires and additional questions, as explained in the information sheet and I am aware that the questionnaires will take approximately 30 minutes to complete.

11. I understand that study personnel may review my child’s medical records at the Women’s and Children’s Hospital and any other hospital my child may be transferred to and from.

12. I understand that my own and my child’s information will be kept confidential as explained in the information sheet, except where there is a requirement by law for it to be divulged.

13. I understand that I may be contacted about possible involvement in future follow-up studies conducted by the Child Nutrition Research Centre.

Signed:  ........................................................

Relationship to Patient:  ......................................................

Full name of patient:  .........................................................

Dated: .......................... 

I certify that I have explained the study to the parent and consider that he/she understands what is involved.

Signed:  ........................................................ Title:  ...................................................

Dated: ..........................
Appendix 3: Updated Contact Details Form

Updated Contact Details

6 Year Allergy follow-up of Children who participated in the DOMInO Study

As this is an on-going study, please complete this form with your current details and return to the address below (no stamp required):

DOMInO 6 Study
Child Nutrition Research Centre (64)
Women’s & Children’s Hospital
Reply Paid 60668, NORTH ADELAIDE SA 5006

DATE ____________________________ STUDY I.D. __ __ __ __ __

Child’s Details

Full Name:

Mother’s Details

Full Name:
Address:
Post Code:
Home Ph: Work Ph: Mobile:
E-mail:

Father’s Details

Full Name:
Address:
Post Code:
Home Ph: Work Ph: Mobile:
E-mail:

Alternative Contact Details (i.e. Grandparent, Aunty, friend)

Full Name:
Relationship to child:
Address:
Post Code:
Home Ph: Work Ph: Mobile:
E-mail:

Alternative Contact Details (i.e. Grandparent, Aunty, friend)

Full Name:
Relationship to child:
Address:
Post Code:
Home Ph: Work Ph: Mobile:
E-mail:

Thank you for your time ☺
Appendix 4: Appointment Confirmation Letter

Dear Parent Name,

Thank you again for participating in the follow up of the DOMInO Study. This letter provides details for Child Name’s 6 year appointment for the Allergy Follow-up phase of the DOMInO Study, as arranged by phone.

At this appointment a nurse will ask you some questions and do a skin prick test on Child Name. We will also ask that you complete a brief child health questionnaire. You will need to allow approximately 45 minutes for this appointment. Your appointment details are:

When: Tuesday, 3rd December 2013

Time: 09:00

Where: Child Nutrition Research Centre, 1st Floor, Rieger Building, Women’s and Children’s Hospital

Enter the hospital from Kermode St, turn left at Rainbows Kiosk, take these lifts to the 1st floor. When you exit the lift, the CNRC reception is right in front of you. It is helpful to bring this letter in case you need to ask for directions.

Please do not give Child Name any medications containing anti-histamines for 4 days prior to this appointment (this includes Avil, Claramax, Claratyne, Clarinase, Dilosyn, Dimetapp, Dramamine, Fenamine, Fexotabs, Lorastyne, Periactin, Phenergan, Polaramine, Relaxa-tabs, Telefast, Travacalm, Unisom Sleepgels, Vallergan, Xergic, Zadine and Zyrtec).

It is also important that you do not apply any creams to your child’s skin on the day of the appointment (this includes moisturisers and steroid creams). You will receive $20 to help with travel costs for this appointment. If you have any queries or regarding this appointment please telephone 8161 8045.

Kind Regards,

The DOMInO Study Team
Appendix 5: HREC Amendment Request to use Facebook

03/04/2014

Dr Tamara Zutlevics
Chair, WCHN Human Research Ethics Committee

Dear Dr Zutlevics,

Re: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children? Six year allergy follow-up of children who participated in the DOMInO study; REC2435/12/14

I write to seek your approval for an amendment to the above mentioned study. The DOMInO 6 year follow up has been granted HREC approval to contact DOMInO study participants by letter to inform them of the six year allergy follow up and invite participation. Unfortunately, a number of families are no longer contactable with the telephone and address details that we have on record. In an effort to ensure all participants are provided the opportunity to take part in this study, we would like to request permission to contact parent/s of the child by using the social media platform, ‘Facebook’.

Purpose
The DOMInO Study Facebook page is multi-purpose and includes the following;

- to keep participants up to date with study activity and enhance retention using a platform congruent with current communication preferences
- a method of contacting participants to ascertain if they would like to participate in DOMInO study follow up
- an avenue for participants to contact study staff if they wish to do so
- dissemination of study results

Method
We aim to contact participants via Facebook private messaging service to advise them of the study and ascertain if they would like to participate. A tracing message will be used in the first instance as there may be more than one person with the same name. The wording of this message will be as follows;
Hello “Participant name”, we are trying to contact women who took part in the DOMInO Study in South Australia to let them know about an allergy follow up study for their child. If you took part in the DOMInO Study and would like to receive this information, please reply to this message with your updated telephone and/or address details and we can send the information to you. Or you can call one of the DOMInO Study team on 08 8161 8045. If you did not take part and have received this message in error, sincere apologies for any inconvenience. “The DOMInO Team”

The parent/s then has the choice to respond via private messaging service, the contents of which, will only be visible to the page administrator. If updated contact details are received, the family will be sent the HREC approved, study invitation pack.

Page Management
Page administrators will have access to the page to post general information and will be assigned the responsibility for monitoring and maintaining DOMInO study page content. There will always be at least two administrators to ensure coverage if one staff member is unable to monitor the page. A list of individuals with administrative rights will be maintained within the department and new administrators will require approval from the WCHRI Operations manager. Page administrators will be required to keep abreast of changes in policies or functionality of Facebook and maintain compliance with terms of service.

Page administrators;
• will communicate at all times with integrity, respect, and accountability in accordance with the Mandatory Policy Directive, Media Protocols – SA Health¹ and the ‘Code of Ethics’ South Australian Public Sector²
• will ensure content is current and accurate
• are responsible for ensuring consent of all involved parties for the right to distribution or publication of photo’s or images as per WCHN Media policy
• are responsible for regularly checking postings and comments to monitor content submitted and will remove any inappropriate content
• will not submit any content advertising or endorsing any products or services of any other organisation

Privacy

Appropriate safeguards will exist to protect the rights and welfare of research participants. No content will be submitted by staff that personally identifies any other person without their express consent. Participants who have already completed the DOMInO 6 year follow up will be invited to “like” the ‘DOMInO Study’ page so they receive related ‘posts’ to their timeline to keep informed of study progress and eventually study results. If they chose to do so, information regarding the limitations to privacy will be communicated clearly by the use of the warning below;

“Depending on your own Facebook privacy settings, your privacy from other people cannot be guaranteed. For example:

• if you ‘like’ this page the study page will appear on your own Facebook page
• if you choose to ‘like’ any comments or photos on the study page other people will be able to view your name and/or profile
• if you write a comment (post) for the study page other people will be able to view your name and/or profile”

Please find a summary of proposed Facebook settings attached for your reference. If you require any additional information, please don’t hesitate to contact me.

Yours Sincerely,

Karen Best RN, RM, PhD Candidate
Child Nutrition Research Centre
(a division of the Women's and Children's Health Research Institute)
Ph: 8161 7154
Mobile: 0434243404
Email: karen.best@adelaide.edu.au

Professor Maria Makrides
Director, Women's & Children's Health Research Institute
Level 7, CRB, Women's and Children's Hospital
72 King William Road
North Adelaide SA 5006
Ph: 8161 6067
Mobile: +61 (0)418 837 482
Email: maria.makrides@health.sa.gov.au

CC Professor Maria Makrides
APPENDIX 1 – HREC Amendment to use Facebook
About

The DOMInO Study (Docosahexaenoic Acid to Optimise Mother Infant Outcome) is run by the Child Nutrition Research Centre.

Description

The Child Nutrition Research Centre (CNRC) is a joint venture of the Women’s & Children’s Health Research Institute (WCHRI) Flinders Medical Centre (FMC) of the South Australian Health Service and Women’s and Children’s Hospital (WCH) of the Children, Youth and Women’s Health Service.

Children born to mothers who took part in the DOMInO study have been attending follow up appointments since 2007 to see if dietary omega 3 (EPA) fatty acids, in particular docosahexaenoic acid (DHA), influenced their growth and development or risk of allergies.

Basic Info

Started: 2005

Contact Info

Website: http://www.wchri.com.au/Appledatumon...
<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
<th>Edit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Page Visibility</td>
<td>Page unpublished</td>
<td></td>
</tr>
<tr>
<td>Posting Ability</td>
<td>Anyone can post to my Page timeline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anyone can add photos and videos to my Page timeline</td>
<td></td>
</tr>
<tr>
<td>Post Visibility</td>
<td>Posts to Page are hidden from my Page timeline</td>
<td></td>
</tr>
<tr>
<td>Post Targeting and Privacy</td>
<td>Privacy control for posts is turned on</td>
<td></td>
</tr>
<tr>
<td>Messages</td>
<td>People can contact my Page privately.</td>
<td></td>
</tr>
<tr>
<td>Tagging Ability</td>
<td>Only Page admins can tag photos posted on my Page.</td>
<td></td>
</tr>
<tr>
<td>Notifications</td>
<td>On Facebook and Email</td>
<td></td>
</tr>
<tr>
<td>Country Restrictions</td>
<td>Page is visible to everyone.</td>
<td></td>
</tr>
<tr>
<td>Age Restrictions</td>
<td>Page is shown to everyone.</td>
<td></td>
</tr>
<tr>
<td>Page Moderation</td>
<td>No words are being blocked from the Page.</td>
<td></td>
</tr>
<tr>
<td>Profanity Filter</td>
<td>Set to strong</td>
<td></td>
</tr>
<tr>
<td>Similar Page Suggestions</td>
<td>Choose whether your Page is recommended to others</td>
<td></td>
</tr>
<tr>
<td>Replies</td>
<td>Comment replies are not yet turned on for your Page</td>
<td></td>
</tr>
<tr>
<td>Remove Page</td>
<td>Delete your Page</td>
<td></td>
</tr>
</tbody>
</table>
# DOMInO Study

<table>
<thead>
<tr>
<th><strong>Name</strong></th>
<th>DOMInO Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Facebook Web Address</strong></td>
<td><a href="http://www.facebook.com/DOMInOStudy">www.facebook.com/DOMInOStudy</a></td>
</tr>
<tr>
<td><strong>Category</strong></td>
<td>Other : Community</td>
</tr>
<tr>
<td><strong>Start Info</strong></td>
<td>Started on 2005</td>
</tr>
<tr>
<td><strong>Short Description</strong></td>
<td>The DOMInO Study (Docosahexaenoic Acid to Optimise Mother Infant Outcome) is run by the Child Nutrition Research Centre</td>
</tr>
<tr>
<td><strong>Long Description</strong></td>
<td>The Child Nutrition Research Centre (CNRC) is a joint venture of the Women's &amp; Children's Health Research Institute (WCHR) and the Flinders Medical Centre (FMC) ...</td>
</tr>
<tr>
<td><strong>Official Page</strong></td>
<td>Enter the official brand, celebrity or organization your Page is about</td>
</tr>
<tr>
<td><strong>Facebook Page ID</strong></td>
<td>256611409795423</td>
</tr>
</tbody>
</table>
DOMino Study

All Page admins can have a different role assigned to them, depending on what they need to work on.

Learn more about different kinds of admin roles.

Karen Best
Manager
Karen can manage admin roles, send messages and create posts as the Page, see which admin created a post or comment, create ads, and view insights.

Add Another Admin

Store  Cancel

Facebook © 2014 · English (US)
Appendix 6: Skin Prick Test SOP

CNRC_SOP_028_V1_Skin Prick Testing (SPT) – Adults and Children

1. INTRODUCTION AND PURPOSE

The objective of this SOP is to detail the procedures required when performing adult and child skin prick testing on study participants involved in clinical trials within the Child Nutrition Research Centre (CNRC) or in collaboration with the CNRC.

Skin prick testing to common allergens is performed to determine whether the participant is atopic and to identify the specific allergen causing the clinical reactivity.

The Skin Prick Testing (SPT) procedures outlined in this SOP are based on the 2009 ASCIA Skin Prick Testing for the Diagnosis of Allergic Disease: A manual for practitioners. www.ascia.com.au

2. SCOPE/APPLICABILITY

This SOP applies to all clinical research staff within the CNRC who are expected to perform or assist with adult and child skin prick testing. This test should cause minimal discomfort and has a low risk of adverse side effects including anaphylactic reaction when performed on participants who are well.

The skin prick testing procedures outlined are a guide for measurements performed in relation to CNRC clinical trials, there may be study specific requirements for individual clinical trials which require additional procedures, please refer to your trial protocol.

3. PROCEDURE

3.1 Contraindications:

3.1.1 Pregnancy: Do not perform this procedure on a pregnant woman.
3.1.2 Some medication such as beta blockers or monoamine inhibitors or high dose corticosteroids: Check list in Appendix 1 and then consult specific study protocols to assess washout period required
   *Appendix 1 to be reviewed annually
3.1.3 An individual suffering symptoms of influenza or gastroenteritis: defer appointment for at least 1 week.
3.1.4 Anaphylaxis within 2 weeks prior to testing

3.2 Precautions:

3.2.1 The skin at the test site must be intact with no eczema, infection or rash evident.
3.2.2 If an individual has had a history of fainting during previous procedures, lay participant in supine position before commencing SPT procedure.
3.2.3 Emergency equipment and drugs should always be readily available in case of anaphylaxis. See page 5.
3.2.3 Allergen extracts should be obtained from reliable commercial sources, stored at 2-8°C when not in use, and discarded after the use-by date.

3.3 Equipment:

- Detergent wipes
- Short clear ruler
- Baby wipes – non allergenic
- Timer
- Skin prick lancets
- Hand wash
- Aquim hand gel
- 3M Scotch tape
- Marker pen for the skin
- Pillow
- Allergens (including positive – histamine - and negative controls)
3.4 Method

3.4.1 Inform the participant of the procedure and ask about any history of anaphylaxis or reactions to procedures, e.g. fainting. Ensure the participant has given written consent for the procedure. Also enquire about their general health today.

3.4.2 Check that the participant has not had any antihistamine medications within the last 4 days.

3.4.3 For adults and children 11 years and over, sit the participant on a chair with the arm extended on pillow on table or desk, or lie on a bed on their back. For children 10 years and under, sit the participant in a comfortable position (assisted by parent/guardian if necessary) with the back at a convenient height for the practitioner to do the test, or lie on their chest to give access to their back.

3.4.4 Inspect the skin in the area where testing is to be undertaken.

3.4.5 Wash hands and prepare skin. If recent application of sunblock or moisturizer reported then clean area with non-soap substance e.g. Dermaveen wash or use plain water.

3.4.6 If using tape, mark a strip of low allergenic tape with the number of skin pricks planned (the allergens, plus positive and negative controls). Numbers should be a minimum of 2cmms apart. If using the forearm, apply the tape so that it is ≥ 5cmms from wrist and ≥ 3cmms from elbow crease and mark the numbers as shown in Figure 1 to provide maximum space between each allergen. If using the back, use the upper back and mark the numbers as shown in Figure 2.

Figure 1: Positioning of numbers on tape if using forearm

Figure 2: Positioning of numbers on tape if using back

3.4.7 If using a marker, positions for skin pricks should be marked by numbers on the skin to identify the allergen. Ensure it is a marker appropriate for use on skin.

3.4.7 Include negative and positive controls.

3.4.8 Squeeze a small drop of test solution onto the skin adjacent to the corresponding marked number. Do not touch the skin with the dropper. In co-operative participants, all drops may be deposited prior to prickng the skin. *The allergens should be stored in a temperature-monitored refrigerator and left out for as short a time as necessary to conduct the test.

3.4.9 Place the sharp end of a sterile lancet in the centre of the droplet, penetrate the skin to allow a prick and lift action. The aim is to prick and lift the first 1-2 layers of skin only
without drawing blood. Repeat with a new lancet for each droplet of extract. Dispose of the used lancet in the sharps container. Start the timer.

3.4.10 The droplets must be left in contact with the skin and can then be blotted off after 1 minute without affecting the readings. Ensure there is no wiping movement that could transfer the extract.

3.4.11 Read the histamine control reaction at 10 minutes.

3.4.12 Read the remaining reactions after a further 5 minutes.

3.4.13 A reading represents the mean diameter of the wheal size in mm. See Appendix 2 for a complete description of measuring technique. Record this figure only on the recording form next to the specific allergen name.

3.4.14 If a wheal > 3mm is not evident for histamine result, repeat test on alternate forearm. *Have another staff member verify borderline results.

3.4.15 Do not include pseudopodia in the measurements. Note its presence with a (P).

3.4.16 The flare (erythema) is not recorded.

3.4.17 After reading the results wash and dry the skin. Apply Eurax if required. (Topical corticosteroids have been shown not to be useful). Advise participant that itch and wheal will settle over the next 2-4 hours.

3.4.18 Record date and time of SPT and name of person performing test.

3.4.19 There should be a post test holding time of 40 minutes, i.e. 20 minutes after completion of readings. It is unnecessary to detain a participant if all SPT results (except histamine) were negative.

3.4.20 Clean all equipment using detergent wipes.

3.5 Reporting results to participants.
The participant is only to be given a formatted letter detailing results if he/she asks for it. Staff must fill in the blank spaces and hand to the participant. Advice on the interpretation of the results should come from his/her GP and that is the only referral we advise following a positive SPT.

3.6 Complications/action.

3.6.1 Safety/risks of skin prick testing
Skin prick testing is an extremely safe procedure, with minimal discomfort. Rarely, adverse events can occur; these can be classified into allergic, test-related non allergic, and non-specific. Examples of test-related non-allergic might include transmission of infection (theoretical but never documented); examples of non-specific are syncope, headache etc. Vasovagal fainting is relatively common and if the test is done on the participant in the sitting position, facilities should be available for the participant to lie down if feeling faint. The expected reaction to a skin prick test is a localised wheal and flare. Delayed local skin swelling (the late phase response) which is often tender or painful may occur uncommonly as a result of an IgE-mediated late-phase reaction. Rarely this can cause quite marked swelling and discomfort, however it does not usually last more than 36 hours. Systemic introduction of allergen may occur as an unintended consequence of the skin prick. Systemic reactions from skin prick testing have been recorded, including the typical manifestations of anaphylaxis such as generalised urticaria, angioedema including airway angioedema, bronchospasm, and
hypotension. However these reactions are generally mild and respond to treatment. All asthmatics should have an appropriate action plan in place, particularly where there are multiple strong positive skin prick test reactions.

3.6.2 Safety measures and safety equipment required
3.6.2.1 Skin prick testing must always be performed in a medical setting with the ready availability of appropriate resuscitation equipment and medical practitioners competent to treat generalised allergic reactions. It is recommended that participants who have undergone skin prick testing and have positive results and have asthma or a history of anaphylaxis, should remain in the centre for at least 20 minutes following completion of the skin prick test (total of 40 minutes after skin pricking).

3.6.2.2 Call a medical emergency (e.g. code blue 33# at WCH and FMC) for all episodes of generalised reaction – symptoms and signs of a generalised reaction include one or more of the following – generalised skin rash (usually urticaria), angioedema (facial or generalised), persistent cough, wheeze, stridor, hoarse voice, difficulty breathing, collapse with low blood pressure. Collapse may occur due to “fainting” but recovery occurs when the patient is supine. A medical (MO) needs to assess all children who have a generalised reaction and a code should still be called if it is unclear if the collapse is due to anaphylaxis or occurring due to a vaso-vagal event.

4. TRAINING


4.3 Observe a staff member performing skin prick testing

4.4 Be observed performing 20 skin prick tests on adult volunteers

4.4.1 Arrange a suitable time to be observed by a registered nurse who is authorised to provide SPT training and sign off competency in SPT.

4.4.2 Organise adult volunteers by sending an email request with date and time to the Operations Manager for approval. This must include an information sheet about the procedure and any adverse effects (Appendix 3). The Operations Manager will arrange for the email to be circulated to the WCHRI staff. Please note that it is not ethical to ask for volunteers on an individual basis.

4.4.3 Download a copy of the SPT Procedure Training Record (Appendix 4) and the SPT Procedure Competency checklist (Appendix 5).

4.4.4 Complete the SPT Procedure Training Record and the SPT Procedure Competency checklist during the observation period, and have it signed.

4.4.5 Provide the completed forms to the Operations Manager (OM) or Document Controller (DC) for filing in the Staff Training folder

4.5 If you are required to perform skin prick testing on children, you first need to be credentialled using adult volunteers. You need to then:

4.5.1 Arrange to observe skin prick testing on children of a range of different ages. (Make an appointment to visit Allergy Clinic as an observer).
4.5.2 Arrange to be observed performing skin prick testing on 5 children by a registered nurse who is authorised to provide SPT training in children and sign off competency in SPT in children.

4.5.3 Complete the SPT Procedure Training Record (appendix) and the SPT Procedure Competency checklist (appendix) during the observation period, and have it signed. These forms should be given to the Operations Manager (OM) or Document Controller (DC) for filing in the Staff Training folder.

5. GLOSSARY
Anaphylaxis
Any acute onset of hypotension or bronchospasm or upper airway obstruction where anaphylaxis is considered possible, even if typical skin features are not present.

OR
Any acute onset illness with typical skin features (urticarial rash or erythema/flushing, and/or angioedema), PLUS involvement of respiratory and/or cardiovascular and/or persistent severe gastrointestinal symptoms (particularly for insect allergy)

Symptoms/signs of respiratory/cardiovascular involvement are:

Respiratory:
- Difficulty/noisy breathing
- Swelling of tongue
- Swelling/tightness in throat
- Difficulty talking and/or hoarse voice
- Wheeze or persistent cough

Cardiovascular:
- Loss of consciousness
- Persistent dizziness
- Pale and floppy (in young children)
- Hypotension

Pseudopods
Irregular linear extensions of the wheal.

Operations Manager
Manager for Operation within the CNRC, directly reports to Director of WCHRI.

Document Controller
A person responsible for the distribution and maintenance of SOP’s.

6. REFERENCES
1. Note for guidance on Good Clinical Practice (CPMP/ICH/135/96) annotated with TGA comments DSEB, July 2000, sections 1 and 5.
Appendix 7: Skin prick test results for parents

Date

Name:
The above mentioned child underwent a skin prick test (SPT) as part of the DOMInO (DHA to Optimise Maternal and Infant Outcomes) study. Please find the result of the SPT below.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Weal (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Negative control</td>
<td></td>
</tr>
<tr>
<td>2 Whole Hens Egg</td>
<td></td>
</tr>
<tr>
<td>3 Peanut</td>
<td></td>
</tr>
<tr>
<td>4 Cashew Nut</td>
<td></td>
</tr>
<tr>
<td>5 Ryegrass pollen</td>
<td></td>
</tr>
<tr>
<td>6 Olive tree pollen</td>
<td></td>
</tr>
<tr>
<td>7 D. pteronyssinus</td>
<td></td>
</tr>
<tr>
<td>8 D. farinae</td>
<td></td>
</tr>
<tr>
<td>9 Cat</td>
<td></td>
</tr>
<tr>
<td>10 Alternaria tenuis</td>
<td></td>
</tr>
<tr>
<td>11 Dog</td>
<td></td>
</tr>
<tr>
<td>12 Histamine</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 8: Certificate for child

CERTIFICATE OF APPRECIATION

presented to

Child Name

For your valuable participation in the DOMInO 6 year Allergy follow up

With thanks from the Child Nutrition Research Centre
Appendix 9: GP letter with skin prick test results

Date

Dr. Name
Address
Address

Re: Child Name DOB:

Child Name is participating in the 6 year allergy follow up phase of the DOMInO study.

The DOMInO study (DHA to Optimise Mother Infant Outcome) was a randomized, placebo controlled trial that was designed to test the effect of tuna oil supplementation in pregnancy on postnatal depression and early childhood neurodevelopment. Participants were enrolled between 18 and 21 weeks gestation and randomly allocated to receive either tuna oil capsules or control vegetable oil capsules from enrolment until birth. Families where a parent or sibling of the fetus had medically diagnosed allergies were invited to join the allergy follow up.

Emerging data suggest that dietary n-3 long chain polyunsaturated fatty acids (LCPUFA), found in tuna oil, have immuno-modulatory benefits on the developing immune system and that these may be greatest in utero and before allergic responses are established. The aim of the 6 year allergy follow up study is to determine whether n-3 LCPUFA supplementation in pregnancy, with a fetus at high hereditary risk of atopic disease will result in fewer children with allergic disease (defined as asthma or rhinitis or eczema) with sensitisation at 6 years of age.

Child Name attended an appointment at the Women’s & Children’s Hospital where skin prick testing was performed. Please find the results below for your records;

Date of SPT:

<table>
<thead>
<tr>
<th>Extract</th>
<th>Wheal</th>
<th>Extract</th>
<th>Wheal</th>
<th>Extract</th>
<th>Wheal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>mm</td>
<td>Ryegrass pollen</td>
<td>mm</td>
<td>Cat</td>
<td>mm</td>
</tr>
<tr>
<td>Whole Hens Egg</td>
<td>mm</td>
<td>Olive tree pollen</td>
<td>mm</td>
<td>Alternaria tenuis</td>
<td>mm</td>
</tr>
<tr>
<td>Peanut</td>
<td>mm</td>
<td>D. pteronyssinus</td>
<td>mm</td>
<td>Dog</td>
<td>mm</td>
</tr>
<tr>
<td>Cashew Nut</td>
<td>mm</td>
<td>D. farinae</td>
<td>mm</td>
<td>Histamine</td>
<td>mm</td>
</tr>
</tbody>
</table>

If you have any queries regarding this research study or the results from this appointment please telephone 8161 7154.

Yours sincerely,

Karen Best

PhD Candidate
Child Nutrition Research Centre (a division of the Women’s and Children’s Health Research Institute)
72 King William Road
North Adelaide SA 5006
Mob: 0434243404
Email: karen.best@adelaide.edu.au
Appendix 10: HREC Amendment Request – In home skin prick testing

External Skin Prick Testing Proposal

Background

All living children enrolled in the 1 & 3 year nested allergy follow-up (Australian New Zealand Clinical Trials Registry: ACTRN12610000735055) of DOMInO children at high-hereditary risk of developing allergic disease, whose parent/carer have not withdrawn consent, will be invited to participate in 6 year allergy follow up – DOMInO 6 study. Approval of the DOMInO 6 study protocol has been granted by the Human Research Ethics Committees of each centre, Women’s and Children’s Hospital Network (WCHN), (REC2435/12/14) and Southern Area Health Service (SAHS REC 146.12). Six year follow up assessments commenced in April 2012 and completion of all assessments is by July 2014 is anticipated.

Rationale for External Appointments

Since enrolling in the DOMInO study 6-7 years ago a number of families have re-located to regional or rural areas, interstate or overseas. Of the 667 participants eligible to be invited to the 6 year follow up, 53 families (13%) now reside outside of the metropolitan area. Whilst we endeavour to obtain data via a parent completed case report form that is posted to these participants, it is critical that we also complete as many skin prick tests as possible to optimise the quality and accuracy of the DOMInO study primary outcome (incidence of allergic disease with sensitisation).

Our aim is to complete as many 6 year appointments (including skin prick testing) as possible in the Child Nutrition Research Centre at the Women’s and Children’s Hospital (WCH) or the Flinders Medical Centre (FMC). A number of the families we have contacted have consented to participation in the 6 year follow up but are either unable or unwilling to travel long distances to attend an appointment at WCH or FMC. The flexibility to conduct the 6 year follow up assessment in a setting closer to the home of the family, in a local/regional hospital or SA Health GP Plus clinic will decrease the potential effects of participant attrition bias and any possible impact this may have on study outcomes.

Safety of Skin Prick Testing (SPT)
Children participating in the DOMInO 6 allergy follow up have previously undergone SPT as part of the 1 & 3 year nested allergy follow-up assessments. Over 1300 SPT’s were conducted at 1 and 3 years of age with no incidence of anaphylaxis. All of these appointments were conducted in a hospital setting although there is more recent evidence of the safety of this procedure in a non-hospital setting.

The HealthNuts study is a population-based study of paediatric food allergy which was conducted in Melbourne in 2010 (19). A total of 2464 12 month old infants presenting to community clinics for routine scheduled immunisation were enrolled. In total, 5120 infants underwent SPT to egg, peanut, sesame and either cow’s milk (n=2715) or shrimp (n=2405) with no adverse reaction or anaphylaxis. Overall 29,760 individual skin prick tests to a food were performed with no adverse reactions.

**DOMInO 6 Methods**

Eligible families are invited to participate in the 6 year allergy follow up when their child is 5 years and 9 months of age. Following verbal consent to participate, an appointment is made at WCH or FMC, whichever is most convenient for the participant.

If the participant resides in a regional, rural or interstate location, CNRC staff will ascertain if the family have scheduled a visit to Adelaide before the child turns 7 years of age and becomes ineligible. If they have not, and the child meets external appointment criteria, the family will be invited to attend the appointment at a local GP Plus Clinic or local hospital so that the assessment can be conducted.

**External Appointment Criteria**

To be eligible for SPT in a local GP Plus Clinic or local hospital the child must meet the following criteria;

- No history of anaphylaxis
- No current or recent (within 7 days) history of unstable asthma
- No antihistamines within the last 4 days

The appointment will be conducted according to current approved protocol ‘6 Year Allergy Follow-up of children who participated in the DOMInO Study Version 1_17/11/2011’. SPT will be conducted in accordance with the ASCIA guidelines.
Personnel and Equipment

DOMInO study PhD Student Investigator, Karen Best will be conducting all external appointments. Ms Best is a Registered Nurse & Midwife and has undergone the following additional training;

- Basic Life Support updated 11th December 2012 (WCHN Centre for Education)
- ASCIA Anaphylaxis Training – Online 22nd March 2013
- Observation in the Medical Day Unit at the WCH to observe symptoms and treatment of anaphylaxis (ongoing)
- Completion of more than 150 SPT’s (WCH & FMC)
- Epipen training

In the event of anaphylaxis, an age appropriate Epipen will be immediately available for the child. Treatment will be administered according to the Australian Society of Clinical Immunology and Allergy (ASCIA) Action Plan for Anaphylaxis. (Appendix 4)

Detailed records of any adverse reactions will be documented and immediately reported the WCHN HREC.

Follow Up

A letter detailing the results of the skin prick test will be forwarded to the child’s GP as is our standard practice with all study participants. Any additional follow up required will be arranged by the child’s GP.

References
20 March 2013

Ms K Best  
Child Nutrition Research Centre  
Women’s and Children’s Health Research Institute  
72 King William Rd  
North Adelaide SA 5006

Dear Karen,

RE: DOMinO 6 – six year allergy follow up of children who participated in the DOMinO study. REC 2435/12/14

Thank you for arranging for the meeting on March 4 2013 with Maria Makrides to discuss your proposed changes to the process to conduct skin prick tests (SPT).

Please accept this letter as confirmation of our agreement at the meeting. The agreement was that all SPT are to be conducted in a health care environment, that is, a local hospital or GP Plus Centre (SA Health). Performing SPT in the home is not supported. The basis of this decision is related to the risk to a child that has a reaction to the SPT rather than the prevalence of a reaction (or the risk of this occurring), and to ensure that the appropriate emergency equipment is available and that there are established processes to access a higher level of care if required.

This stipulation extends to the SPT proposed for participants that now reside in Victoria should you be able to make the necessary arrangements.

Mr John Markic, Manager Insurance Services, SA Health agrees with this amendment to the DOMinO study REC2435/12/14. Further he has agreed that Ms K Best will be covered under the SA Health professional indemnity program for the SPT conducted in Victoria.

Yours sincerely

Julie Paholski  
Clinical Risk Manager  
Women’s and Children’s Health Network

CC Dr T Zulavica, Chair WCHN Human Research Ethics Committee
Dr Tamara Zutlevics  
Chair, WCHN Human Research Ethics Committee

Dear Dr Tamara Zutlevics,

Re: DOMInO 6 - Six year allergy follow-up of children who participated in the DOMInO study. REC2435/12/14

Thank you for your response dated 10th October 2012. Following consultation with the Women's and Children's Health Network Clinical Governance Unit we would like to amend our previous request to conduct the DOMInO 6 year follow up assessment in the home of the child. We would instead like to request approval to conduct the 6 year assessment including skin prick test (SPT), in a health care environment, that is, a local hospital or SA Health GP Plus Clinic.

Please find supplementary information including a detailed external appointment proposal for the conduct of these assessments in Appendix 3- External Appointment Proposal.

In response to your queries raised;

WCHN HREC Query 1:

1. Clarification on the procedure that will be followed if an anaphylactic reaction occurs in a participant's home as a result of the skin prick test. Please also provide a comment if the particular intervention (e.g. the use of an Epipen) is allowed under Hospital policy for use by non-medical or nursing staff.

Systemic allergic reactions are extremely rare with prick testing despite extensive use. A sample (N = 16,204) of the U.S. population, 6 to 74 years of age, was examined in the National Health and Nutrition Examination Survey NHANES II. Participants were skin prick tested to common allergens with no incidence of anaphylaxis. In 2006 -2010, 865 six year old children born to mothers in the Northhampton Women’s survey, underwent SPT to common allergens in their home, with no documented adverse reactions.

More recently and closer to home, the HealthNuts study, a population-based study of paediatric food allergy, was conducted in Melbourne in 2010. A total of 5120 infants underwent SPT to foods in a community setting with no adverse reaction or anaphylaxis.

The children that we are proposing to SPT in a local hospital or SA Health GP Plus Clinic, have previously undergone SPT as a part of the DOMInO 1 & 3 year nested allergy follow up. Over 1300 SPT’s were conducted at 1 and 3 years of age with no incidence of anaphylaxis or other adverse reaction.

As an added safety precaution, any child with a history of anaphylaxis or unstable asthma at the time of the assessment will not undergo skin prick testing. Nevertheless, in the event of an anaphylactic reaction, treatment will be administered by Karen Best (RN, RM) in accordance with the Australian Society of Clinical Immunology and Allergy (ASCIA) Action Plan for Anaphylaxis. (see Appendix 2 of the planned process for external appts)
WCHN HREC Query 2:

2. As the possible risk to the WCHN may be altered by the proposed modification to the study please obtain the written approval of Ms Anita Conroy in the Clinical Governance Unit.

Please find a letter attached from Clinical Risk Manager Ms Julie Paholski (Appendix 1) documenting support from John Markic of the attached External Appointment Proposal as well as Australia wide professional indemnity for Karen Best (Appendix 2 - Email correspondence).

If you require any additional information, please don’t hesitate to contact me.

Yours Sincerely,

Karen Best RN, RM, PhD Candidate

Child Nutrition Research Centre
(a division of the Women's and Children's Health Research Institute)
72 King William Road
North Adelaide SA 5006
Ph: +61 8 8161 6848Mob: 0434243404
Email: karen.best@adelaide.edu.au
16 April 2013

Professor Maria Makrides  
WCHRI  
Level 7, Rieger Building  

Dear Maria  

Re: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children? (Six year allergy follow-up of children who participated in the DOMInO study). REC2435/12/14  

Thank you for your letter of 21 March 2013 in which you responded to queries raised in my letter of 10 October 2012. I advise that following review by the Clinical Risk Manager at WCHN and by the Department of Health Insurance Services approval is given to conduct the 6 year assessment including the skin prick test (SPT) in a health care environment that is a local hospital or SA Health GP Plus Clinic. The detailed external appointment proposal for the conduct of the assessments was also noted.  

Yours sincerely  

TAMARA ZUTLEVİČS (DR)  
CHAIR  
WCHN HUMAN RESEARCH ETHICS COMMITTEE
SECTION A: FAMILY INFORMATION

A1 Whom does the child live with? (Please one only)
☐ Intact family (Natural parents living together)
☐ Separated Parents (Divided Care)
☐ Sole Parent - Mother
☐ Sole Parent - Father
☐ Fostered
☐ Adopted
☐ Other, specify ________________________________

A2 Who is the primary carer of the child? (Please one only)
☐ Mother
☐ Father
☐ Other, specify ________________________________

A3 How many days per fortnight is the child in the care of the primary carer?
☐ Full-time or ___ days per fortnight

A4 What is the primary carer’s date of birth?
__ __ / __ __ / __ __ __ (dd/mm/yyyy)

A5 Did the primary carer complete secondary school (Year 12 or equivalent)?
☐ Yes
☐ No
☐ Unknown

A6 Has the primary carer completed any further study?
☐ Yes
☐ No (Go to Question A7)
☐ Unknown (Go to Question A7)

A6.1 What is the highest qualification that the primary carer has completed? (Please one only)
☐ Certificate/Diploma
☐ Degree
☐ Higher Degree
☐ Other, specify ________________________________
☐ Unknown

A7 How many years has the primary carer spent in full time education since Year 1?
(include primary school, secondary school and any further study, even if not completed. Do not include reception or equivalent)
___ ___ years or ☐ Unknown
SECTION A: FAMILY INFORMATION – Primary Carer

A8 Has the primary carer been in the workforce at any time in the last 12 months?
   □ Yes (Go to Question A8.1)
   □ No (Go to Question A8.4)

A8.1 What is the primary carer’s usual or regular occupation?

A8.2 List main tasks of regular occupation

A8.3 Is the primary carer currently employed?
   □ Yes (Go to Question A8)
   □ No (Go to Question A8)

A8.4 What has been the main activity of the primary carer during this time? (Cross one only)
   □ Actively seeking work
   □ Home duties (and not actively seeking work)
   □ Student (and not actively seeking work)
   □ Disability pension
   □ Carer’s pension
   □ Other, please specify ____________________________
SECTION A: FAMILY INFORMATION – Secondary Carer

A9 Who is the secondary carer of the child?
☐ Mother
☐ Father
☐ Other, specify_____________________________________________________
☐ Not applicable (go to Section 5)

A10 How many days per fortnight is the child in the care of the secondary carer? (For divided care, total number of days per fortnight for primary carer [Question A3] and secondary carer [Question A10] should add up to 28)
___ days per fortnight

A11 What is the secondary carer’s date of birth?
___ / ___ / _______ (dd/mm/yyyy)

A12 Did the secondary carer complete secondary school (Year 12 or equivalent)?
☐ Yes
☐ No
☐ Unknown

A13 Has the secondary carer completed any further study?
☐ Yes
☐ No (go to Question A14)
☐ Unknown (go to Question A15)

A13.1 What is the highest level that the secondary carer has completed? (Please one only)
☐ Certificate/Diploma
☐ Degree
☐ Higher Degree
☐ Other, specify_____________________________________________________
☐ Unknown

A14 How many years has the secondary carer spent in full time education since Year 1? (Include primary school, secondary school and any further study, even if not completed. Do not include reception or equivalent)
___ ___ years or ☐ Unknown

A15 Has the secondary carer been in the workforce at any time in the last 12 months?
☐ Yes (go to Question A15.1)
☐ No (go to Question A15.4)
SECTION A: FAMILY INFORMATION – Secondary Carer cont.

A15.1 What is the secondary carer’s usual or regular occupation?

A15.2 List main tasks of regular occupation

A15.3 Is the secondary carer currently employed?
   □ Yes (go to Section B)
   □ No (go to Section E)

A15.4 What has been the main activity of the secondary carer during this time?
   □ Actively seeking work
   □ Home duties (and not actively seeking work)
   □ Student (and not actively seeking work)
   □ Disability pension
   □ Carer’s pension
   □ Other, please specify

SECTION B: HOME ENVIRONMENT

B1 How many adults (≥ 16 years) live in the home of the primary carer?

B2 How many children (< 16 years) other than the study child live in the home of the primary carer?

B3 What is the position, by age, of this child in the family?

B4 What is the primary language spoken in the home of the primary carer?
   □ English
   □ Other, specify

B5 Does anyone living in the home of the primary carer, smoke cigarettes?
   □ Yes
   □ No (go to Question 85)
   □ Unknown (go to Question 85)
B5.1 If yes, specify (cross all that apply)
- Primary Carer
- 1 person (other than primary carer)
- 2 people (other than primary carer)
- 3 or more people (other than primary carer)

B6 Are there any pets in or around the home of the primary carer?
- Yes
- No (go to Question B7)

B6.1 If yes, specify (cross all that apply)
- Dog
- Cat
- Bird
- Rabbit/Guinea Pig
- Mouse/Rat
- Reptiles
- Other, specify ____________________________

B7 In the home of the primary carer, what fuel is usually used for cooking? (cross all that apply)
- Electricity
- Gas
- Other _________________________________

B8 In the home of the primary carer, what fuel is usually used for heating?
- Electricity
- Gas/Kerosene/Paraffin
- Wood/Coal/Oil
- No heating
- Other _________________________________

B9 Is there a free-standing gas heater without a chimney in the home of the primary carer?
- Yes
- No

B10 Does the child have a house dust mite protector or plastic cover on their bed mattress, in the home of the primary carer?
- Yes
- No
B11 Does the child have a house dust mite protector or plastic cover on their pillow, in the home of the primary carer?

☐ Yes
☐ No
☐ No pillow

B12 What year did the child commence full time schooling?

☐ Not Commenced

SECTION C: DIETARY INFORMATION

C1 How many fish meals (50 to 80 grams of fish, equivalent to one small can of tuna or 4 fish fingers) did the child consume within the last month?

☐ No fish
☐ Unknown

C2 How often within the last month did your child eat DHA fortified foods?

☐ No DHA enriched foods
☐ Unknown
### C3  Does your child take any dietary (vitamin/mineral) supplements?

- [ ] Yes (Please complete table)
- [ ] No (Go to Question C4)
- [ ] Unknown (Question C4)

<table>
<thead>
<tr>
<th>Supplement Name</th>
<th>Enriched with DHA</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4-6 days/week</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>2-3 days/week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once per week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once a fortnight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less than once a fortnight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown</td>
</tr>
</tbody>
</table>

|                 | Yes               | Daily                      |
|                 | No                | 4-6 days/week              |
|                 | Unknown           | 2-3 days/week              |
|                 |                   | Once per week              |
|                 |                   | Once a fortnight           |
|                 |                   | Less than once a fortnight |
|                 |                   | Unknown                    |
SECTION C: DIETARY INFORMATION cont.

C4  In the last 12 months, how often, on average, did your child eat or drink the following? (Please cross one box per column)

<table>
<thead>
<tr>
<th>Food</th>
<th>Never or occasionally</th>
<th>Once or twice per week</th>
<th>Three or more times a week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat (e.g. beef, lamb, chicken, pork)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses (peas, beans, lentils)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal (including bread)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast Food/Take away</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### SECTION D: PARENTAL HEALTH DATA

#### D1 Have any ‘immediate family’ members ever been *medically diagnosed* with asthma?

<table>
<thead>
<tr>
<th></th>
<th>D1.1 Biological Mother</th>
<th>D1.2 Biological Father</th>
<th>D1.3 Biological Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>□ Yes</td>
<td>□ Yes</td>
<td>□ Yes</td>
</tr>
<tr>
<td></td>
<td>□ No</td>
<td>□ No</td>
<td>□ No</td>
</tr>
<tr>
<td></td>
<td>□ Unknown</td>
<td>□ Unknown</td>
<td>□ Unknown</td>
</tr>
</tbody>
</table>

#### D2 Have any ‘immediate family’ members ever been *medically diagnosed* with eczema?

<table>
<thead>
<tr>
<th></th>
<th>D2.1 Biological Mother</th>
<th>D2.2 Biological Father</th>
<th>D2.3 Biological Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td>□ Yes</td>
<td>□ Yes</td>
<td>□ Yes</td>
</tr>
<tr>
<td></td>
<td>□ No</td>
<td>□ No</td>
<td>□ No</td>
</tr>
<tr>
<td></td>
<td>□ Unknown</td>
<td>□ Unknown</td>
<td>□ Unknown</td>
</tr>
</tbody>
</table>

#### D3 Have any ‘immediate family’ members ever been *medically diagnosed* with hay fever?

<table>
<thead>
<tr>
<th></th>
<th>D3.1 Biological Mother</th>
<th>D3.2 Biological Father</th>
<th>D3.3 Biological Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay Fever</td>
<td>□ Yes</td>
<td>□ Yes</td>
<td>□ Yes</td>
</tr>
<tr>
<td></td>
<td>□ No</td>
<td>□ No</td>
<td>□ No</td>
</tr>
<tr>
<td></td>
<td>□ Unknown</td>
<td>□ Unknown</td>
<td>□ Unknown</td>
</tr>
</tbody>
</table>
SECTION E: CHILD HEALTH INFORMATION – ASTHMA

E1 Has your child EVER had wheezing or whistling in the chest at any time in the past?
   - Yes
   - No (go to Question 6)

E2 Has your child had wheezing or whistling in the chest in the past 12 months?
   - Yes
   - No (go to Question 6)

E3 How many attacks of wheezing or whistling has your child had in the past 12 months?
   - None
   - 1 to 3
   - 4 to 12
   - More than 12

E4 In the past 12 months, how often on average has your child's sleep been disturbed due to wheezing or whistling?
   - Never woken with wheezing
   - Less than one night per week
   - One or more nights per week

E5 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

E6 Has your child EVER had asthma?
   - Yes
   - No

E7 In the past 12 months, has your child's chest sounded wheezy during or after exercise?
   - Yes
   - No

E8 In the past 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 (If No to Question 6 and to Question 6, go to Question E3)
SECTION E: CHILD HEALTH INFORMATION – ASTHMA cont.

E9 In the past 12 months, how often has your child had wheezing or whistling first thing in the morning?
  - One or more mornings per week
  - Less than one morning per week
  - Never

E10 In the past 12 months, how often were your child’s activities affected or limited by cough, wheeze or shortness of breath whilst they were playing or at school?
  - Daily
  - Weekly
  - Monthly
  - Less than monthly
  - Never

E11 In the past 12 months, how often were your child’s sporting activities limited by cough or wheeze or shortness of breath?
  - Daily
  - Weekly
  - Monthly
  - Less than monthly
  - Never

E12 In the past 12 months, has your child been treated with Ventolin (Salbutamol)?
  - Yes
  - NO (Go to Question E13)

E12.1 If “YES”, please provide frequency
  - Daily
  - Weekly
  - Monthly
  - Less than monthly

E12.2 Did Ventolin (Salbutamol) improve your child’s symptoms?
  - Yes
  - No
  - Unsure

Salbutamol Trade Names:
Aironir, Asmol, Butamol, Salmol, Univent, Ventolin
SECTION E: CHILD HEALTH INFORMATION – ASTHMA cont.

E13 **In the last 12 months**, has your child been treated with regular use of inhaled preventer medications (such as steroids) for wheezing, coughing or asthma?
- [ ] Yes
- [ ] No
- [ ] Unknown

E14 **In the last 12 months**, has your child been treated with oral steroids for wheezing, coughing or asthma?
- [ ] Yes
- [ ] No
- [ ] Unknown

E15 Does your child currently use any asthma medication?
- [ ] Yes
- [ ] No
- [ ] Unknown

E15.1 If “YES”, (Please cross all that apply)
- [ ] preventer, Specify______________________________
- [ ] reliever, Specify______________________________
- [ ] other, Specify______________________________

E16 In which of the last 12 months did this wheezing or whistling problem occur?
*If answer at E2 was “No” to wheezing or whistling in the chest in the past 12 months, please go to E17*
- [ ] January
- [ ] April
- [ ] July
- [ ] October
- [ ] February
- [ ] May
- [ ] August
- [ ] November
- [ ] March
- [ ] June
- [ ] September
- [ ] December
E17 In the last 12 months, has your child had a problem with a wet cough *(NOT associated with wheeze)*?

- Yes
- No *(Go to Question F1)*

E17.1 Complete the following table for each wet coughing episode:

<table>
<thead>
<tr>
<th>Episode</th>
<th>Duration of cough (nights). Any part of night = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Episode 1</td>
<td></td>
</tr>
<tr>
<td>Episode 2</td>
<td></td>
</tr>
<tr>
<td>Episode 3</td>
<td></td>
</tr>
<tr>
<td>Episode ≥4</td>
<td></td>
</tr>
</tbody>
</table>

SECTION F: CHILD HEALTH INFORMATION - RHINITIS

F1 Has your 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 *(Go to Question F5)*

F2 In the last 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 *(Go to Question F5)*

F3 In the last 12 months, has this nose problem been accompanied by itchy-watery eyes?

- Yes
- No

F4 In which of the last 12 months did this nose problem occur? *(Please cross all which apply)*

- January
- April
- July
- October
- February
- May
- August
- November
- March
- June
- September
- December

F5 In the last 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

F6 Has your child EVER had hay fever?

- Yes
- No *(Go to F8)*
SECTION F: CHILD HEALTH INFORMATION – RHINITIS cont.

F7 In the last 12 months, has your child received any treatments for hay fever?

☐ Yes
☐ NO (Go to Question F8)

F7.1 If “YES”, please provide details (cross all that apply)

☐ Antihistamines
☐ Steroid nasal sprays
☐ Non-medicated nasal sprays or eye drops
☐ Other, specify:______________________________________________

F8 Does your child snore?

☐ Yes
☐ NO (Go to Section G)

F8.1 If “Yes”, how often?

☐ < 3 nights per week
☐ ≥ 3 nights per week
SECTION G: CHILD HEALTH INFORMATION - ECZEMA

G1  Has your child EVER had an itchy rash which was coming and going for at least 6 months?
   Yes
   No (Go to Question G7)

G2  Has your child had this itchy rash at any time in the last 12 months?
   Yes
   No (Go to Question G7)

G3  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

G4  At what age did this itchy rash first start to occur?
   Under 2 years
   Age 2-4
   Age 5 or more

G5  Has this rash cleared completely at any time during the last 12 months?
   Yes
   No

G6  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

G7  Has your child EVER had eczema?
   Yes
   No (Go to Section H)

G7.1 If yes, in the last 12 months has your child had any steroid cream/s for eczema?
   Yes
   No
## SECTION H: CHILD HEALTH INFORMATION – FOOD ALLERGY

### H1 In the last 12 months, has your child had an allergic reaction* to any of the following foods?

<table>
<thead>
<tr>
<th>Food</th>
<th>Yes</th>
<th>No</th>
<th>Not consumed</th>
<th>If YES, Medically Diagnosed?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Not consumed</td>
<td>Yes</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Egg</td>
<td>Yes</td>
<td>No</td>
<td>Not consumed</td>
<td>Yes</td>
</tr>
<tr>
<td>Fish</td>
<td>Yes</td>
<td>No</td>
<td>Not consumed</td>
<td>No</td>
</tr>
<tr>
<td>Shellfish</td>
<td>Yes</td>
<td>No</td>
<td>Not consumed</td>
<td>Yes</td>
</tr>
<tr>
<td>Peanut</td>
<td>Yes</td>
<td>No</td>
<td>Not consumed</td>
<td>No</td>
</tr>
<tr>
<td>Other Nut</td>
<td>Yes</td>
<td>No</td>
<td>Not consumed</td>
<td>No</td>
</tr>
<tr>
<td>Wheat</td>
<td>Yes</td>
<td>No</td>
<td>Not consumed</td>
<td>No</td>
</tr>
</tbody>
</table>

**Other Food Allergy?**

<table>
<thead>
<tr>
<th>Food</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### H2 Has your child EVER had any severe allergic reactions (anaphylaxis)?

*Anaphylaxis: multi-system involvement which must include circulatory and/or respiratory involvement.*

- Yes (please specify trigger/s) __________________________
- No

### H3 Has your child EVER been prescribed an Epipen?

- Yes (please specify what for) __________________________
- No

263
SECTION I: CHILD HEALTH INFORMATION - GENERAL

I1  How many times per week does your child engage in vigorous physical activity long enough to make him/her breathe hard?
   ☐ Never or occasionally
   ☐ Once or twice per week
   ☐ Three or more times per week

I2  During a normal week, how many hours a day (24 hours) does your child watch television (including playing electronic games)?
   ☐ ≤ 1 hour
   ☐ > 1 - < 3 hours
   ☐ ≥ 3 - < 5 hours
   ☐ ≥ 5 hours

I3  In the last 12 months has your child had a hospital admission >24 hours duration?
   ☐ Yes
   ☐ No (Go to Question I4)

<table>
<thead>
<tr>
<th>Event</th>
<th>Primary Reason</th>
<th>Secondary Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I3.1 Were any of these admissions to an Intensive Care Unit?
   ☐ Yes (Complete a SAE form for each ICU admission)
   ☐ No
SECTION I: CHILD HEALTH INFORMATION – GENERAL cont.

14 Has your child EVER been given paracetamol for pain or fever?
   - Yes
   - No (Go to Question 15)

14.1 How old was your child when they first received paracetamol?
   - ≤ 12 months
   - > 1 - < 3 years
   - ≥ 3 - < 5 years
   - ≥ 5 years

14.2 In the last 12 months, how often on average has your child been given paracetamol?
   - At least once per week
   - At least once per fortnight
   - At least once per month
   - Less than once per month
   - Never, in the last 12 months

---

Paracetamol Trade Names: Anadol, Bexylin, Codalgin, Codaphen, Control, Comforal, Disnap, Disparol,
Dymadon, Quatral, Ebedal, Hedacol, Ipseol, Lemprin, Logicin, Morsydol, Norgesic, Barcal, Ranol, Panodeine,
Panadol, Panamox, Paramax, Paracet, Panol, Panped, Prince, Quishan, Quimol, Tramadol.

---

15 Has your child EVER been given ibuprofen for pain and fever?
   - Yes
   - No (Go to Section I)

15.1 How old was your child when they first received ibuprofen?
   - ≤ 12 months
   - > 1 - < 3 years
   - ≥ 3 - < 5 years
   - ≥ 5 years

15.2 In the last 12 months, how often on average has your child been given ibuprofen?
   - At least once per week
   - At least once per fortnight
   - At least once per month
   - Less than once per month
   - Never, in the last 12 months

---

Ibuprofen Trade Names;
Advil, Act-3, AFT, Brufen, Bugesic, Butafen, Dimetapp, Fenpaed, Gofex, Hedafen, Ibupac, Logicin,
Matrix, Nurofen, Panaflex, Proven, Rufen, Tri-profen
## SECTION J: CHILD PHYSICAL ASSESSMENT

### HEIGHT

<table>
<thead>
<tr>
<th>J1</th>
<th>Date measured</th>
<th>__ <strong>/</strong> <strong>/</strong>____ (dd/mm/yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J1.1</td>
<td>1\textsuperscript{st} measure</td>
<td>__ __ : __ cm</td>
</tr>
<tr>
<td>J1.2</td>
<td>2\textsuperscript{nd} measure</td>
<td>__ __ : __ cm  □ Not Done</td>
</tr>
</tbody>
</table>

*If difference between 1\textsuperscript{st} and 2\textsuperscript{nd} measure is >0.5cm, then perform 3\textsuperscript{rd} measurement*

| J1.3 | 3\textsuperscript{rd} measure | __ __ : __ cm  □ Not Done |

**J1.4 Who took this measurement?**

- □ CNRC Staff
- □ Other Health Professional
- □ Parent/Carer
- □ Unknown
- □ Not Done, Reason ____________________________
- □ Other, specify ____________________________

### WEIGHT

<table>
<thead>
<tr>
<th>J2</th>
<th>Date measured</th>
<th>__ <strong>/</strong> <strong>/</strong>____ (dd/mm/yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J2.1</td>
<td>1\textsuperscript{st} measure</td>
<td>__ __ : __ kg</td>
</tr>
<tr>
<td>J2.2</td>
<td>2\textsuperscript{nd} measure</td>
<td>__ __ : __ kg  □ Not Done</td>
</tr>
</tbody>
</table>

*If difference between 1\textsuperscript{st} and 2\textsuperscript{nd} measure is >100 grams, perform 3\textsuperscript{rd} measurement*

| J2.3 | 3\textsuperscript{rd} measure | __ __ : __ kg  □ Not Done |

**J2.4 Who took this measurement?**

- □ CNRC Staff
- □ Other Health Professional
- □ Parent/Carer
- □ Unknown
- □ Not Done, Reason ____________________________
- □ Other, specify ____________________________
SECTION K: SKIN PRICK TESTING

K1  Has the SPT been completed?
  
  ☐ Yes
  ☐ No, If No please enter reason on MIS *(Go to Section L)*

K1.1  Date of SPT  ___/___/______

K1.2  Where was the skin prick test performed?
  
  ☐ CNRC clinic
  ☐ External clinic or practice *(i.e. not WCH or FMC, please specify)*

  ☐ Other, please specify ________________________________

K1.3  Who performed the skin prick test?
  ________________________________ *(Please Print)*

K1.4  Skin prick test results

<table>
<thead>
<tr>
<th>Extract</th>
<th>Weal (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Negative control</td>
<td>mm</td>
</tr>
<tr>
<td>2  Whole Hens Egg</td>
<td>mm</td>
</tr>
<tr>
<td>3  Peanut</td>
<td>mm</td>
</tr>
<tr>
<td>4  Cashew Nut</td>
<td>mm</td>
</tr>
<tr>
<td>5  Ryegrass pollen</td>
<td>mm</td>
</tr>
<tr>
<td>6  Olive tree pollen</td>
<td>mm</td>
</tr>
<tr>
<td>7  D. pteronyssinus</td>
<td>mm</td>
</tr>
<tr>
<td>8  D. farinae</td>
<td>mm</td>
</tr>
<tr>
<td>9  Cat</td>
<td>mm</td>
</tr>
<tr>
<td>10 Alternaria tenuis</td>
<td>mm</td>
</tr>
<tr>
<td>11 Dog</td>
<td>mm</td>
</tr>
<tr>
<td>12 Histamine</td>
<td>mm</td>
</tr>
</tbody>
</table>

K1.5  Was the Skin Prick Test completed per protocol?
  
  ☐ Yes
  ☐ No, Please Specify ________________________________
SECTION L: CHILD HEALTH QUESTIONNAIRE

L1 Has the CHQ been completed?
   ☐ Yes
   ☐ No, if No please enter reason on MIS

SECTION M: SERIOUS ADVERSE EVENT (SAE)

M1 Has the child experienced a new SAE?

   For the purposes of the DOMINO Study follow up, SAE is defined as Intensive Care Unit admission, diagnosis of Congenital Abnormality or Anaphylaxis.

   ☐ Yes (if Yes, complete SAE Form and record on MIS)
   ☐ NO (Go to CR? sign off)

M1.1 Date of SAE
   ___ / ___ / ______ (dd/mm/yyyy)

M1.2 SAE Number
   ___ ___

CNRC STAFF NOTES BOX
(Information written within this box is for internal use only and will not entered by data entry staff. Please update the MIS as required)

6 year follow up completed by (Print): 6 year follow up completed by (Sign): Date

6 year follow up checked by (Print): 6 year follow up checked by (Sign): Date
Appendix 12: Child Health Questionnaire (CHQ)

CHILD HEALTH QUESTIONNAIRE (CHQ-PF50)
PARENT FORM – 50 ENGLISH (AUSTRALIA)

INSTRUCTIONS: This form asks about your child's health and well-being. Your responses will be treated confidentially. There are no right or wrong responses. If you are unsure how to answer a question, give the best response you can. It is very important that you fill in each question. Please use blue or black ink.

Correct Marks: ■ ☑ ☑ ☑

SECTION 1: YOUR CHILD’S GLOBAL HEALTH

1.1. In general, would you say your child’s health is:

   Excellent ☐
   Very good ☐
   Good ☐
   Fair ☐
   Poor ☐

SECTION 2: YOUR CHILD’S PHYSICAL ACTIVITIES

The following questions ask about physical activities your child might do during a day.

2.1. During the past 4 weeks, has your child been limited in any of the following activities due to health problems?

Yes, a lot ☐
Yes, somewhat ☐
Yes, a little ☐
No, not limited ☐

a. Doing things that take a lot of energy, such as playing soccer, or running?

b. Doing things that take some energy such as riding a bike or roller blading?

c. Ability (physically) to get around the neighborhood, playground, or school areas?

d. Walking one block or climbing one flight of stairs?

e. Bending, lifting, or stooping?

f. Taking care of himself/herself, that is, eating, dressing, bathing, or going to the toilet?
SECTION 3: YOUR CHILD’S EVERYDAY ACTIVITIES

3.1. During the past 4 weeks, has your child’s school work or activities with friends been limited in any of the following ways due to emotional difficulties or problems with his/her behaviour?

<table>
<thead>
<tr>
<th></th>
<th>Yes, limited a lot</th>
<th>Yes, somewhat limited</th>
<th>Yes, limited a little</th>
<th>No, not limited</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Limited in the KIND of schoolwork or activities with friends he/she could do</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Limited in the AMOUNT of time he/she could spend on schoolwork or activities with friends</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Limited in PERFORMING schoolwork or activities with friends (a few extra short)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2. During the past 4 weeks, has your child’s school work or activities with friends been limited in any of the following ways due to problems with his/her physical health?

<table>
<thead>
<tr>
<th></th>
<th>Yes, limited a lot</th>
<th>Yes, somewhat limited</th>
<th>Yes, limited a little</th>
<th>No, not limited</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Limited in the KIND of schoolwork or activities with friends he/she could do</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Limited in the AMOUNT of time he/she could spend on schoolwork or activities with friends</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SECTION 4: PAIN

4.1. During the past 4 weeks, how much bodily pain or discomfort has your child had?

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Very mild</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Very severe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2. During the past 4 weeks, how often has your child had bodily pain or discomfort?

<table>
<thead>
<tr>
<th></th>
<th>None of the time</th>
<th>Once or twice</th>
<th>A few times</th>
<th>Fairly often</th>
<th>Very often</th>
<th>Every day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SECTION 6: BEHAVIOUR
Below is a list of items that describe children's behaviour or problems they sometimes have.

5.1. How often during the past 4 weeks did each of the following statements describe your child?

<table>
<thead>
<tr>
<th>Statement</th>
<th>Very often</th>
<th>Fairly often</th>
<th>Sometimes</th>
<th>Almost never</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Argued a lot?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Had difficulty concentrating or paying attention?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Lied or cheated?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Stole things inside or outside the home?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Had temper tantrums or a hot temper?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.2. Compared to other children your child's age, in general would you say his/her behaviour is:

<table>
<thead>
<tr>
<th>Quality</th>
<th>Excellent</th>
<th>Very good</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SECTION 6: WELL-BEING
The following phrases are about children's moods.

6.1. During the past 4 weeks, how much of the time do you think your child
      felt

<table>
<thead>
<tr>
<th>Feeling</th>
<th>All of the time</th>
<th>Most of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Felt like crying?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Felt lonely?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Felt nervous?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Felt bothered or upset?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Felt cheerful?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SECTION 7: SELF-ESTEEM
The following ask about your child’s satisfaction with self, school, and others. It may be helpful if you keep in mind how other children your child’s age might feel about these areas.

7.1. During the past 4 weeks, how satisfied do you think your child has felt about:

<table>
<thead>
<tr>
<th></th>
<th>Very satisfied</th>
<th>Somewhat satisfied</th>
<th>Neither satisfied nor dissatisfied</th>
<th>Somewhat dissatisfied</th>
<th>Very dissatisfied</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. His/her school ability?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. His/her athletic ability?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. His/her friendships?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. His/her looks/appearance?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. His/her family relationships?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. His/her life overall?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SECTION 8: YOUR CHILD’S HEALTH
The following statements are about health in general.

8.1. How true or false is the statement for your child?

<table>
<thead>
<tr>
<th>Statement</th>
<th>Definitely true</th>
<th>Mostly true</th>
<th>Don’t know</th>
<th>Mostly false</th>
<th>Definitely false</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. My child seems to be less healthy than other children I know</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. My child has never been seriously ill</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. When there is something going around my child usually catches it</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. I expect my child will have a very healthy life</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. I worry about my child’s health more than other people worry about their children’s health</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.2 Compared to one year ago, how would you rate your child’s health now.

<table>
<thead>
<tr>
<th></th>
<th>Much better now than 1 year ago</th>
<th>Somewhat better now than 1 year ago</th>
<th>About the same as 1 year ago</th>
<th>Somewhat worse now than 1 year ago</th>
<th>Much worse now than 1 year ago</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SECTION B: YOU AND YOUR FAMILY

9.1. During the past 4 weeks, how much emotional worry or concern did each of the following cause you?

<table>
<thead>
<tr>
<th>None at all</th>
<th>A little bit</th>
<th>Some</th>
<th>Quite a bit</th>
<th>A lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Your child's physical health</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>b. Your child's emotional well-being or behavior</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>c. Your child's attention or learning abilities</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

9.2. During the past 4 weeks, were you limited in the amount of time you had for your own needs because of:

<table>
<thead>
<tr>
<th>Yes, limited a lot</th>
<th>Yes, somewhat limited</th>
<th>Yes, limited a little</th>
<th>No, not limited</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Your child's physical health</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>b. Your child's emotional well-being or behavior</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>c. Your child's attention or learning abilities</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

9.3. During the past 4 weeks, how often has your child's health or behaviour:

<table>
<thead>
<tr>
<th>Very often</th>
<th>Fairly often</th>
<th>Sometimes</th>
<th>Almost never</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Limited the types of activities you could do as a family?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>b. Interupted various everyday family activities (eating meals, watching tv)</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>c. Limited your ability as a family to &quot;pick up and go&quot; on a moment's notice</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>d. Caused tension or conflict in your home?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>e. Been a source of disagreements or arguments in your family?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>f. Caused you to cancel or change plans (personal or work) at the last minute?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

9.4. Sometimes families may have difficulty getting along with one another. They do not always agree and they may get angry. In general, how would you rate your family's ability to get along with one another?

<table>
<thead>
<tr>
<th>Excellent</th>
<th>Very good</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>
Appendix 13: CRF completion Instructions

DOMInO 6 year follow up

CRF Completion Instructions

Prepared by Karen Best, 13/02/2013
**GENERAL INSTRUCTIONS**

1. Document the study ID at the top right hand side of every proceeding page.

2. All writing must be in black ball point pen and must be legible. If extra space is needed to clarify a response it shall be written as close as possible to the relevant question.

3. Any corrections or changes made in the CRF shall be crossed through with a single line, initialled and dated. Corrections should not obscure the original entry. Correction fluid or tape must NOT be used.

4. The final page contains a signature box that shall be signed and dated by the individual completing each section.

5. The final pages contains a ‘checked by’ box and is to be completed by the staff member nominated to check that the all applicable entries in the CRF have been completed.

6. Acceptable abbreviations include;

   - UNK = unknown
   - NA = not applicable
   - ND = not done

7. Lines provided for documenting numbers are required to have all spaces filled. If not required, preceding boxes shall be filled with a zero. For example; 0 0 8.

8. When you select a closed box □, mark it with an X. Not a tick (√) as this can be difficult to read.

9. Dates shall be written in the allocated space as dd/mm/yyyy, where dd = day, mm = month, yyyy = year. e.g. 31/08/2007

10. The CRF is to be completed as the information is received, at the time of the appointment.
SECTION A: FAMILY INFORMATION

A1 Whom does the child live with?
Select the option provided that best represents the family structure for the child.

A2 Who is the primary carer of the child?
The primary carer is the person who provides the majority of the care of the child. If both primary and secondary carer's provide an equal amount of care, the primary carer will be the parent/carer who attended the appointment with the child. Both may be recorded as full time carer’s.

A3 How many days per fortnight is the child in the care of the primary carer?
If the selection at Q. A1 was “Intact family living together”, select “full time”. If the selection entered at Q. A1 indicated divided care, record how many days or nights per fortnight the child is in the care of the primary carer.

A4 What is the primary carer’s date of birth?
Record the date of birth of the primary carer.

A5 Did the primary carer complete secondary school?
Record whether the primary carer has completed the entire curriculum of secondary schooling. In SA this will be year 12, in other states or countries this may differ. Ascertain if equivalent completion of secondary school studies has occurred.

A6 Has the primary carer completed any further study?
The certificate/degree diploma etc. must be a completed course. Do not include partially completed qualifications.

A6.1 What is the highest qualification that the primary carer has completed?
Ascertain the highest qualification completed, only one box should be selected. A trade or an apprenticeship is classified as a certificate/diploma.

A7 How many years has the primary carer spent in full time education since Year 1?
Record the number of years (full time equivalent) that the primary carer has spent in formal education from year 1. Include primary school, secondary school and any further study, even if not completed. (Do not include reception or equivalent). Include any time spent in further study (degree, trade certificate etc.,) even if un-finished. If study is done part time, calculate the full time equivalent value.
A8 Has the primary carer been in the workforce at any time in the last 12 months?
The questions regarding occupation & employment have been carefully worded by the statisticians and are a standard set of questions to ascertain the Australia New Zealand Standard Classification of Occupations (ANZSCO). If yes, continue to question A8.1 to provide details of the type work that the carer has undertaken within the last 12 months. If no, we need to ascertain the main activity of the carer during the last 12 months, please enter this at Q A8.4.

A8.1 What is the primary carer’s usual or regular occupation?
Specify the occupation of the primary carer. All occupations will be coded by data entry personnel at the Data Management and Analysis Centre (DMAC) according to the Australian Standard Classification of Occupations (ASCO). If you are unsure whether the occupation provides adequate information about the role/job and level of responsibility, please provide a brief description.

A8.2 List main tasks of regular occupation
List the main tasks of the occupation to enable accurate coding of responsibility in their occupation. For example, a “Manager” could manage a small business or multi-national corporation and may generate different coding. List main tasks of the carer’s occupation including details of the level of responsibility the carer has, especially if occupation title is ambiguous.

A8.3 Is the primary carer currently employed?
If yes, proceed to Q.A9. If No, but the carer has been in the workforce within the last 12 months (and this information is recorded at A8 -8.2) proceed to Q.A9.

A8.4 What has been the main activity of the primary carer during this time?
Complete one option which most accurately describes the activity of the carer if they have not been in the workforce in the past 12 months. If the person has not been employed at any time in the past 12 months the category selected will be used assign the ANZSCO code.

A9 Who is the secondary carer of the child?
The secondary carer of the child is the person who, after the primary carer, takes responsibility for the care of the child. The child may live with the secondary carer all of the time (e.g. intact family living together) or only some of the time (e.g. separated parents). If the child has no secondary carer (e.g. in a single-parent family) then place a cross in the ‘Not applicable’ box and go straight to section B, leaving the rest of the section blank.

A10 How many days per fortnight is the child in the care of the secondary carer?
Record how many days per fortnight the child is in the care of the secondary carer. In the case of an intact family where mother, father and live in same household, the answer to this question would be 14. For divided care, total number of days per fortnight for primary carer [Question A3] and secondary carer [Question A10] should add up to 14.
A11 What is the secondary carer’s date of birth?
Record the date of birth of the secondary carer.

A12 Did the secondary carer complete secondary school (Year 12 or equivalent)?
Record whether the secondary carer has completed the entire curriculum of secondary schooling. In SA this will be year 12, in other states or countries this may differ. Ascertain if equivalent completion of secondary school studies has occurred.

A13 Has the secondary carer completed any further study?
The certificate/degree diploma etc. must be a completed course. Do not include partially completed qualifications.

A14 How many years has the secondary carer spent in full time education since Year 1?
Record the number of years (full time equivalent) that the secondary carer has spent in formal education from year 1. Include primary school, secondary school and any further study, even if not completed. (Do not include reception or equivalent). Include any time spent in further study (degree, trade certificate etc.,) even if un-finished. If study is done part time, calculate the full time equivalent value.

A15 Has the secondary carer been in the workforce at any time in the last 12 months?
The questions regarding occupation & employment have been carefully worded by the statisticians and are a standard set of questions to ascertain the Australia New Zealand Standard Classification of Occupations (ANZSCO). If yes, continue to question A15.1 to provide details of the type work that the carer has undertaken within the last 12 months. If no, we need to ascertain the main activity of the carer during the last 12 months, please enter this at Q A15.4.

A15.1 What is the secondary carer’s usual or regular occupation?
Specify the occupation of the secondary carer. All occupations will be coded by data entry personnel at the Data Management and Analysis Centre (DMAC) according to the Australian Standard Classification of Occupations (ASCO). If you are unsure whether the occupation provides adequate information about the role/job and level of responsibility, please provide a brief description.

A15.2 List main tasks of regular occupation
List the main tasks of the occupation to enable accurate coding of responsibility in their occupation. For example, a “Manager” could manage a small business or multi-national corporation and may generate different coding. List main tasks of the carer’s occupation including details of the level of responsibility the carer has, especially if occupation title is ambiguous.
A15.3 Is the secondary carer currently employed?
   If yes, proceed to B1. If No, but the carer has been in the workforce within the last 12 months (and this information is recorded at A15 -15.2) proceed to Q.B1

A15.4 What has been the main activity of the secondary carer during this time?
   Complete one option which most accurately describes the activity of the carer if they have not been in the workforce in the past 12 months. If the person has not been employed at any time in the past 12 months the category selected will be used assign the ANZSCO code.

B1 How many adults (≥ 16 years) live in the home of the primary carer?
   Record the number of people aged 16 years or over who live in the home of the primary carer the majority of the time (i.e. more than 7 days a fortnight).

B2 How many children (< 16 years) other than the study child live in the home of the primary carer?
   Record the number of children under the age of 16 years who live in the home of the primary carer most of the time (i.e. more than 7 days a fortnight). Do not include the DOMInO study child. If the child in the DOMInO study is an only child, then the answer to this question would be ‘0’.

B3 What is the position, by age, of this child in the family?
   Record the birth order of the child taking into account all children who live in the home. Do not include parents.

B4 What is the primary language spoken in the home of the primary carer?
   Record the predominant language spoken at home.

B5 Does anyone living in the home of the primary carer, smoke cigarettes?
   Record all people who smoke cigarettes regardless of where they smoke, i.e. only outside, only at work etc. Please be sensitive when asking this question as some parent do not want the children to be aware of this information. If yes, record all person that smoke cigarettes.

B6 Are there any pets in or around the home of the primary carer?
   Record all pets whether they are inside or outside of the home. Include all pets using the “other” option if required.

B7 In the home of the primary carer, what fuel is usually used for cooking?
   Enter all types of fuel used for cooking inside the home. Do not include BBQ cooking outside.

B8 In the home of the primary carer, what fuel is usually used for heating?
   Enter all types of fuel used for heating.

B9 Is there a free-standing gas heater without a chimney in the home of the primary carer?
   Ascertain if any gas heaters are portable as this will mean that there is no flue or chimney.
B10 Does the child have a house dust mite protector or plastic cover on their bed mattress, in the home of the primary carer?
The plastic mattress protector or house dust mite mattress cover needs to completely enclose/cover the whole mattress top and bottom. Do not include standard fabric mattress protectors.

B11 Does the child have a house dust mite protector or plastic cover on their pillow, in the home of the primary carer?
The plastic pillow protector or house dust mite pillow cover needs to completely enclose/cover the whole pillow. Do not include standard fabric pillow protectors.

B12 What year did the child commence full time schooling?
Enter the year that the child started school including reception or equivalent

C1 How many fish meals (60 to 80 grams of fish, equivalent to one small can of tuna or 4 fish fingers) did the child consume within the last month?
Advise the parents that tuna and/or fish fingers can be counted. A fish meal generally contains 60-80 grams of fish.

C2 How often within the last month did your child eat DHA fortified foods?
Parents may be more aware of “omega 3” than DHA fortified foods. If they are unsure, show the list of fortified foods in the D6 folder. Enter the number of times the child ate a meal containing DHA fortified foods.

C3 Does your child take any dietary (vitamin/mineral) supplements?
If the parent is unsure of whether the supplement contains DHA, obtain as much information as you can regarding brand, appearance of bottle to enable look up.

C4 In the last 12 months, how often, on average, did your child eat or drink the following?

D1 Have any family members ever been medically diagnosed with asthma, eczema or hay fever?
Enquire whether a medical doctor has diagnosed the either the mother father or biological siblings of the child with asthma, eczema or hay fever. This question related to genetic predisposition to allergy and therefore must only include biological family members directly related to the child.
SECTION E: CHILD HEALTH INFORMATION – ASTHMA

Questions E1 to E8 are taken from the International Study of Asthma and Allergies in Childhood (ISAAC). ISAAC is a worldwide epidemiological research programme established to investigate asthma, rhinitis and eczema in children. The questions are designed as a minimum set for inclusion in self-completed or interview-administered questionnaires used in population surveys of respiratory disease in children. Note that enquiry about symptoms proceeds from the relatively mild to the relatively severe, and precedes enquiry about diagnosis. It is important that these questions are asked as listed below i.e. verbatim and in order.

E1 Has your child EVER had wheezing or whistling in the chest at any time in the past?
It does not mention “attacks” of wheezing, in order to identify children with persistent symptoms which are not obviously characterised as episodes or attacks.

E2 Has your child had wheezing or whistling in the chest in the past 12 months?
Limitation to a 12 month period reduces errors of recall and (at least in theory) should be independent of month of completion.

E3 How many attacks of wheezing or whistling has your child had in the past 12 months?
Record the number of episodes of wheezing or whistling in the chest.

E4 In the past 12 months, how often on average has your child's sleep been disturbed due to wheezing or whistling?
E3 & E4 offer two alternative quantitative measures of the frequency of wheezing. Problems with the concept of attacks (see above) and difficulty in quantifying the frequency of recurrent asthma lead to the inclusion of question 4 to identify and quantify persistent wheeze.

E5 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?
The aim of this question is to measure severity of asthma.

E6 Has your child EVER had asthma?
All participants are asked about diagnosed asthma, as occasionally asthma may be diagnosed in the absence of wheeze (on the basis of recurrent nocturnal cough etc.).

E7 In the past 12 months, has your child's chest sounded wheezy during or after exercise?
Wheeze after exercise may be an alternate presentation of asthma.

E8 In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection?
Nocturnal cough is widely accepted as an alternative presentation of asthma, and this question has been included to increase the overall sensitivity of the questionnaire. Questions E9 to E14 are in addition to the ISAAC questionnaire and are designed to assess severity of asthma only if asthma is present (i.e. Yes to E6, E7, or E8)
E9  In the past 12 months, how often has your child had wheezing or whistling first thing in the morning?
If the answer to E2 - Has your child had wheezing or whistling in the chest in the past 12 months? Is No, this answer should be ‘Never’.

E10  In the past 12 months, how often were your child’s activities affected or limited by cough, wheeze or shortness of breath whilst they were playing or at school?
Note, this question includes ‘cough’ so may be no wheeze in last 12 months, but activity could be limited due to cough.

E11  In the past 12 months, how often were your child’s sporting activities limited by cough or wheeze or shortness of breath?
As above.

E12  In the past 12 months, has your child been treated with Ventolin (Salbutamol)?
If the child has been treated with Ventolin, record how often and in the parent opinion, whether or not its administration helped to relieve the child’s symptoms.

E13  In the last 12 months, has your child been treated with regular use of inhaled preventer medications (such as steroids) for wheezing, coughing or asthma?
Record if any inhaled preventer medication has been used.

E14  In the last 12 months, has your child been treated with oral steroids for wheezing, coughing or asthma?
Record if oral steroids have been prescribed for the child’s wheeze, coughing or asthma.

E15  Does your child currently use any asthma medication?
Record all medications that the child is currently taking for prevention or treatment of asthma.

E16  In which of the last 12 months did this wheezing or whistling problem occur?
Cross all months that asthma, wheezing or whistling in the chest has occurred over the past 12 months. If the answer to E2 - Has your child had wheezing or whistling in the chest in the past 12 months? Is No, disregard this question and proceed to E17.

E17  In the last 12 months, has your child had a problem with a wet cough (not associated with wheeze).
This question aims to collect frequency of non-asthmas related respiratory conditions I.e. bronchitis.
SECTION F: CHILD HEALTH INFORMATION – RHINITIS

Questions F1 to F6 are taken from ISAAC, a worldwide epidemiological research program established to investigate asthma, rhinitis and eczema in children. It is important that these questions are asked as listed below i.e. verbatim and in order.

F1 Has your 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?
If the child has NEVER had any symptoms of sneezing, runny nose or blocked nose (in the absence of cold or flu) go to F6.

F2 In the last 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?
If yes to F1, ascertain if these symptoms have occurred in the past 12 months.

F3 In the last 12 months, has this nose problem been accompanied by itchy-watery eyes?

F4 In which of the last 12 months did this nose problem occur? (Please cross all which apply)
Cross all months that sneezing, runny nose or blocked nose, (in the absence of cold or flu) occurred in the past 12 months.

F5 In the last 12 months, how much did this nose problem interfere with your child’s daily activities?
Ascertain if the child’s behaviour was affected in any way by the presence of the symptoms.

F6 Has your child EVER had hay fever?
This may be parental report, does not have to have been doctor diagnosed.

F7 In the last 12 months, has your child received any treatments for hay fever?
Record any treatment that he child has received to alleviate symptoms of hay fever.

F8 Does your child snore?
If yes, enter how often the child snores, on average.
SECTION G: CHILD HEALTH INFORMATION – ECZEMA

Questions G1 to G7 are taken from ISAAC, a worldwide epidemiological research program established to investigate asthma, rhinitis and eczema in children. It is important that these questions are asked as listed below i.e. verbatim and in order.

G1 Has your child EVER had an itchy rash which was coming and going for at least 6 months?
G2 Has your child had this itchy rash at any time in the last 12 months?
G3 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?
G4 At what age did this itchy rash first start to occur?
G5 Has this rash cleared completely at any time during the last 12 months?
G6 In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?
G7 Has your child EVER had eczema?
If yes, document if any steroid creams have been used to treat the child’s eczema.

SECTION H: CHILD HEALTH INFORMATION – FOOD ALLERGY

H1 In the last 12 months, has your child had an allergic reaction* to any of the following foods?
The definition of allergic reaction is development of symptoms within 2 hours of the food being ingested. The reaction must include at least 3 concurrent non-contact urticarial lesions persisting for at least 5 minutes and/or generalised skin erythema and/or vomiting. If the parent confirms that an allergic reaction to food has occurred, ascertain if it was medically diagnosed by SPT or food challenge. Only record allergic reactions experienced within the past 12 months. If the child is avoiding a food, enter “Not Consumed”.

H2 Has your child EVER had any severe allergic reactions (anaphylaxis)?
Anaphylaxis is defined as multi-system involvement which must include circulatory and/or respiratory involvement. If yes, record what the trigger for anaphylaxis was.
If anaphylaxis occurred prior to the DHALLERGY 3 year appointment, this information should have been recorded on an SAE form, please double check. If anaphylaxis has occurred within the last 3 year please complete a SAE form. *If a SAE form is required, please let Karen Best know immediately.

H3 Has your child EVER been prescribed an Epipen?
Ascertain if the child has been prescribed an auto-injector. Brands may include Anapen or Epipen. Record what allergic reaction the auto injector has been prescribed for.
SECTION I: CHILD HEALTH INFORMATION - GENERAL

I1 How many times per week does your child engage in vigorous physical activity long enough to make him/her breathe hard? This question aims to establish the general physical activity of the child.

I2 During a normal week, how many hours a day (24 hours) does your child watch television (including playing electronic games)? This question aims to establish sedentary behaviour of the child.

I3 In the last 12 months has your child had a hospital admission >24 hours duration? Do not include admission to day surgery or accident and emergency unless the stay was for longer than 24 hours. If the child spent any time of their admission in intensive care, complete a SAE form. *If a SAE form is required, please let Karen Best know immediately.

I4 Has your child EVER been given paracetamol for pain or fever? Ask if the child has ever received paracetamol. Brands containing paracetamol may include the following: Aerodol, Benylin, Codalgin, Codaphane, Codral, Comforal, Dismol, Disprol, Dymadon, Duatrol, Febridol, Hedanol, Ipomol, Lemsip, Logicin, Masydol, Norgesic, Pacimol, Pamol, Panadeine, Panadol, Panamax, Paramax, Paracaps, Paralgin, Parapaed, Prodeine, Quiphen, Quimol, Tramadol If yes, ask what age the child was when they first receive paracetamol and on average, how often the child has been given paracetamol in the past 12 months.

I5 Has your child EVER been given ibuprofen for pain and fever? Ask if the child has ever received ibuprofen. Brands containing ibuprofen may include the following: Advil, Act 3, AFT, Brufen, Bugesic, Butafen, Dimetapp, Fenpaed, Gofex, Hedafen, Ibupac, Logicin, Motrin, Nurofen, Panafes, Proven, Rafen, Tri-profen. If yes, ask what age the child was when they first receive ibuprofen and on average, how often the child has been given ibuprofen in the past 12 months.
SECTION J: CHILD PHYSICAL ASSESSMENT

J1-J2  Growth Assessment

Growth Assessments should be conducted following the following Standard Operating Procedures.
CNRC_SOP_004_D.V4_Weight Measurement (Appendix 4)
CNRC_SOP_015_D.V4_Height Measurement (Appendix 5)
Two measurements shall be taken and a third only if the difference between 1st and 2nd measure is >0.5cm/100gms.

SECTION K: SKIN PRICK TESTING

The skin prick test results are the primary outcome for the DOMInO 6 study. It is crucial that this procedure is performed and measured accurately and consistently. Refer to the Australasian Society of Immunology ad Allergy (ASCIA) “Skin Prick Testing for the Diagnosis of Allergic Disease – A Manual for Practitioners”. SPT for DOMInO 6 children is generally performed on their back but may be done on the forearm if this is not possible i.e. eczema on back or child refusal.

K1  Has the SPT been completed?
If the SPT has not been completed this will need to be recorded on the MIS as Primary Outcome 'Not Achievable'. Please advise Karen Best to follow up.

K1.1  Date of SPT  __ __/__ __/__ __ __ __

K1.2  Where was the skin prick test performed?
Occasionally SPT will be performed by a private allergist. Enter the location that the SPT was performed.

K1.3  Who performed the skin prick test?

K1.4  Skin prick test results
Enter all results to one decimal place i.e. 6.0mm. If a result is negative, enter 0.

K1.5  Was the skin prick test completed per protocol?
If for any reason the SPT could not be completed or the results read in accordance with the ASCIA guidelines and DOMInO 6 protocol record the reason here i.e. “child scratched SPT area, wheal for cat unreadable”.

SECTION L: CHILD HEALTH QUESTIONNAIRE

L1  Has the CHQ been completed?
The child health questionnaire (CHQ) is a paediatric quality of life questionnaire for completion by parents. This should be given to parents to complete during the appointment. Once complete, please check that there are no duplicate answers (multiple answers selected for one question) or omissions before the parent leaves the appointment.
SECTION M: SERIOUS ADVERSE EVENT (SAE)

This is a follow-up study with no active intervention. Nevertheless, all hospital admissions will be documented as possible adverse events and the frequency of events will be compared between the treatment and control groups. Admission to intensive (Level 3) care, anaphylaxis or death will be treated as possible serious adverse events (SAE) and will be reviewed by an Independent SAE Committee.

M1 Has the child experienced a new SAE?

If the child has experienced an SAE according to the protocol definition described above since the previous DOMInO follow up appointment complete a SAE form.

M2 SAE Number

SAE number will be generated by the study Coordinator.
Appendix 14: HREC Amendment Request – Verbal Consent

20th May 2013

Dr Tamara Zutlevics
Chair, WCHN Human Research Ethics Committee

Dear Dr Zutlevics,

Re: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children? Six year allergy follow-up of children who participated in the DOMInO study; REC2435/12/14

I write to seek your approval for an amendment to the current approved protocol.

The majority of the 667 families participating in the DOMInO 6 year follow up will attend either the Women’s & Children’s Hospital or Flinders Medical Centre for assessment. This assessment consists of a skin prick test, weight, height and parent interview to obtain environmental data and clinical symptoms of allergy.

Some of our participants living interstate or in country areas (or locally but do not wish to attend a clinic appointment) agree to forgo the skin prick test component of the assessment and complete the study questionnaire at home. In this instance a consent form and questionnaire are mailed to their home.

A small number of these participants have difficulty getting around to returning the questionnaire and consent form to us (for a variety of reasons), although they are happy to be interviewed and complete the questions over the phone.

It is, at times, difficult to obtain the signed consent form from these participants within the specified eligibility period for the study, rendering the information they have provided to us unusable.

To enable us to include these families with a willingness to take part in DOMInO 6 follow up, I would like to propose that WCHN HREC give consideration to waiving the need for written consent for these participants. As these participants are completing the case report form questions only, which do not contain any sensitive information (Appendix 1), I respectfully suggest that voluntary completion of the questions by interview over the phone or return of the questionnaire by mail constitute sufficient consent and comply with section 2.2.5 of the National Statement;
Consent may be expressed orally, in writing or by some other means (for example, return of a survey, or conduct implying consent), depending on:
(a) the nature, complexity and level of risk of the research; and
(b) the participant’s personal and cultural circumstances.

Your consideration of this request is greatly appreciated. If you require any additional information, please don’t hesitate to contact me.

Yours Sincerely,

Karen Best RN, RM, PhD Candidate
Child Nutrition Research Centre
(a division of the Women’s and Children’s Health Research Institute)
72 King William Road
North Adelaide SA 5006
AUSTRALIA
Ph: +61 8 8161 6848
Mob: 0434243404
Email: karen.best@adelaide.edu.au

*National Statement on Ethical Conduct in Human Research (2007-updated February 2013). The National Health and Medical Research Council, the Australian Research Council and the Australian Vice-Chancellors’ Committee. Commonwealth of Australia, Canberra
27th June 2013

Ms K Best
Child Nutrition Research Centre
WCHRI

Dear Ms Best

Re: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children? Six year allergy follow-up of children who participated in the DOMInO study REC 2435/12/2014

I refer to your letter received 22nd May 2013 requesting an amendment to allow families who have not returned the consent form and questionnaire to answer the case report form questions (Parent CRF V4_22052013) over the telephone, without written consent. At its meeting on 26th June 2013, the WCHN Human Research Ethics Committee approved the amendment subject to clarification on how participants will indicate their preference to complete the questionnaire by telephone. I look forward to your response and remind you that you should not proceed until you receive a letter of full approval.

TAMARA ZUTLEVICS (DR)
CHAIR
WCHN HUMAN RESEARCH ETHICS COMMITTEE
Dear Dr Zutlevics,

Re: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children? Six year allergy follow-up of children who participated in the DOMInO study; REC2435/12/14

Thank you for your letter dated 27th June in response to our request to waive written consent for questionnaires returned by mail or completed by phone interview. In response to your query regarding how participants will indicate their preference to complete the questionnaire, please find the following clarification;

As per approved protocol, an information pack containing the following is mailed to the family's home;

- Participant information sheet
- Consent Form
- Updated Contact Details form

A follow up phone call is made to the family to answer any questions regarding the information sent, ascertain if they would like to participate and if so schedule a clinic appointment. Families who wish to participate but are unable to attend an appointment are offered the option of completing the DOMInO Parent Case Report Form and Child Health Questionnaire at home. If they chose this option, the information is posted to their home.

If the participant does not return the completed forms, they are telephoned and asked whether they would still like to participate in the study and if their preference is to return forms by post or to complete the questions over the phone at a convenient time.
All DOMInO 6 study processes and contact with participants is logged in real time in to our web based Management Information System (MIS) designed by the Data Management and Analysis Centre (DMAC) at University of Adelaide. This system provides tracking of information sent and received at CNRC, assessments and an ongoing record of participant contact and preferences. Participants who provide their responses over the telephone or return forms by post omitting the signed consent form will be the only participants without written consent. This detail will be logged in the MIS and a separate log maintained to document study ID numbers of the participants with verbal consent only.

If you require any additional information, please don’t hesitate to contact me.

Yours Sincerely,

Karen Best RN, RM, PhD Candidate
Child Nutrition Research Centre
(a division of the Women’s and Children’s Health Research Institute)
72 King William Road
North Adelaide SA 5006
AUSTRALIA
Ph: +61 8 8161 6848
Mob: 0434243404
Email: karen.best@adelaide.edu.au
23 July 2013

Ms K Best
Child Nutrition Research Centre
WCHRI

Dear Ms Best

Re: Does n-3 LCPUFA supplementation in pregnancy reduce asthma and allergies in school age children? Six year allergy follow-up of children who participated in the DOMInO study REC 2435/12/2014

I refer to your letter dated 17 July 2013 in response to my letter of 27 June 2013 and advise approval to allow families who have not returned the consent form and questionnaire to answer the case report form questions (Parent CRF V4_22052013) over the telephone. I note that verbal consent will be obtained from this group at the follow-up phone call.

TAMARA ZUTLEVICS (DR)
CHAIR
WCHN HUMAN RESEARCH ETHICS COMMITTEE
Appendix 15: Statistical Analysis Plan

DOMInO 6
Statistical Analysis Plan

Analysis plan authors
Karen Best (Study Coordinator, Student Investigator)
Thomas Sullivan (Statistician)
Maria Makrides (Principal Investigator)
Debbie Palmer (Chief Investigator)

Date
04/07/2014
Preface

This statistical analysis plan (SAP) describes the planned analyses and reporting for the 6 year allergy follow up of the DOMInO trial (DOMInO 6). The DOMInO 6 study is being conducted to determine whether docosahexaenoic acid (DHA) supplementation of pregnant women with a fetus at higher hereditary risk of atopic disease will result in fewer children with allergic disease with sensitisation at 6 years of age. It continues on from the nested allergy follow up of DOMInO children at 1 and 3 years in the DHAllergy study.

The following documents were reviewed in preparation of this SAP:
- DOMInO 6 Background and Research Plan (NHMRC Application ID 1027710).
- DOMInO, DHAllergy and DOMInO 6 Case Report Forms (CRFs) and other data collection tools.

Study Methods

1. Study design and eligibility for DOMInO6

DOMInO was a randomised controlled double-blind multicentre trial that investigated whether DHA supplementation of women during pregnancy reduced the incidence of postnatal depression and improved infant neurodevelopment. Consenting women were randomised to a DHA or control group and were asked to consume three DHA-rich fish oil capsules or three vegetable oil capsules per day, respectively, from trial entry until delivery. Following randomisation into DOMInO, women presenting at the South Australian centres were screened to assess the atopy status of their family. Women were eligible for the allergy follow up if their unborn baby had a mother, father or sibling with a history of medically diagnosed allergic disease (asthma, allergic rhinitis or eczema). Informed consent for the nested allergy follow up at 1 and 3 years was sought during pregnancy once eligibility was confirmed. For participation in DOMInO 6, further informed consent was sought prior to the child turning 6 years of age.

2. Method of treatment assignment

The randomisation schedule for DOMInO was produced by an independent statistician using ralloc.ado version 3.3 in Stata Release 9. Randomisation was performed using randomly permuted blocks of sizes 2, 4, 6 and 8 in proportions of 0.125, 0.375, 0.375 and 0.125 respectively. The randomisation was stratified by centre (Women’s and Children’s Hospital, Flinders Medical Centre, Sunshine Hospital, Campbelltown Hospital, Royal Brisbane Women’s Hospital and SA private hospitals) and parity (first pregnancy > 20 weeks gestation, subsequent pregnancy > 20 weeks gestation). A telephone randomisation service was used to allocate consenting women to receive DHA or placebo supplementation.

3. Blinding

Participants, research staff and investigators in the original DOMInO trial were blinded to treatment group allocation. To facilitate blinding, the DHA and placebo capsules were
identical in appearance. Although the blinding was broken during the analysis of the DOMInO trial, with the exception of the study statistician all research staff and investigators involved in DOMInO 6 were not aware of individual group allocations. Participating families were able to request details of group allocation at any stage after completion of the initial DOMInO trial. The number of families requesting to be unblinded in each group will be reported on as a trial quality outcome.

**Sequence of Planned Analyses**

1. **Interim analyses**
   There are no planned interim analyses or formal stopping rules for this study.

2. **Final analyses and reporting**
   Once the study has been completed and all data have been entered, a review of the data will be conducted and final changes will be made to this SAP. No statistical analyses will be performed until the final version of this SAP has been approved.

   Unblinded treatment codes will be included in the database and analysis of all primary and secondary outcomes will be performed unblinded to treatment group. Results of the statistical analyses will be made available to the Chief Investigators and key Associate Investigators. Any post-hoc, exploratory analyses which were not identified in this SAP will be clearly identified in the final report. Any deviations from the planned analyses detailed in this SAP will be documented with reasons in a post-analysis version of the SAP.
### Outcome Variables

#### 1. Primary outcome variable

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Hypothesis: DHA supplementation of pregnant women with a fetus at high-risk of atopic disease will:</th>
<th>CRF reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic disease symptoms with sensitisation</td>
<td>Reduce the proportion of children with allergic disease symptoms with sensitisation</td>
<td>Current wheeze (E2) or rhinitis (F2) or eczema (G2, G3) with sensitisation* to at least one allergen (K1.4) at 6 years</td>
</tr>
</tbody>
</table>

* Sensitisation for eczema is defined as wheal size ≥3mm than control, as measured by skin prick test, to at least one allergen extract (Whole hens egg, Peanut, Cashew nut, Ryegrass pollen, Olive tree pollen, D. pteronyssinus, D. farinae, cat, Alternaria tenius, Dog). Sensitisation for wheeze and rhinitis is defined as wheal size ≥3mm than control, as measured by skin prick test, to at least one aero-allergen extract (Ryegrass pollen, Olive tree pollen, D. pteronyssinus, D. farinae, cat, Alternaria tenius, Dog).

#### 2. Secondary outcome variables – 6 years

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Hypothesis: DHA supplementation of pregnant women with a fetus at high-risk of atopic disease will:</th>
<th>CRF Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Any sensitisation</td>
<td>Reduce the proportion of children with sensitisation</td>
<td>Sensitisation to at least one allergen extract (K1.4)</td>
</tr>
<tr>
<td>2.2 Sensitisation by extract</td>
<td>Reduce the proportion of children with sensitisation to individual extracts</td>
<td>Sensitisation to individual allergen extracts (K1.4)</td>
</tr>
<tr>
<td>2.3 Sensitisation no allergic disease</td>
<td>Reduce the proportion of sensitised children without symptoms of allergic disease</td>
<td>Sensitisation (K1.4) with no symptoms of current wheeze (E2), rhinitis (F2), or eczema (G2, G3)</td>
</tr>
<tr>
<td>2.4 Any current wheeze</td>
<td>Reduce the proportion of children with symptoms of current wheeze</td>
<td>Current wheeze (E2)</td>
</tr>
<tr>
<td>2.5 Current wheeze with sensitisation</td>
<td>Reduce the proportion of children with symptoms of current wheeze and sensitisation</td>
<td>Current wheeze (E2) with sensitisation to aero-allergens (K1.4)</td>
</tr>
<tr>
<td>2.6 Frequency of current wheeze</td>
<td>Reduce the frequency of symptoms of current wheeze</td>
<td>Current wheeze (E2) and number of attacks (E3) ≥ 4</td>
</tr>
<tr>
<td>2.7 Wheeze disturbs sleep</td>
<td>Reduce the severity of asthma symptoms</td>
<td>Current wheeze (E2) and Sleep disturbed (E4) ≥ 1</td>
</tr>
<tr>
<td>2.8 Severe wheeze limiting speech</td>
<td>Reduce the severity of asthma symptoms</td>
<td>Current wheeze (E2) and speech limited (E5)</td>
</tr>
<tr>
<td>2.9 Exercise wheeze</td>
<td>Reduce symptoms of exercise wheeze</td>
<td>Exercise wheeze (E7)</td>
</tr>
<tr>
<td>2.10 Night cough</td>
<td>Reduce symptoms of night cough</td>
<td>Night cough (E8)</td>
</tr>
<tr>
<td>2.11</td>
<td>Ever had asthma</td>
<td>Reduce parent report of asthma “ever”</td>
</tr>
<tr>
<td>2.12</td>
<td>Ever had asthma and current wheeze</td>
<td>Reduce parent report of asthma with current wheeze symptoms</td>
</tr>
<tr>
<td>2.13</td>
<td>Any allergic rhinitis symptoms</td>
<td>Reduce the proportion of children with symptoms of allergic rhinitis</td>
</tr>
<tr>
<td>2.14</td>
<td>Allergic rhinitis with sensitisation</td>
<td>Reduce the proportion of children with symptoms of allergic rhinitis with sensitisation</td>
</tr>
<tr>
<td>2.15</td>
<td>Severity of rhinitis symptoms</td>
<td>Reduce severity of allergic rhinitis symptoms</td>
</tr>
<tr>
<td>2.16</td>
<td>Any allergic rhinitis with ocular symptoms</td>
<td>Reduce severity of allergic rhino-conjunctivitis symptoms</td>
</tr>
<tr>
<td>2.17</td>
<td>Any allergic rhinitis with ocular symptoms with sensitisation</td>
<td>Reduce severity of allergic rhino-conjunctivitis symptoms</td>
</tr>
<tr>
<td>2.18</td>
<td>Parent reported hay fever ever</td>
<td>Reduce the proportion of children with parent reported hay fever</td>
</tr>
<tr>
<td>2.19</td>
<td>Parent reported hay fever required treatment last 12 months</td>
<td>Reduce severity of allergic rhinitis symptoms</td>
</tr>
<tr>
<td>2.20</td>
<td>Any eczema symptoms</td>
<td>Reduce the proportion of children with a eczema symptoms</td>
</tr>
<tr>
<td>2.21</td>
<td>Eczema symptoms with sensitisation</td>
<td>Reduce the proportion of children with eczema symptoms and sensitisation</td>
</tr>
<tr>
<td>2.22</td>
<td>Eczema severity - rash cleared last 12 months</td>
<td>Reduce the severity of symptoms of eczema</td>
</tr>
<tr>
<td>2.23</td>
<td>Eczema severity - awake at night</td>
<td>Reduce the severity of symptoms of eczema</td>
</tr>
<tr>
<td>2.24</td>
<td>Parent report eczema ever</td>
<td>Reduce the proportion of children with parent reported eczema</td>
</tr>
<tr>
<td>2.25</td>
<td>Parent report eczema with treatment last 12 months</td>
<td>Reduce the proportion of children requiring treatment for eczema</td>
</tr>
<tr>
<td>2.26</td>
<td>Snoring</td>
<td>Reduce the incidence of snoring</td>
</tr>
<tr>
<td>2.27</td>
<td>Anaphylaxis ever</td>
<td>Reduce the proportion of children with history of anaphylaxis</td>
</tr>
<tr>
<td>2.28</td>
<td>Prescribed epipen ever</td>
<td>Reduce the proportion of children requiring an epipen</td>
</tr>
<tr>
<td>2.29</td>
<td>Parent reported asthma - activities affected at school</td>
<td>Reduce the proportion of children whose activities are affected at school by asthma symptoms</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.30</td>
<td>Parent reported asthma - sporting activities affected</td>
<td>Reduce the proportion of children whose sporting activities are affected by asthma symptoms</td>
</tr>
<tr>
<td>2.31</td>
<td>Parent reported asthma - Salbutamol last 12 months</td>
<td>Reduce the proportion of children requiring Salbutamol for asthma symptoms</td>
</tr>
<tr>
<td>2.32</td>
<td>Parent reported asthma - regular inhaled preventer</td>
<td>Reduce the proportion of children requiring preventer medication for asthma symptoms in the last 12mths</td>
</tr>
<tr>
<td>2.33</td>
<td>Parent reported asthma - oral steroids</td>
<td>Reduce the proportion of children requiring oral steroids for asthma symptoms in the last 12 months</td>
</tr>
<tr>
<td>2.34</td>
<td>Parent reported asthma - current asthma medications</td>
<td>Reduce the proportion of children requiring current asthma medication for asthma symptoms</td>
</tr>
<tr>
<td>3. Secondary outcome variables – 1, 3 and 6 years combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td><strong>Hypothesis: DHA supplementation of pregnant women with a fetus at high-risk of atopic disease will:</strong></td>
<td><strong>CRF Reference</strong></td>
</tr>
<tr>
<td><strong>3.1</strong></td>
<td>Incidence of allergic disease symptoms and sensitisation at 1, 3 &amp; 6 years</td>
<td>Reduce the proportion of children with sensitisation and allergic disease symptoms over time</td>
</tr>
<tr>
<td><strong>3.2</strong></td>
<td>Incidence of any sensitisation at 1, 3 &amp; 6 years</td>
<td>Reduce the proportion of children with sensitisation over time</td>
</tr>
<tr>
<td><strong>3.3</strong></td>
<td>Incidence of sensitisation to individual extracts at 1, 3 &amp; 6 years</td>
<td>Reduce the proportion of children with sensitisation to individual extracts over time</td>
</tr>
<tr>
<td><strong>3.4</strong></td>
<td>Persistent wheeze</td>
<td>Reduce the proportion of children with persistent wheeze</td>
</tr>
<tr>
<td><strong>3.5</strong></td>
<td>Persistent wheeze with sensitisation at 1, 3 &amp; 6 years</td>
<td>Reduce the proportion of children with persistent wheeze with sensitisation</td>
</tr>
<tr>
<td><strong>3.6</strong></td>
<td>Transient wheeze</td>
<td>Reduce the proportion of children with transient wheeze</td>
</tr>
<tr>
<td><strong>3.7</strong></td>
<td>Transient wheeze with sensitisation</td>
<td>Reduce the proportion of children with transient wheeze and sensitisation</td>
</tr>
<tr>
<td><strong>3.8</strong></td>
<td>Late-onset wheeze</td>
<td>Reduce the proportion of children with late-onset wheeze</td>
</tr>
<tr>
<td><strong>3.9</strong></td>
<td>Late-onset wheeze with sensitisation</td>
<td>Reduce the proportion of children with late-onset wheeze and sensitisation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>3.10</strong></td>
<td>Incidence of rhinitis at 3 &amp; 6 years</td>
<td>Reduce the proportion of children diagnosed with rhinitis over time</td>
</tr>
<tr>
<td></td>
<td>Rhinitis symptoms at 3 years (W10.1) and 6 years (F2)</td>
<td></td>
</tr>
<tr>
<td><strong>3.11</strong></td>
<td>Incidence of rhinitis with sensitisation at 3 &amp; 6 years</td>
<td>Reduce the proportion of children diagnosed with rhinitis and sensitisation over time</td>
</tr>
<tr>
<td></td>
<td>Rhinitis symptoms with sensitisation at 3 years (W10.1, W7.3) and 6 years (F2, K1.4)</td>
<td></td>
</tr>
<tr>
<td><strong>3.12</strong></td>
<td>Incidence of rhino-conjunctivitis at 3 &amp; 6 years</td>
<td>Reduce the proportion of children diagnosed with rhino-conjunctivitis over time</td>
</tr>
<tr>
<td></td>
<td>Rhino-conjunctivitis symptoms at 3 years (W10.1, W10.2) and 6 years (F2, F3)</td>
<td></td>
</tr>
<tr>
<td><strong>3.13</strong></td>
<td>Incidence of rhino-conjunctivitis with sensitisation at 3 &amp; 6 years</td>
<td>Reduce the proportion of children diagnosed with rhino-conjunctivitis and sensitisation over time</td>
</tr>
<tr>
<td></td>
<td>Rhino-conjunctivitis symptoms with sensitisation at 3 years (W10.1, W10.2, W7.3) and 6 years (F2, F3, K1.4)</td>
<td></td>
</tr>
<tr>
<td><strong>3.14</strong></td>
<td>Incidence of eczema at 1, 3 &amp; 6 years</td>
<td>Reduce the proportion of children diagnosed with eczema between over time</td>
</tr>
<tr>
<td></td>
<td>Eczema symptoms at 1 year (S8.3, S8.4), 3 year (W8.3, W8.4), 6 year (G2, G3)</td>
<td></td>
</tr>
<tr>
<td><strong>3.15</strong></td>
<td>Incidence of eczema with sensitisation at 1, 3 &amp; 6 years</td>
<td>Reduce the proportion of children diagnosed with eczema with sensitisation over time</td>
</tr>
<tr>
<td></td>
<td>Eczema symptoms with sensitisation at 1 year (S8.3, S8.4, S7.3), 3 years (W8.3, W8.4, W7.3) and 6 years (G2, G3, K1.4)</td>
<td></td>
</tr>
<tr>
<td><strong>3.16</strong></td>
<td>New cases of any allergic disease with sensitisation at 6 years of age</td>
<td>Reduce the proportion of children with a new diagnosis of allergic disease with sensitisation at 6 years of age</td>
</tr>
<tr>
<td></td>
<td>Current wheeze (E2) or rhinitis (F2) or eczema (G2, G3) with sensitisation (K1.4) at 6 years, with no diagnosis of allergic disease with sensitisation at 1 year (eczema (S8.3, S8.4) with sensitisation (S7.3)) or 3 years (current wheeze (W9.2) or rhinitis (W10.1) or eczema (W8.3, W8.4) with sensitisation (W7.3))</td>
<td></td>
</tr>
<tr>
<td><strong>3.17</strong></td>
<td>New cases of eczema with sensitisation at 6 years of age</td>
<td>Reduce the proportion of children with a new diagnosis of eczema with sensitisation at 6 years</td>
</tr>
<tr>
<td></td>
<td>Current eczema (G2, G3) with sensitisation (K1.4) at 6 years, with no diagnosis of eczema with sensitisation at 1 (S8.3, S8.4, S7.3)) or 3 years (W8.3, W8.4, W7.3)</td>
<td></td>
</tr>
<tr>
<td><strong>3.18</strong></td>
<td>New cases of rhinitis with sensitisation at 6 years of age</td>
<td>Reduce the proportion of children with a new diagnosis of rhinitis with sensitisation at 6 years</td>
</tr>
<tr>
<td></td>
<td>Current rhinitis (F2) with sensitisation (K1.4) at 6 years, with no diagnosis of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhinitis with sensitisation at 3 years (W10.1, W7.3)</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
<td>--------------------------------------------------</td>
</tr>
</tbody>
</table>

* Denotes a longitudinal outcome defined at 1, 3 and 6 years
4. Secondary outcome variables – trial quality

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Hypothesis</th>
<th>CRF Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Allergic disease without sensitisation</td>
<td>There will be no difference between the groups</td>
<td>Current wheeze (E2), rhinitis (F2) or eczema (G2, G3) without sensitisation (K1.4)</td>
</tr>
<tr>
<td>4.2 Hospitalisations in last 12 months</td>
<td>There will be no difference between the groups</td>
<td>Hospitalisations (I3)</td>
</tr>
<tr>
<td>4.3 Serious adverse events in last 12 months</td>
<td>There will be no difference between the groups (Anaphylaxis, ICU admissions)</td>
<td>Serious adverse events (M1)</td>
</tr>
<tr>
<td>4.4 Child Health Questionnaire</td>
<td>There will be no difference between the groups</td>
<td>Physical score</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Psychosocial score</td>
</tr>
<tr>
<td>4.5 Completion of SPT</td>
<td>There will be no difference between the groups</td>
<td>Completed SPT (K1)</td>
</tr>
<tr>
<td>4.6 SPT per protocol</td>
<td>There will be no difference between the groups</td>
<td>SPT completed according to protocol (K1.5)</td>
</tr>
<tr>
<td>4.7 Proportion of families unblinded before the 6 year assessment</td>
<td>There will be no difference between the groups</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

5. Outcomes for association with cord blood plasma phospholipids

Total n-3 LCPUFA and proportion DHA from cord blood plasma phospholipids will be associated with the following outcomes at 1, 3 and 6 years:

- Allergic disease with sensitisation
- Any sensitisation
- Any allergic disease
Descriptive Statistics

1. Flow of participants through the allergy follow up

Information will be presented by treatment group (where appropriate) on:

DOMInO Study
- The number of mothers randomised into the DOMInO trial
- The number of mothers randomised into DOMInO in the Women's and Children's Hospital, Flinders Medical Centre and SA private hospitals

1 & 3 year follow up in DHAllergy
- Number of mothers eligible for 1 & 3 year allergy follow up
- Number of mothers not approached to participate in the 1 & 3 year allergy follow up
- Number of mothers who did not give consent for the 1 & 3 year allergy follow up
- Number of mothers that consented to the 1 & 3 year allergy follow up
- Number of mothers/children withdrawn during the 1 & 3 year allergy follow up

Intermediate
- Number of mothers/children withdrawn from the allergy cohort since completion of 3 year follow up but prior to 6 year follow up

6 year follow up
- Number of mothers/children eligible for DOMInO 6 participation
- Number of mothers/children lost to follow up at 6 years
- Number of mothers approached but refused participation in DOMInO 6, by reason
- Number of mothers that consented to the 6 year follow up study
- Number of mothers/children withdrawn from DOMInO 6 after giving consent
- Number of mothers/children that completed 6 year follow up
- Number of children that completed the primary outcome (skin prick test)

2. Baseline Characteristics

A descriptive comparison of the randomised groups will be conducted on the following baseline characteristics:

- Enrolling Centre
- Maternal history of allergic disease (eczema, asthma, hay fever) (DOMInO CRF B10)
- Paternal history of allergic disease (eczema, asthma, hay fever) (DOMInO CRF B10)
- Infant sex (DOMInO CRF G1)
- Maternal smoking during pregnancy (DOMInO CRF B3)
- Maternal BMI (DOMInO CRF B7 and B8)
- Maternal age at trial entry (DOMInO CRF A1)
- Parity (DOMInO CRF B12)

Means and standard deviations, or medians and interquartile ranges, will be reported for continuous baseline characteristics. Frequencies and percentages will be reported for categorical baseline characteristics.
### 3. Post Randomisation Characteristics

A descriptive comparison of the randomised groups will be conducted on the post-randomisation characteristics presented in the following table.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Categories</th>
<th>CRF Ref.</th>
</tr>
</thead>
</table>
| Socio-Economic Indexes for Areas (SEIFA) Index of Relative Socio-Economic Disadvantage (IRSD) | Lowest < 937.0  
Low ≥ 937.0 to < 980.8  
Middle ≥ 980.8 to < 1020.0  
High ≥ 1020.0 to < 1063.0  
Highest ≥ 1063.0 | Choir MIS - Postcode |
| Primary carer occupation                                  | Professional/managerial (ANZSCO 1-2)  
Semi-skilled/trade/unskilled (ANZSCO 3-8)  
Other | A8.1 |
| Secondary carer occupation                                | Professional/managerial (ANZSCO 1-2)  
Semi-skilled/trade/unskilled (ANZSCO 3-8)  
Other | A15.2 |
| Primary Carer completed secondary school                   | Yes  
No | A5 |
| Secondary Carer completed secondary school                 | Yes  
No | A12 |
| Primary carer further education                            | No further study  
Certificate/Diploma  
Degree  
Higher Degree | A6.1 |
| Secondary carer further education                          | No further study  
Certificate/Diploma  
Degree  
Higher Degree | A13.1 |
| Child height, weight and BMI                               | - | J1, J2 |
| Number of adults in home                                   | 1  
2  
≥ 3 | B1 |
| Number of other children in home                           | 0  
1  
2  
≥ 3 | B2 |
| Position of child in family                                | 1  
2  
≥ 3 | B3 |
| Smoker in household                                        | Yes  
No | B5 |
<p>| Cat as pet                                                 | Yes | B6.1 |</p>
<table>
<thead>
<tr>
<th><strong>Item</strong></th>
<th><strong>Yes/No</strong></th>
<th><strong>B</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog as pet</td>
<td>Yes/No</td>
<td>B6.1</td>
</tr>
<tr>
<td>Farm animals (Sheep, Horse, Cow, Pig, Alpaca, Chicken, Other)</td>
<td>Yes/No</td>
<td>B6.1</td>
</tr>
<tr>
<td>Gas fuel used in home</td>
<td>Yes/No</td>
<td>B7 or B8</td>
</tr>
<tr>
<td>Dust Mite Protector mattress</td>
<td>Yes/No</td>
<td>B10</td>
</tr>
<tr>
<td>Dust Mite Protector pillow</td>
<td>Yes/No, No pillow</td>
<td>B11</td>
</tr>
<tr>
<td>Year commenced schooling</td>
<td>_ _ _ _ Not commenced</td>
<td>B12</td>
</tr>
<tr>
<td>DHA intake fish meals</td>
<td>_ _ _ No fish</td>
<td>C1</td>
</tr>
<tr>
<td>DHA intake fortified foods</td>
<td>_ _ _ No DHA enriched foods</td>
<td>C2</td>
</tr>
<tr>
<td>DHA intake via supplements</td>
<td>Yes/No</td>
<td>C3</td>
</tr>
<tr>
<td>Meat</td>
<td>Never or occasionally</td>
<td>C4</td>
</tr>
<tr>
<td>Fruit</td>
<td>Once or twice per week</td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td>Three or more times per week</td>
<td></td>
</tr>
<tr>
<td>Pulses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast food/take away</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol ever</td>
<td>Yes/No</td>
<td>I4</td>
</tr>
<tr>
<td>Age first received Panadol</td>
<td>≤ 12 months</td>
<td>I4.1</td>
</tr>
<tr>
<td></td>
<td>&gt; 1 - &lt; 3 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 3 – 5 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 5 years</td>
<td></td>
</tr>
<tr>
<td>Paracetamol last 12 months</td>
<td>At least once per week</td>
<td>I4.2</td>
</tr>
<tr>
<td></td>
<td>At least once per fortnight</td>
<td></td>
</tr>
</tbody>
</table>
Means and standard deviations, or medians and interquartile ranges, will be reported for continuous post-randomisation characteristics, while frequencies and percentages will be reported for categorical characteristics. Differences between study groups will be assessed using t-tests or Wilcoxon tests for continuous variables and Chi-square tests or Fisher exact tests for categorical variables.
General Analysis Issues

**1. Analysis software**

All analyses will be performed using SAS® version 9.3 or later, or Stata Release 12 or later.

**2. Analysis approach**

The planned analysis of the two randomised groups will be performed using an intention-to-treat (ITT) approach; participants will be analysed according to the treatment they were randomised to receive irrespective of compliance with the protocol. Note the study design was consistent with the ITT principle since women and children were followed up regardless of compliance.

**3. Methods for withdrawals, missing data and outliers**

All data collected on children and their families up until the time of withdrawal will be included in the statistical analysis. For each outcome variable, missing data will be summarised descriptively by treatment group.

Multiple imputation will be used to create 100 complete datasets for analysis, even if only a small percentage of data are missing, in order to meet the requirements for publishing in top-ranking journals. Use of 100 imputations ensures that the loss of power compared to full information maximum likelihood methods is less than 1%, even when the fraction of missing information is high (Graham et al., 2007). Imputation will be performed separately by treatment group using the fully conditional specification method, also known as chained equations. Imputation models will include a range of variables, including baseline characteristics collected as part of the DOMInO trial and other relevant auxiliary variables collected during the allergy follow up. For each outcome, covariates pre-specified for adjustment in the analysis model and variables that are strong predictors of the outcome, particularly those that also predict missingness on the outcome, will be prioritised for inclusion into the imputation model. Given that some outcomes make use of data collected at 1, 3 and 6 years, imputed datasets will include all families that consented to participate in the allergy follow up (i.e. not just those families that consented to 6 year follow up). Results will not be imputed for children that die during follow up however, since these data aren't meaningful for analysis.

Analyses will be performed on both the raw and imputed data, with conclusions to be drawn based on the results of the imputed analyses. To explore the validity of the missing at random assumption used in multiple imputation, sensitivity analyses on the primary outcome will be performed by only imputing to families that consented to DOMInO 6 and by considering missing not at random mechanisms.

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. Protocol violations and deviations**

No subjects will be excluded from the ITT analyses due to protocol deviations.
5. Data transformations

No data transformations are planned. The statistical analysis methods for post-randomisation characteristics and outcomes 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).

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 and parity were used as stratification variables in the randomisation process, all analyses will be adjusted for centre (Flinders Medical Centre & SA private hospitals, Women’s and Children’s Hospital) and parity (first pregnancy > 20 weeks gestation, subsequent pregnancy > 20 weeks gestation). Flinders Medical Centre and SA private hospitals were combined to form a single category for the purpose of adjustment due to the small number of mothers recruited from private hospitals. Further, a large number of private patients were recruited from Flinders Private Hospital and hence share some of the facilities with Flinders Medical Centre patients. For the analysis of primary and secondary outcomes, excluding trial quality outcomes, adjustment will also be made for the categorical baseline characteristics shown in the table below.

<table>
<thead>
<tr>
<th>Baseline characteristic (CRF reference)</th>
<th>Categories</th>
</tr>
</thead>
</table>
| Maternal history of allergic disease (DHAllergy B10) | Yes if the mother has a medical diagnosis of asthma, eczema or hay fever  
No otherwise |
| Infant gender (DHAllergy G1) | Male  
Female |

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.
7. Planned treatment by covariate interactions

No treatment by covariate interactions have been pre-specified. Any unplanned treatment by covariate interactions are to be considered exploratory and will be clearly identified in the final report.

8. Multiple comparisons and multiplicity

Multiple hypothesis tests will be performed to assess the effectiveness of DHA due to multiple secondary outcomes, unadjusted and adjusted analyses and analyses based on raw and imputed data. No adjustment will be made for the number of secondary analyses performed 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 the imputed 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.

9. Statistical significance

For each outcome variable, statistical significance will be assessed at the 0.05 level using a two-sided comparative test of treatment effect.
Analysis Methods

1. Binary outcomes

Outcomes 1, 2.1 - 2.34, 3.4 - 3.9, 3.16-3.18, 4.1 - 4.3, 4.5 - 4.7

For binary outcomes, the groups will be compared using log binomial regression models, with the effects of treatment described as relative risks with 95% confidence intervals. If any of the models fail to converge, a log Poisson regression model with robust variance estimation will be used instead. If the number and percentage of participants experiencing the binary outcome of interest is considered too small to reliably estimate the relative risk, a Fisher exact test on the unimputed data will be performed.

2. Longitudinal binary outcomes

Outcomes 3.1 - 3.3, 3.10 - 3.15

For binary outcomes measured separately at 1, 3 and 6 years (or just at 3 and 6 years), the groups will be compared using log binomial generalised estimating equations (GEEs). The models will make use of an independence working correlation matrix to adjust for the dependence within subjects due to repeated measurements over time. In the models the effect of treatment group, time and the interaction between treatment group and time will be assessed. Since the functional form for the association between time and outcome will be difficult to establish based on only 3 time periods of measurement, time will be included in the models as a categorical predictor variable. Separate estimates of treatment effect will be obtained at each time point, independent of whether the interaction effect is statistically significant or not. If the interaction term is not statistically significant, a second model excluding the interaction term will be fitted so that the main effect of treatment over the three time points can be assessed. The effect of treatment, whether at an individual time point or across all time points, will be described using relative risks and 95% confidence intervals. If models fail to converge, a log Poisson GEE using an independence working correlation matrix will be used instead. If the number and percentage of participants experiencing the binary outcome of interest is considered too small to reliably estimate the relative risk at any of the time points, separate Fisher exact tests at each time point will be performed on the unimputed data.

3. Continuous outcomes

Outcome 4.4

For continuous outcomes, the groups will be compared using linear regression models, with the effects of treatment described as mean differences with 95% confidence intervals.

4. Cord blood plasma phospholipids

Associations between cord blood plasma phospholipids and allergy outcomes will be assessed using log binomial regression models. The effect of a one unit increase in total n-3 LCPUFA or proportion DHA on the risk of allergy will be described using relative risks with 95% confidence intervals. If model fit is poor, cord blood plasma phospholipids will be categorised as appropriate. To allow for possible effect modification by DHA
supplementation, a treatment group by cord blood plasma phospholipid interaction effect will be included in statistical models. Should there be no statistical evidence of effect modification (interaction p-value > 0.05), the interaction term will be excluded from the relevant statistical model.

References


1. **INTRODUCTION AND PURPOSE**

Two task specific Westinghouse 130L refrigerators have been purchased for the specific purpose of storage of allergen extracts. Refrigerators are stored in the general office area at both WCH and FMC to enable easy access at all times and are clearly marked “Allergen Storage only”.

*Refrigerators are for allergen extract storage only. No other material shall be stored in the refrigerators (with the exception of bottled Normal Saline).*

The purpose of this guideline is to document the procedure for storage of allergen extracts and monitoring the temperature of CNRC refrigerators used for the specific purpose of storing allergen extracts.

2. **SCOPE/ APPLICABILITY**

This applies to all CNRC Staff members who may on occasion be required to monitor the temperature of the ‘Allergen Refrigerators’.

3. **ALLERGEN STORAGE**

Allergen extracts should be stored in a refrigerator between (+2°C and 8°C) to reduce the rate of potency loss.

The potency of allergen extracts is affected by several factors, including the passage of time, temperature, concentration, number of allergens in a vial, volume of the storage vial, and presence of stabilizers and preservatives.

4. **REFRIGERATOR SET UP AND MAINTENANCE**

In the absence of specific storage guidelines for domestic refrigerators, CNRC guidelines for allergen storage have been adapted from the Australian Government Department of Health and Ageing National Vaccine Storage Guidelines.
The refrigerator:
- Must be positioned out of direct sunlight. Be aware of seasonal changes in temperature that may affect refrigerator temperature.
- Must used exclusively for storage of allergens
- Seals must be in good condition and the door close properly
- Must have adequate ventilation around the refrigerator with clearances of;
  - 30mm on each side
  - 25mm above
  - 50 – 75mm behind
- Water bottles are to be stored in the refrigerator compartment and door to assist with stabilisation of temperature by increasing ‘cold mass’.
- Store allergens at least 4 cm away from the walls of the refrigerator
- Allow adequate space between allergen containers for circulation
- Allergens must never be stored in the door of the refrigerator

NB: The manufacturer states that the rear liner of the Westinghouse 130L refrigerator at times may appear frosty or wet. This is normal and demonstrates that the refrigerator is functioning correctly.

5. TEMPERATURE MONITORING AND RECORDING PROCEDURE

The EMT 888 thermometer is used to monitor refrigerator temperature (Appendix 1) and shall be recorded by CNRC staff daily at the beginning of the day.

1. Read current temperature (largest number) from the display.
2. Record the current temperature on the Temperature Monitoring Record (Appendix 2)
3. Reset the EMT 888 by pressing the ‘Reset’ button

6. DATA LOGGING

Data logging allows the recording of temperature patterns over time and in conjunction with the electronic thermometer (EMT888) will ensure quality temperature monitoring. Periodic data logging will be undertaken by K Best and results downloaded and stored on the WCHN Network.
7. TROUBLESHOOTING PROCEDURE

Should the EMT 888 record a temperature less than 2⁰ C increase the refrigerator temperature by a small increment and notate this action on the Temperature Monitoring Record

Should the EMT 888 record a temperature greater than 8⁰ C reset the EMT 888 and perform the following checks;

- Check connections on the EMT888
- Check EMT 888 probe position in the refrigerator
- Check that the power cord of the refrigerator is plugged into the power outlet properly and that the outlet is switched on
- Check the refrigerator door is closing properly

Frequent opening or prolonged periods with the door open may cause an increase in temperature. After performing the following checks ensure that the refrigerator door is kept for 30 minutes prior to reading the current temperature.

If the refrigerator temperature is still outside of required parameters (2⁰ C and 8⁰) relocate allergens to an alternate refrigerator and notify Karen Best 0434243404 or Anna Seamark 0410 143 564.

8. REFERENCES

2. SmPC Staloral®

9. APPENDICES

Appendix A. Data sheet for electronic thermometer EMT 888

Appendix B. CNRC Temperature Monitoring Re
Appendix 17: ASCIA Abstract

Australasian Society of Clinical Immunology and Allergy (ASCIA), 2014

ABSTRACT

EFFECT OF MATERNAL DIETARY LONG CHAIN POLYUNSATURATED FATTY ACID INTAKE DURING PREGNANCY ON CLINICAL OUTCOMES OF ALLERGIC DISEASE IN THE OFFSPRING: A SYSTEMATIC REVIEW

Karen Best₁, ₂, Maria Makrides₁, ₂

₁ Women’s and Children’s Health Research Institute, Adelaide, Australia
₂ School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia

Background: The worldwide epidemic of allergic disease may be a consequence of our changing environment. Western diets have changed dramatically over the last 30-40 years, now comprising an overwhelming predominance of omega-6 (n-6) polyunsaturated fatty acid (PUFA) at the expense of omega-3 (n-3) PUFA. This change coincidently parallels the increasing prevalence of allergy, suggesting the imbalance may have a causal relationship.

Methods: Included publications were prospective randomised controlled trials (RCTs) that evaluated an intervention modifying maternal n-3 long chain poly-unsaturated fatty acid (LCPUFA) intake and observational studies that observed the association of maternal n-3 LCPUFA intake and clinical outcomes of allergic disease.

Results: A total of 13 publications from 10 observational studies and 7 publications representing five unique randomised controlled trials (RCT) were included in the review. Decreased incidence of allergic disease symptoms was evident in eight of thirteen observational studies. Incidence of eczema was reported in four RCTs with one study reporting a significant reduction in cumulative incidence of IgE associated eczema and another reporting significant reduction in severity of disease. Symptoms of recurrent wheeze, persistent cough and diagnosed asthma were reduced in one trial but did not reach statistical significance. There was no difference in asthma at 6 months and 0-24 months or 3 years. A registry based follow up reported a significant reduction in ‘any asthma’ and ‘allergic asthma’ at 16 years. Four trials assessing sensitisation by skin prick test all reported a significant protective effect; three of these reported a significant reduction in sensitisation to egg.

Discussion: Findings from this review suggest that increased supply of n-3 LCPUFA to the fetus through maternal intake may influence development and progression of allergic disease. Congruence between epidemiological evidence and RCTs, further supports this nutrient-disease association.
Appendix 18: PSANZ Abstract

Six Year Follow Up of Children at High Hereditary Risk of Allergy, Born To Mothers Supplemented With Docosahexaenoic Acid (DHA) in the DOMInO Trial

Best K1,2, Sullivan T6, Gold M1, Kennedy D4,5, Martin J4,5, Palmer D1,7, Makrides M1,2,3

1Women’s & Children’s Health Research Institute, University of Adelaide, North Adelaide, Australia
2School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia
3Healthy Mothers, Babies and Children, South Australian Health and Medical Research Institute, Adelaide, Australia
4Discipline of Paediatrics, University of Adelaide, Adelaide, Australia
5Department of Respiratory and Sleep Medicine, Women’s & Children’s Hospital, North Adelaide, Australia
6School of Population Health, University of Adelaide, Adelaide, Australia
7School of Paediatrics and Child Health, University of Western Australia, Subiaco, Australia

Email: karen.best@adelaide.edu.au

Background: Dietary omega-3 (n-3) long chain polyunsaturated fatty acids (LCPUFA) modulate neonatal markers of the immune response but there is uncertainty regarding the effect on clinical allergy outcomes. This double blind, randomised controlled trial aimed to determine whether supplementation with DHA rich fish oil during pregnancy to women with a fetus at high risk of allergic disease, will reduce the risk of allergy in the child.

Method: Between 2005 and 2008, pregnant women between 18-21 weeks gestation were randomly assigned to consume capsules containing ~1 g/d of DHA or a blended vegetable oil (no DHA) until birth, the DOMInO trial. From 2012-2014, 603 children (90% of eligible) born to mothers in the DOMInO trial, with a family history of allergic disease completed a six year of age follow up assessment. History of asthma, allergic rhinitis and eczema were assessed, along with food and aero-allergen sensitisation by skin prick testing.

Results: Preliminary results show no difference in the overall percentage of children with IgE-mediated allergic disease between the DHA and control groups (75/279 (26.8%) vs 79/263 (30.0%); RR 0.95; 95% CI 0.73,1.23; P=0.69), although there were fewer children with parent reported hay fever in the DHA group (68/314 (21.6%) vs 84/289 (29.1%); RR 0.75; 95%CI 0.57, 0.99; P=0.04) and fewer children were sensitised to house dust mite, D.Farinae, in the DHA group 31/246 (12.6%) vs 49/231 (21.2%) control; RR 0.61 (0.41, 0.92); P=0.019.

Conclusions: DHA supplementation in pregnancy did not reduce the overall incidence of IgE-mediated allergies at six years of age although parent reported hay fever and D.Farinae sensitisation were lower.